CONFIDENTIAL

PROTOCOL

Protocol Title: A Phase 2, Randomized, Open-Label Trial to Evaluate the Safety, Preliminary Efficacy, and Biomarker Response of Host Directed Therapies added to Rifabutin-modified Standard Antimicrobial Therapy in Adult Patients with Drug-Sensitive Smear-Positive Pulmonary Tuberculosis (TB HDT)

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4. Synopsis

Table 1. Protocol synopsis.

Study/PI/Sponsor	**TB HDT/Wallis/Aurum Institute**
Protocol Title:	A Phase 2, Randomized, Open-Label Trial to Evaluate the Safety, Efficacy, and Biomarker Response of Host Directed Therapies added to Rifabutin-modified Antimicrobial Therapy in Adult Patients with Drug-Sensitive Smear-Positive Pulmonary Tuberculosis (TB HDT)
Investigational host-directed agents:	CC-11050 (Celgene)
Non-investigational host-directed agents (approved for other indications):	Everolimus (Tradename: Certican), auranofin (Tradename: Ridauara), vitamin D (Tradename: Lennon)
Antimicrobial TB therapy:	Isoniazid plus pyridoxine (vitamin B_6), rifabutin, ethambutol, and pyrazinamide
Treatment Indication:	Drug-sensitive pulmonary tuberculosis (TB)
Study Purpose:	To examine the safety and preliminary efficacy of multiple adjunctive host directed TB therapies (TB HDT), to assess their potential to shorten TB treatment and/or prevent permanent lung damage.
Trial Objectives:	To determine the safety and preliminary efficacy of 4 TB HDT candidates: 1. Safety (treatment emergent serious adverse events and SUSARs) 2. Microbiologic effects in sputum (culture conversion, change in MGIT TTP) and blood (WBA) 3. PET/CT imaging 4. Serum markers of inflammation (CRP and neopterin) 5. Effects on *Mtb*-specific and general immune function 6. Pulmonary effects (spirometry, 6MWT, O_2 saturation, and St. George Respiratory Symptom Questionnaire) In each case, TB HDT effects will be determined by comparison to patients treated with standard TB therapy alone with regard to a common set of primary and secondary endpoints.
Primary Endpoint 1. Safety	a. For auranofin, everolimus, and vitamin D: the proportions of patients experiencing suspected unexpected serious adverse reactions (SUSARs). b. For CC-11050: the proportion of patients experiencing treatment emergent serious adverse events (SAEs).
Secondary Endpoints 1. Safety (cont'd)	c. TEAEs other than SAEs, categorized according to severity, drug relatedness, and leading to early withdrawal. d. Quantitative and qualitative clinical safety laboratory measurements, including observed and change from baseline. e. Quantitative and qualitative measurement of ECG results (heart rate, RR interval, PR interval, QRS interval, QT interval and QTc interval), including observed and change from baseline. f. Proportion of patients with disease exacerbation (change from baseline) at multiple time points using IRIS criteria

Study/PI/Sponsor	**TB HDT/Wallis/Aurum Institute**
Secondary endpoints (contd) 2. Microbiology:	a. Proportion of patients with positive sputum cultures on solid culture medium after 8 weeks of treatment b. The proportion of subjects with positive cultures at other time points, using solid and liquid medium c. Proportion of subjects with multiple positive cultures in liquid or solid medium after day 112 of treatment d. Time to stable culture conversion in liquid medium e. Change in MGIT TTP over time as examined by nonlinear regression f. Whole blood bactericidal activity expressed as a cumulative effect over the dosing interval (most likely only for sites in Gauteng). g. Baseline mycobacterial isolates will be tested for susceptibility to INH, RIF EMB, and PZA in MGIT. Patients whose treatment has failed (repeated positive cultures after day 112 to day 540) will have DST repeated. Those with new resistance will have deep sequencing performed on baseline and failure isolates to determine if mixed infection was present initially
3. ^{18}F-FDG PET/CT imaging (change from baseline to 2 months):	a. Maximum standardized uptake values (SUV) b. Mean SUV c. Additional PET and CT parameters (Exploratory) [1]
4. Serum markers of inflammation (change from baseline to 2 months):	a. Neopterin [2, 3] b. CRP
5. Mtb-specific immune function (change from baseline to 2 months):	a. Quantiferon gold in-tube (quantitative)
6. Pulmonary function (change from baseline to 2 and 6 months):	a. FEV_1 (% of expected value) b. 6 minute walk test (distance) (6MWT) c. Oxygen saturation at conclusion of 6 minute walk test (%) d. SGRSQ score
7. Exploratory Endpoints:	a. Change from baseline to 2 and 6 months in gene expression, proteomic, and metabolomic profiles b. PD-1 expression on CD4 and CD8 lymphocytes c. Urine for high-sensitivity LAM assay
Trial Design:	This Phase 2, experimental medicine trial will be a randomized, open-label, 5-arm, parallel group trial in drug sensitive TB patients with moderately advanced or far advanced pulmonary disease by chest X-ray. Willing patients providing informed consent will undergo screening evaluations to establish eligibility. Patients meeting all the inclusion and none of the exclusion criteria will be randomized to one of five treatment arms no more than 5 days from completing screening. All patients will receive 6 months of rifabutin-substituted standard therapy. Those randomized to one of the four HDT arms will concurrently receive TB HDT during the first 4 months of therapy. During the treatment period patients will undergo safety, efficacy, and biomarker assessments no less than monthly. All subjects will receive a final safety evaluation at the end of standard TB treatment (6 months). The study will be conducted in Africa under the direction of Robert Wallis, MD FIDSA (Aurum Institute).
Subject Population:	A total of 200 HIV-uninfected patients (40 per arm) with moderately or far advanced drug sensitive smear-positive pulmonary TB aged 18 to 65 years will be enrolled.

Study/PI/Sponsor	**TB HDT/Wallis/Aurum Institute**
Inclusion Criteria:	1. Willing and able to provide signed written consent or witnessed oral consent in the case of illiteracy, prior to undertaking any trial-related procedures. 2. Aged 18 to 65 years, male (willing to use condoms), or if female, either not of reproductive potential (post-menopause, or status-post surgical sterilization) or with a copper intrauterine contraceptive device (ParaGard ® T 380A / MULTILOAD ® cu250 / cu375) in place at least 24 hours prior to initiation (day 1) of TB treatment and or adjunctive Host Directed Therapy (HDT). IUD should remain in place up to day 180 (TB treatment completion) at a minimum 3. Body weight (in light clothing without shoes) between 40 and 90 kg. 4. Pulmonary tuberculosis diagnosed by positive sputum AFB smear microscopy scores ≥1+ with subsequent culture confirmation OR positive Xpert TB/RIF with Ct <20 [4]. 5. RIF susceptibility diagnosed by Xpert TB/RIF OR Hain test 6. Chest radiograph meeting criteria for moderate or far advanced pulmonary tuberculosis [5]. 7. HIV-1 seronegative 8. HBsAg negative
Exclusion Criteria:	1. Any condition for which participation in the trial, as judged by the investigator, could compromise the well-being of the subject or prevent, limit or confound protocol specified assessments 2. Current or imminent treatment for malaria. 3. 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. 4. TB treatment within the preceding 12 months. 5. TB meningitis or other forms of severe tuberculosis with high risk of a poor outcome as judged by the investigator. 6. History of allergy or hypersensitivity to any of the trial therapies or related substances, including known allergy or suspected hypersensitivity to rifampin or rifabutin. 7. Having participated in other clinical trials with investigational agents within 8 weeks prior to trial start or currently enrolled in an investigational trial. 8. Subjects with any of the following at screening: a. Cardiac arrhythmia requiring medication; b. Prolongation of QT/QT_c interval with QTcF (Fridericia correction) >450 ms; c. History of additional risk factors for Torsade de Pointes, (e.g., heart failure, hypokalemia, family history of Long QT Syndrome); d. Any clinically significant ECG abnormality, in the opinion of the investigator. e. Patients requiring concomitant medications that prolong the QT interval. 9. Random blood glucose >140 mg/dL (7.8 mmol/L), or history of unstable Diabetes Mellitus which required hospitalization for hyper- or hypo-glycaemia within the past year prior to start of screening. 10. Use of systemic corticosteroids within the past 28 days. 11. Subjects with any of the following abnormal laboratory values: a. creatinine >2 mg/dL b. haemoglobin <8 g/dL c. platelets <100x10^9 cells/L d. serum potassium <3.5 e. aspartate aminotransferase (AST) ≥2.0 x ULN f. alkaline phosphatase (AP) >5.0 x ULN g. total bilirubin >1.5 mg/dL **NB:** All of the inclusion and none of the exclusion criteria must be met

Study/PI/Sponsor	**TB HDT/Wallis/Aurum Institute**
Investigational and non-investigational HDT agents and doses:	1. CC-11050: 200 mg BID with food 2. Everolimus 0.5 mg QD 3. Auranofin 3 mg QD for 1 week, then 6 mg QD, with dose reduction permitted for tolerability 4. Vitamin D, a total of 3 doses: 5 mg initially (day 0), then 2.5 mg Q month for 2 doses (days 28 and 56).
Standard Treatment:	All patients will receive once daily antimicrobial TB chemotherapy consisting of: 1. Intensive phase (weeks 1-8): rifabutin 300 mg plus isoniazid 300 mg plus pyrazinamide 25 mg/kg plus ethambutol 20 mg/kg (see table for weight adjustments) plus pyridoxine 25 mg; 2. Continuation phase (weeks 9-26): rifabutin 300 mg plus isoniazid 300 mg pluse pyridoxine 25 mg.
Statistical and Sample Size Considerations:	A sample size of 40 per arm has been proposed for this trial. Mathematical modelling predicts that if a new 4-month regimen reduces the proportion positive after 2 months of treatment to 1%, it will reduce to 10% the risk of a relapse rate >10% in a trial with N=680 per arm [6, 7]. Poisson analysis indicates a 94% likelihood that the proposed target for the proportion culture positive at month 2 (1%) would result in ≤1 subject being positive out of 40. In contrast, the culture positive rates expected in the control arm (15%-20%) are very unlikely to yield this result (1.5%-0.3%), indicating the sample size will have adequate power.
Interim analysis	The DSMB will review preliminary safety and efficacy signals at regular intervals during the trial. A formal interim analysis will be performed when data through 2 months (including culture) are available from half the expected enrolment. The board will give specific attention to the proportion of subjects with serious adverse events, the proportion culture positive on solid medium at 2 months, and changes in FEV1 from baseline to 2 months. The DSMB will be directed to discontinue enrolment and treatment for arms in which any parameter appears likely to be worse than in the control arm. Specific guidance is provided to the DSMB to assist this assessment.
Pharmacokinetics (PK):	Blood samples will be collected using a sparse sampling approach for determination of drug concentration in plasma. Analyses will focus on drugs with greatest known intersubject PK variability. Most likely only for Gauteng province sites.
Pharmacokinetics-Pharmacodynamics (PK-PD):	Plasma concentrations will be used to build a population PK model to evaluate the effects of patient covariates on trial drug pharmacokinetics and to confirm adequate exposures to HDT with background rifabutin. Where possible, PK samples from the proposed trial will be pooled with high resolution PK data from other trials to inform the PK model. Most likely only for Gauteng province sites. A population PK-PD approach will be applied to the full time course of pharmacodynamic endpoints (e.g., immunologic biomarkers, transcriptomic signals, and culture conversion, and WBA) to estimate the temporal nature and magnitude of HDT effects.
Trial Timeline:	An enrolment rate of 6 subjects per week is targeted to provide an interim TLR on half of subjects in 9 months, a full TLR in 14 months, and a total study clinical duration of 18 months. There will be a further sputum collection at day 360 and 540 to assess TB symptoms, recurrence or re-infection taking the total study duration including treatment period to about 30 months.

5. Schedule of events

Table 2. Schedule of events.

	-3 to -1[1]	Day[2]															Early with-drawal
		0	1	7	14	21	28	35	42	56	84	112	140	180	360	540	
Informed Consent	X														X	X[3]	
Demog/Hist	X																
Incl/Excl	X																
HIV Test[4]	X										X						
Pregnancy Test[5]	X	X								X							X
IUD insertion/check[6]	X						X										
Lab Safety Tests[7]	X	X		X	X		X			X	X	X	X	X			X
12-lead ECG	X	X			X							X					X
Chest X-Ray	X						X			X	X	X	X	X			X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Physical Exam[8]	F	F	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
TB Symptoms	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
St George's Respiratory Questionnaire															X	X	
Xpert TB/RIF	X														X	X	
Culture LJ MGIT	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
DST[9]	X											X	X	X			
Con Med Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Randomization		X															
Standard therapy[10]			HRbZE								HRb						
HDT			HDT														
FDG PET/CT		X								X							
Spirometry/6MWT[11]			X		X		X			X	X	X	X	X	X	X	X
WBA 0,1,2,3,4,6,8 hrs									X		X		X				
PK Sampling[12]									X		X		X			X[13]	
Lymphocyte PD-1[14]		X								X				X			
Serum for proteomics and metabolomics		X			X					X				X			
PBMC, WB for gene expression profiling		X			X					X				X			
Serum biomarkers: (CRP and Neopterin)		X			X		X			X	X	X	X	X			
QFN+ (quantitative)		X			X		X			X	X	X	X	X			
Urine stored for LAM HS		X			X		X			X	X	X	X	X			
Adherence Check			X	X	X	X	X	X	X	X	X	X	X	X			
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X			X

[1] All screening procedures do not have to be performed on the same day
[2] Acceptable windows: day 7, ±1 day; day 14, ±2 days; days 21-84, ±3 days; days 112 - 360 later, ±7 days, day 540, 533 to 720..
[3] If necessary
[4] HIV test performed on-site (POC). Repeated at 3 months (day 84) to ensure initial HIV negative results were not due to the HIV latency window period. HIV – seroconversion would result in withdrawal from the study. HIV testing may not be performed by finger prick, but should be performed at POC by the **on-site laboratory** on blood collected from a 2ml EDTA tube.
[5] For female subjects; performed at POC by the **on-site laboratory.**
[6] For female subjects of childbearing potential. Copper IUD (ParaGard ® T 380A / MULTILOAD ® cu250 / cu375) and must be in place at least by day 0. Patients who have IUDs placed to facilitate trial participation will have the presence of the IUD confirmed by examination on day 28. IUD must remain in place until day 180. After day 180, IUD is no longer obligatory as participants will be off study medication.
[7] Lab Safety Tests: FBC (RBC, Hb, HCT, MCV, WBC) incl. Diff & Platelets; Potassium; Creatinine; Total Bilirubin; Alkaline Phosphatase; AST & Random Glucose.
[8] F=full, L=limited. Height will be measured at screening only.
[9] DST on or after day 112 if culture is positive
[10] H=isoniazid; Rb=rifabutin; Z=pyrazinamide; E=ethambutol. All will be administered once daily.
[11] An additional ± 1 day window will be allowed for performing FDG PET/CT scan.
[12] Samples will be obtained for WBA and PK pre-dose (0 hr) and at specified times post dose. The times at which blood samples are obtained will be recorded. The times of the prior day's dose and that given in clinic on the study visit day will be recorded. Administration of study medications must only occur once phlebotomy for 0hr time point for Pk sampling and WBA has been completed, thus administration of treatment must be directly observed (DOTs), after the 0hr time point, on-site at these visits **only**. The 1 hr time point is to occur 1hr post-dose (directly observed) and NOT 1 hr post 0 hr phlebotomy.
[13] If necessary
[14] Everolimus and control arms only

6. Investigators and Institutions

Institution	Name	Role	Telephone	E-mail
The Aurum Institute, South Africa	Prof. Robert S Wallis	International Principal Investigator	+27 (0) 10 590 1322	rwallis@auruminstitute.org
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7. Protocol Title

A Phase 2, Randomized, Open-Label Trial to Evaluate the Safety, Efficacy, and Biomarker Response of Host Directed Therapies added to Rifabutin-modified Antimicrobial Therapy in Adult Patients with Drug-Sensitive Smear-Positive Pulmonary Tuberculosis (TB HDT)

8. Background

Major unmet medical needs exist for all forms of *M tuberculosis* infection. Patients with MDR TB urgently require treatments that quickly eradicate active infection while preventing additional resistance, which otherwise causes failure and death. Half of all TB patients regardless of drug susceptibility require interventions to prevent permanent lung injury, which results in cough, breathlessness, disability, and reduced longevity. All patients need shorter regimens that do not increase the risk of relapse, which to date have been elusive [8-10].

Adjunctive host-directed therapies (HDTs) can help address these needs [11]. Agents promoting the antimicrobial activity of phagocytic cells have the potential to prevent new resistance and accelerate eradication of infection, by promoting vesicular acidification, autophagy, cellular production of antimicrobial peptides, and other effector mechanisms. Alternatively, agents that block or modify inflammatory mechanisms can prevent matrix destruction and lung injury, and enhance antimicrobial drug penetration and action. The spectrum of candidates is broad, as it includes many drugs approved for other indications. There is no single, accepted development path for the repurposing of licensed drugs as TB HDT agents. Indeed, the preclinical evaluation of these candidates has been problematic due to the lack of animal models validated for this purpose. Cyclooxygenase inhibitors, for example, can be beneficial, indifferent, or harmful as TB HDT, depending on the mouse strain in which they are tested [12-14]. As a result, many HDT candidates are best evaluated in clinical trials.

This project will examine 4 candidate TB HDT agents during the first 4 months of antimicrobial chemotherapy for drug-sensitive TB, to determine the extent to which the agents can shorten TB treatment and prevent permanent lung injury. The study is designed as an experimental medicine trial in which multiple endpoints, validated and novel, will be evaluated. Such trials are intended to increase knowledge of disease pathogenesis and cure, and increase experience with novel biomarkers and endpoints, as well as testing specific therapeutic interventions. Experimental medicine trials are most appropriate when 3 criteria are met: ***1)*** ordinary clinical trials have not reached anticipated outcomes; ***2)*** animal models have major shortcomings; and ***3)***.Knowledge regarding the safety profiles of the proposed interventions is sufficient to avoid placing patients at risk. All 3 criteria are met in the present case.

The study will enrol subjects into 4 experimental arms and 1 control arm. All patients will receive antimicrobial TB chemotherapy consisting of 2 months of daily isoniazid (plus pyridoxine), rifabutin, pyrazinamide, plus ethambutol, followed by 4 months of daily isoniazid (plus pyridoxine) plus rifabutin. Rifabutin is substituted for rifampin due to undesirable PK DDIs with 2 study drugs (CC-11050 and everolimus). Patients assigned to the control arm will receive no additional therapy. Patients enrolled into the experimental arms will additionally receive either CC-11050 (a type 4 phosphodiesterase inhibitor with anti-inflammatory properties), everolimus (an mTOR inhibitor that promotes autophagy, thereby reducing intracellular *M. tuberculosis* survival), auranofin (an oral gold preparation with both anti-inflammatory and direct antimicrobial properties) or vitamin D (essential for host defences against many intracellular pathogens). Forty subjects will be studied in each arm. Endpoints will assess effects on sputum microbiology, respiratory function, inflammatory markers in blood, lung inflammation as measured by PET/CT, blood cell gene expression profiles, whole blood bactericidal activity (WBA), and other biomarkers. Patients will be followed until standard treatment

is completed on day 180. An external data safety monitoring board will review preliminary efficacy and safety signals at multiple time points during the trial. An interim analysis will be performed when month 2 data (including cultures) are available from 20 subjects per arm to assess safety and efficacy. This study is being supported by the Bill and Melinda Gates Foundation, which selected the agents to be studied, but has no potential conflicting interest with its possible outcome.

9. Treatment Indication

Adjunctive treatment of drug-sensitive pulmonary tuberculosis

10. Study Purpose

To examine the safety and preliminary efficacy of multiple adjunctive host directed TB therapies (TB HDT), to assess their potential to shorten TB treatment and/or prevent permanent lung damage. The adjunctive agents will include everolimus, vitamin D, auranofin, and CC-11050, given together with rifabutin-modified antimicrobial treatment (2HRbZE/4HRb) in patients with drug sensitive pulmonary TB. As an experimental medicine trial, the study will include multiple novel biomarker endpoints, to increase knowledge regarding these biomarkers and provide insights into the process of TB cure.

11. Study Objectives

To determine the safety and preliminary efficacy of multiple TB HDT agents, and to assess the potential role of multiple novel biomarkers to evaluate these agents.

12. Trial Design

The trial will be open label. Patients will be randomly assigned on day 0 to the 5 arms at a ratio of 1:1:1:1:1 (Figure 1) in blocks of 10. All patients will receive anti-tuberculosis chemotherapy starting on study day 1 consisting of 8 weeks of daily isoniazid (plus pyridoxine), rifabutin, pyrazinamide, plus ethambutol, followed by 18 weeks of daily isoniazid (plus pyridoxine) plus rifabutin. Patients assigned to an experimental arm will additionally receive a host-directed agent, starting on day 1, for 16 weeks.

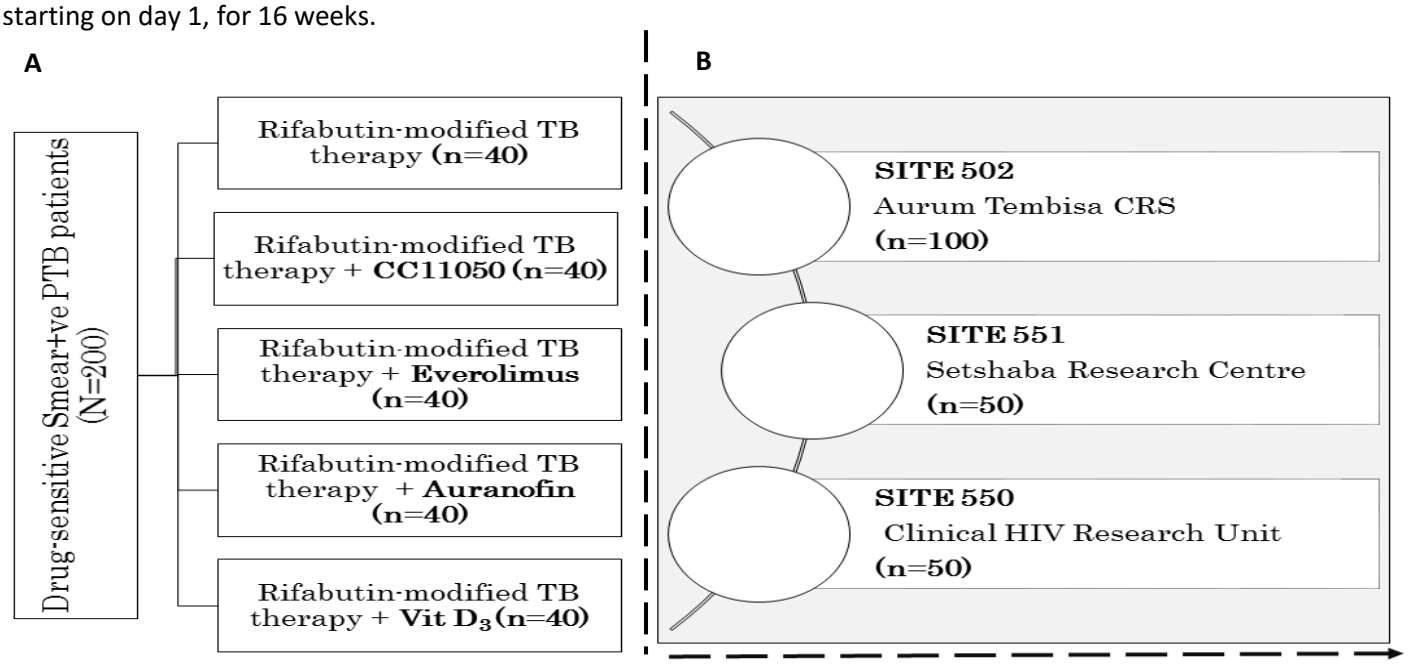


Figure 1. *Study Schematic. **A:** Randomisation of 200 subjects (1:1:1:1:1), 40 per treatment arm. **B** Selected sites and target enrolment.* *Subject Population*

A total of 200 HIV-uninfected patients (40 per arm) with moderately or far advanced drug sensitive smear-positive pulmonary TB aged 18 to 65 years will be enrolled. Patients must meet all inclusion criteria and no exclusion criteria.

12.1. Informed Consent

Informed consent will be sought from potential participants using information sheets available in relevant languages. Written informed consent will be sought, with the assistance of a translator where necessary, using standard consent forms. Participants unable to read or write will be asked to make a mark or thumbprint in the presence of a witness.

12.2. Randomization

Subjects will be randomly assigned to the 5 treatment arms at a ratio of 1:1:1:1:1 (*ie*, 40 per arm). **NB**: In October 2017, enrolment was temporary halted into the auranofin arm due to a drug shortage. During that month, 3 patients who would have been assigned to auranofin were assigned to the next treatment arm. The shortage was relieved by a shipment of auranofin from a new supplier (Sebela Pharma) on 31-October-2017. Enrolment into the auranofin arm resumed shortly thereafter. Following the approval of this protocol revision, a new randomization schedule will be prepared to randomly replace the 3 auranofin recipients among the remaining subjects. This procedure will maintain the original randomization schedule of 40 per arm.

12.3. Inclusion Criteria

12.3.1. Willing and able to provide signed written consent or witnessed oral consent in the case of illiteracy, prior to undertaking any trial-related procedures.
12.3.2. Aged 18 to 65 years, male (willing to use condoms), or if female, either not of reproductive potential (post-menopause, or status-post surgical sterilization) or with a copper intrauterine contraceptive device (ParaGard ® T 380A / MUL-TILOAD ® cu250 / cu375) in place at least 24 hours prior to initiation (day 1) of TB treatment and or adjunctive Host Directed Therapy (HDT). IUD should remain in place up to day 180 (TB treatment completion) at a minimum
12.3.3. Body weight (in light clothing without shoes) between 40 and 90 kg.
12.3.4. Pulmonary tuberculosis diagnosed by positive sputum AFB smear (microscopy score ≥1+) with subsequent culture confirmation OR positive Xpert TB/RIF with Ct <20 [4].
12.3.5. RIF susceptibility diagnosed by Xpert TB/RIF OR Hain test
12.3.6. Chest radiograph meeting criteria for moderate or far advanced pulmonary tuberculosis [5] (Appendix 1).

12.4. Exclusion Criteria:

12.4.1. Any condition for which participation in the trial, as judged by the investigator, could compromise the well-being of the subject or prevent, limit or confound protocol specified assessments
12.4.2. Current or imminent treatment for malaria.
12.4.3. 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.
12.4.4. Tuberculosis treatment within the preceding year.
12.4.5. TB meningitis or other forms of severe tuberculosis with high risk of a poor outcome as judged by the investigator.
12.4.6. History of allergy or hypersensitivity to any of the trial therapies or related substances, including known allergy or suspected hypersensitivity to rifampin or rifabutin.
12.4.7. HIV-1 seropositive
12.4.8. HBsAg positive
12.4.9. Having participated in other clinical trials with investigational agents within 8 weeks prior to trial start or currently enrolled in an investigational trial.
12.4.10. Cardiac arrhythmia requiring medication;
12.4.11. Prolongation of QT/QT_c interval with QTcF (Fridericia correction) >450 ms;
12.4.12. History of additional risk factors for Torsade de Pointes, (e.g., heart failure, hypokalemia, family history of Long QT Syndrome);
12.4.13. Any clinically significant ECG abnormality, in the opinion of the investigator.
12.4.14. Patients requiring concomitant medications that prolong the QT interval.
12.4.15. Random blood glucose >140 mg/dL (7.8mmol/L), or history of unstable Diabetes Mellitus which required hospitalization for hyper- or hypo-glycaemia within the past year prior to start of screening.
12.4.16. Use of systemic corticosteroids within the past 28 days.
12.4.17. Creatinine >176.84 µmol/L
12.4.18. Haemoglobin <8 g/dL
12.4.19. Platelets <$100x10^9$ cells/L
12.4.20. Serum potassium <3.5
12.4.21. Aspartate aminotransferase (AST) ≥2.0 x ULN
12.4.22. Alkaline phosphatase (AP) >5.0 x ULN
12.4.23. Total bilirubin >132.63 µmol/L

13. Investigational HDT agents and doses

13.1. CC-11050 (Celgene) 200 mg BID with food (day 1 up to and including day 112)

13.1.1. Background.

TNF is central to inflammation and lung injury in tuberculosis. Studies of adjunctive anti-TNF therapies in tuberculosis have shown promise, but have been limited by the agents available for testing. Corticosteroids, for example, accelerate the resolution of signs and symptoms in tuberculosis[15]; in high doses (>130 mg/kg prednisolone per day), they reduce TNF production and accelerate sputum culture conversion [16, 17]. However, the safety profile of corticosteroids at such high doses proved to be unacceptable [17]. Thalidomide, a small molecule inhibitor of TNF production [18], has shown benefits in erythema nodosum leprosum, an inflammatory complication of leprosy [19], and in case reports in patients with refractory CNS TB paradoxical reactions [20, 21]. However, the sole randomized controlled trial of adjunctive thalidomide in paediatric TB meningitis was halted by its safety monitoring board due to excess deaths in the thalidomide arm that appeared to be allergic in aetiology [22]. Subsequent studies have shown the effects of thalidomide to be complex, also stimulating CD4 and CD8 T cells and expanding NK T cells. Etanercept (soluble TNF receptor) is a TNF blocker approved by multiple agencies for treatment of rheumatoid arthritis (RA), psoriasis, psoriatic arthritis (PA), ankylosing spondylitis (AS), and juvenile idiopathic arthritis (JIA). In C3HeB/FeJ mice receiving standard TB treatment, human etanercept yielded small benefits on inflammatory pathology and CFU counts[23]. One prospective controlled trial of etanercept in human tuberculosis found it to be safe, with small improvements in sputum culture conversion, chest radiography, and symptoms [24]. However, these small effects are consistent with the relative lack of clinical efficacy of etanercept in other chronic granulomatous inflammatory conditions such as sarcoidosis and Crohn's disease (CD)[25].

In the 1990s, Celgene Corporation initiated R&D to develop orally bioavailable anti-TNF agents. Compounds were identified that reduced TNF expression by inhibiting type 4 phosphodiesterase (PDE_4i), thereby increasing cyclic adenosine monophosphate (cAMP). One early PDE_4 inhibitor, CC-3052, was studied as an adjunct to INH monotherapy in the mouse and rabbit TB models, in which it reduced necrosis, fibrosis, granuloma number and size, and accelerates the INH-driven reduction in mycobacterial burden [26-29]. The TNF IC_{50} of CC-3052 is approximately 3 μM [30]. CC-3052 has never been tested in clinical trials.

Apremilast **CC-11050**

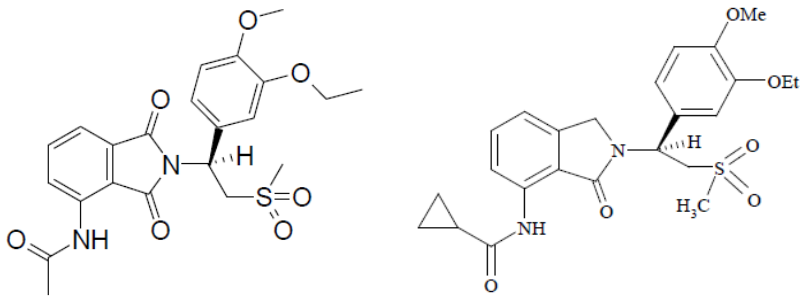


Figure 2. *Chemical structures of apremilast and CC-11050.*

CC-1088, the first PDE_4i to enter human trials, was equally potent to thalidomide in inhibiting TNF production in LPS-stimulated whole blood cultures. While well tolerated, clinical activity required high drug doses (1.5 grams per day). Apremilast (CC-10004, Ortezla®) was the next compound in this series to be evaluated (figure 2). Its TNF IC_{50} is approximately 0.1 μM, more than 5000-fold lower than CC-1088 [31]. Apremilast received US FDA approval in 2014 for the treatment of psoriasis and psoriatic arthritis in adults. Additionally, apremilast has performed well in clinical trials for ankylosing spondylitis, and Behçet's Disease [32]. Celgene is in the process of obtaining supplemental approvals of apremilast for these three indications.

CC-11050, the compound proposed for the present study, was developed at Celgene as a back-up compound should apremilast not perform as anticipated. Like apremilast, CC-11050 blocks the degradation of cAMP via inhibition of PDE_4. Results from various *in vitro* cellular assays demonstrate that CC-11050 can inhibit the key drivers of pathophysiology of several inflammatory and immune diseases. Its TNF IC_{50} is 0.1 to 0.2 μM (50-100 μg/ml). Therapeutic concentrations of CC-11050 also inhibit IL-12, MIP-1α, IL-2, IL-10, and IL-17, but have little effect on IL-1β or RANTES.. Key elements of the IB are summarized here.

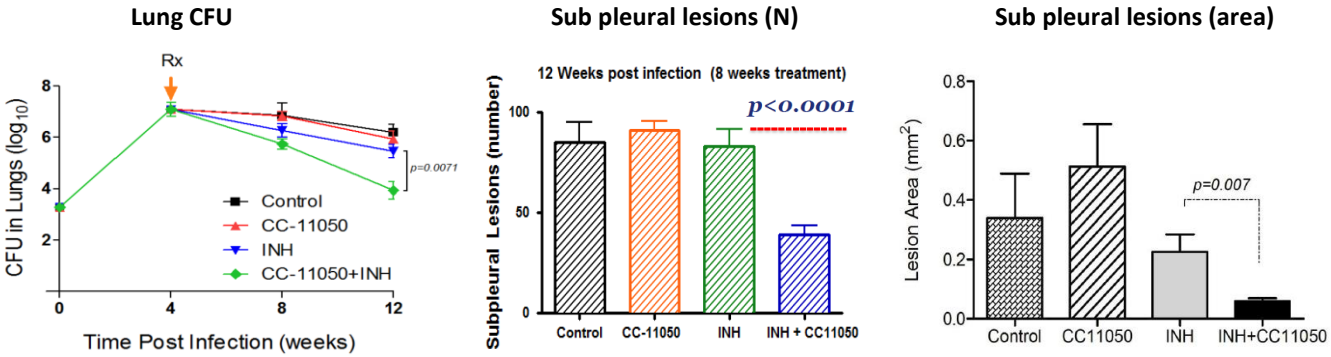


Figure 3. *Effects of CC-11050 in Mtb-infected rabbits.*

13.1.2. Experience in experimental TB models.

In *M. tuberculosis*-infected mice, CC-11050 down-regulates genes in multiple inflammatory networks, including those associated with IL-17, IL-23, and NFkB. CC-11050 accelerates the clearance of *M. tuberculosis* from the lungs when added to isoniazid therapy in chronic infection models in mice and rabbits (left panel, figure 3). In rabbits, CC-11050 similarly reduces the number and size of sub pleural lesions (center and right panels, figure 3). CC-11050 is currently being studied by Dr. J. Flynn as an adjunct to INH in the cynomolgus macaque TB model.

13.1.3. Metabolism and elimination.

In cryopreserved rat, rabbit, monkey, and human hepatocytes, major biotransformation pathways of CC-11050 included O-demethylation, O-deethylation, and N-deacylation, coupled with glucuronidation; ring oxidation (hydroxylation) was a minor pathway. All metabolites formed by the human hepatocytes were also formed by at least 1 of the 3 animal species tested. In human liver microsomes, the oxidative metabolism of CC-11050 is predominantly catalyzed by cytochrome P450 (CYP)3A4/5, with minor contribution by CYP2C8. As a result, drug-drug interaction studies will be required if CC-11050 is to be administered concomitantly with rifampin or, alternatively, rifabutin.

In rats and monkeys, the hepatobiliary route was the predominant clearance route of [^{14}C]-CC-11050, with very minimal urinary excretion. The absorbed fraction of the dose was primarily cleared as metabolites, with a very minor amount eliminated as unchanged parent. The major metabolic pathways of [^{14}C]-CC-11050 in vivo in rats and monkeys were oxidative metabolism, with subsequent glucuronidation. In rats, the major circulating components in plasma were unchanged CC-11050, M7 (O-desmethyl glucuronide CC-11050), and M13 (O-desethyl CC 11050), while 3 other minor metabolites were also detectable. In monkey plasma, unchanged CC-11050, M15 (O-desmethyl CC-11050), and M7 were the major components of circulating [^{14}C]-radioactivity. In monkey, metabolite M15 was the dominant component in plasma and the relative exposures of M15 to the parent CC-11050 were approximately 4- and11-fold higher in male and female monkeys, respectively. The exposure of the other major metabolite (M7) was approximately 50% to 66% of the exposure of CC-11050 in monkey. Other minor metabolites were present in monkey plasma.

CC-11050 is a relatively low clearance compound, with a moderately long terminal half-life in rats and monkeys. The oral bioavailability of a methylcellulose suspension formulation was low and ranged from 10% to 17% in rats and monkeys. In vitro plasma protein binding of CC-11050 was moderately high in rats, monkeys and humans, ranging from ~95% in rats and monkeys to ~97% in humans. Plasma protein binding for M15 (also referred to as CC-16361 or O-desmethyl metabolite of CC-11050), a major human metabolite, was comparable to CC-11050 and ranged from ~87% to ~98%. Following oral dosing of [^{14}C]-CC-11050 to male Long-Evans rats, radioactivity distributed widely into tissues except the brain.

Based on the results of in vitro studies, CC-11050 by itself is not likely to cause drug-drug interactions (DDIs) due to inhibition or induction when co-administered with substrates of CYP enzymes. Metabolite M15 showed approximately 50% inhibition of CYP2C9, CYP2C19, and CYP3A4 activities in human liver microsomes, suggesting modest potential for DDIs.

13.1.4. Preclinical toxicology.

CC-11050 and M15 have been investigated in a series of nonclinical studies to evaluate its safety pharmacology. *In vitro*, there was no significant inhibition of the human ether-à-go-go related gene (hERG) potassium channels by CC-11050 or M15. No adverse effects on cardiovascular or respiratory function were noted in monkeys following acute oral administration of doses up to 1000 mg/kg of CC-11050 nor were there any adverse effects on the central nervous system in rats receiving acute oral doses of up to 1000 mg/kg (male) and 300 mg/kg (females) of CC-11050. CC-11050 is not anticipated to have an effect on neurobehavior, respiratory, or cardiovascular function in humans.

CC-11050 has been evaluated in repeat-dose toxicity studies of up to 6-months duration in rats and 9-months duration in monkeys. The no-observed-adverse-effect levels (NOAELs) for rats were 150 and 45 mg/kg/day for males and females, respectively. The area under the concentration-time curves (AUCs) on Day 180 at the NOAEL doses were 7434 and 13150 ng•hr/mL in male and female rats, respectively. The NOAELs for monkeys was the maximum dose tested, 1000 mg/kg/day, for males and females. The AUCs on Day 177 at the NOAEL doses were 13070 and 21970 ng•hr/mL in male and female monkeys, respectively. The exposure margins based on the maximum clinical dose of 200 mg twice daily (area under the concentration-time curve from time 0 to infinity [AUC0-∞] = 4302 ng•hr/mL) were approximately 1.7-fold for male rats and 3- to 5-fold for female rats and male and female monkeys.

No adverse findings have been observed in monkeys; however, in rats, at exposures that are ~2 to 5-fold the maximum projected exposure in humans, the gastrointestinal tract was identified as the primary target organ in repeat-dose toxicity studies. Toxicity manifests as inflammation of the mucosa, muscularis, and/or serosa in the gastrointestinal tract, primarily in the small intestines (jejunum and ileum). Inflammatory changes were associated with erosions and ulcerations, as well as inflammation of the adjacent arteries. Inflammation was also observed in the mesentery and was associated with neovascularization, inflammation of adjacent arteries and atrophy of the adipocytes, and occasionally extended to the pancreas. These inflammatory changes receded, although residual microscopic findings persisted, after the cessation of dosing.

Preclinical studies of CC-11050 showed only minor distinguishing characteristics relative to apremilast. CC-11050 inhibits angiogenesis in more models than apremilast. In the ferret emesis model, CC-11050 causes less nausea and vomiting than apremilast (therapeutic index of >100:1 versus 30:1). Indeed, apremilast requires a 1-2 week initial dose escalation period to prevent nausea; this does not appear to be necessary for CC-11050.

13.1.5. Mutagenesis and foetal effects.

CC-11050 was nonmutagenic and nonclastogenic in the *in vitro* bacterial (Ames assay) and mammalian (mouse lymphoma assay) mutagenicity assays and the *in vivo* mammalian erythrocyte micronucleus assay. Metabolite M15 was nonmutagenic in the Ames assay.

No test article-related malformations were observed in rat or rabbit fetuses at doses of up to 50 and 500 mg/kg administered to pregnant rats and rabbits, respectively, on Gestation Days (GD) 6 to 17 for rats and 7 to 20 for rabbits. The exposure margins based on the maternal AUCs and the clinical dose of 200 mg twice daily (BID) were approximately 3- and 2-fold for rats and rabbits, respectively. In a rat fertility study, CC-11050 affected male and female fertility at doses of 450 mg/kg and ≥45 mg/kg/day, respectively. Based upon decreases in mating, fertility, and fecundity indices the NOAEL for male reproductive performance and fertility was determined to be 150 mg/kg/day (Day 28 AUC0-24 of 13161 ng•hr/mL). Based upon the decreases in viable embryos, increases in post-implantation loss and total resorptions and decreases in female mating, fertility, and/or fecundity indices, the NOAEL for reproductive performance, fertility, and early embryonic development in females was 15 mg/kg/day(Day 14 AUC0-24 of 6285 ng•hr/mL).

13.1.6. Clinical pharmacology.

In 6 completed clinical Phase 1 studies in healthy male subjects, the pharmacokinetics of CC-11050 were characterized by a moderate rate of absorption with maximum plasma concentration (Cmax) occurring at a median maximum time (Tmax) of approximately 3 hours for all doses studied. Steady-state plasma concentrations were achieved by Day 5 in the multiple-dose study. At the end of 10 days of dosing (50 to 200 mg once daily [QD]), there was a less than dose-proportional increase in Cmax and AUC0-∞. Mean half-life (t½) ranged from 11 to 18 hours following 10-day repeated dose administration, with accumulation ratios of 1.1 to 1.5. Renal elimination of CC-11050 was minimal (<0.5% of the administered dose excreted in the urine following a single dose and at steady state), suggesting that CC-11050 is cleared primarily by nonrenal mechanisms. Based on the biologic activity of achieved plasma concentrations of CC-11050 and its metabolites M15 and M7 in measured phase 1 trials, doses of 200 mg BID were predicted to produce >40% inhibition of TNF for >2/3 of the dosing interval. A robust formulation has since been developed that increases the AUC by over three-fold. It is anticipated that this will produce greater and more sustained TNF inhibition.

Concomitant administration of CC-11050 with ketoconazole, a potent CYP3A4 inhibitor, produced an approximate 54% increase in systemic exposure to CC-11050. Based on this finding, a significant PK DDI was anticipated if CC-11050 is to be co-administered rifampin, a potent CYP3A4 inducer. Celgene therefore conducted a study of the safety, tolerability and PK of CC-11050 after single and multiple doses of CC-11050 and to evaluate CC-11050 PK under fasted and fed conditions and after steady-state administration with isoniazid and rifampin or isoniazid and rifabutin. The study enrolled a total of 24 subjects. Key findings are shown in figure 4 and tables **3** and **4**. Rifampin had a major effect on CC-11050 PK, reducing exposure by 62%, whereas rifabutin had minimal effect on CC-11050 or its main metabolite. Based on these observations, CC-11050 will be administered with food, with rifabutin substituted for rifampin.

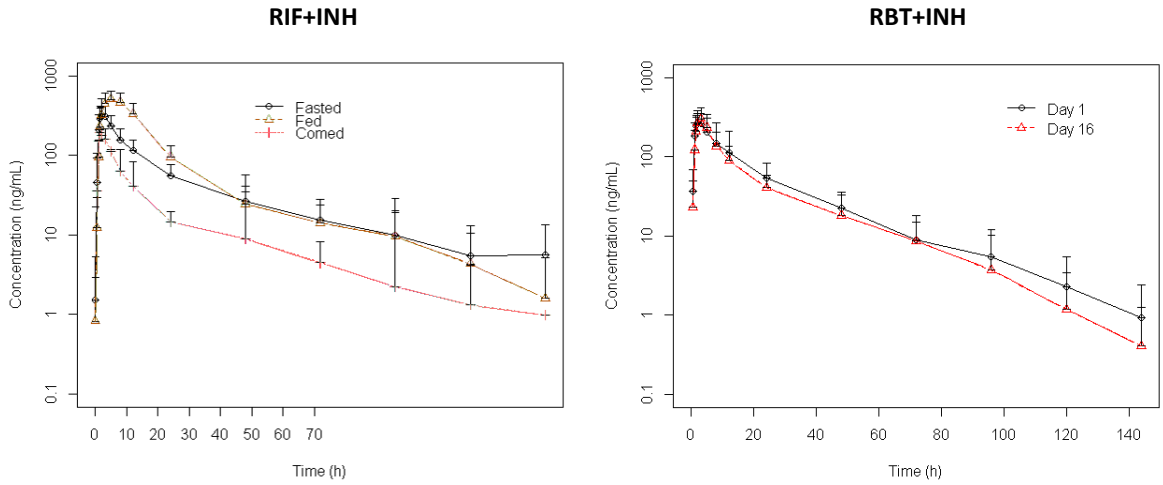


Figure 4. *Effects of TB drugs on CC-11050 PK.*

Table 3. Effects of feeding and rifampin plus isoniazid on CC-11050 PK.

	Fasted			Fed			Fasted, w/ RIF+INH		
Variable	**N**	**Geomean**	**CV%**	**N**	**Geomean**	**CV%**	**N**	**Geomean**	**CV%**
$AUC_{0-\infty}$ (ng*h/mL)	16	5287	37	16	8938	29	15	2035	32
AUC_{0-t} (ng*h/mL)	16	4958	34	16	8695	29	15	1902	31
CL/F (L/h)	16	113	37	16	67	29	15	295	32
C_{max} (ng/mL)	16	340	31	16	540	28	15	195	29
$t_{1/2}$ (h)	16	20	85	16	18	70	15	22	72
t_{max} (h)*	16	2	(1-3)	16	5	(2-8)	15	1.5	(1-3)
Vz/F (L)	16	3349	69	16	1790	73	15	9374	57

Table 4 . Effects of rifabutin plus isoniazid on CC-11050 PK.

	Fasted			Fasted, w/ RBT+INH		
Variable	**N**	**Geomean**	**CV%**	**N**	**Geomean**	**CV%**
$AUC_{0-\infty}$ (ng*h/mL)	16	4174	45	15	3677	44
AUC_{0-t} (ng*h/mL)	16	4080	46	15	3622	45
CL/F (L/h)	16	144	45	15	163	44
C_{max} (ng/mL)	16	281	36	15	299	36
$t_{1/2}$ (h)	16	18	46	15	14	44
t_{max} (h)*	16	2	(1-8)	15	3	(1.5-5)
Vz/F (L)	16	3647	75	15	3229	56

Rifampin additionally markedly reduces exposure to everolimus, also to be tested in this trial. As a result of these findings, rifabutin has been substituted for rifampin in all study arms. The suitability of rifabutin for TB treatment is discussed in section 15.1 below.

Administration of CC-11050 with a high-fat meal resulted in delayed absorption (increased tmax) and increased overall absorption (increased Cmax and AUC). The geometric mean absolute oral bioavailability (Fabs) of CC-11050 compared to IV was 24.59%. A study of orally administered [^{14}C]-radio-labeled CC-11050 showed that CC-11050 is extensively metabolized. Total recovery of [^{14}C]-radiolabel averaged 77.68% of the administered dose, with 73.45% recovered from feces and 4.24% recovered from urine. [^{14}C]-CC-11050 and 9 metabolites were identified in human plasma. The 3 most abundant radioactive components in plasma were metabolite M15 (O-desmethyl CC-11050), followed by unchanged [^{14}C]-CC-11050, and metabolite M7 (O desmethyl glucuronide). The systemic exposure (as AUC0-t) for each metabolite relative to CC-11050 was 17.5% for metabolite M7 and 986% for metabolite M15. Three minor circulating metabolites, M1, M9, and M13, each accounted for between 3% and 6% of exposure relative to [^{14}C]-CC-11050. The remaining 4 circulating metabolites, M2, M10, M17, and M22, were detected at trace levels. Although metabolic profiles were qualitatively similar across the species, there were some quantitative differences.

Comparison of metabolic profiles in plasma indicate that human profiles were similar to monkey profiles in that CC-11050, M15, and M7 were the major circulating components, and exposure of M15 is approximately 4- to 11-fold higher compared to CC-11050 in monkeys. It is calculated that exposure of M15 metabolite in the toxicology studies in

monkeys is approximately equivalent to or exceeds the potential estimated exposure in humans at the maximum proposed clinical dose of 200 mg BID. Pharmacological activity of M15 was also characterized and was found to be less potent (58-fold) compared to CC-11050 on PDE4 inhibition, and exhibited one-fifth of the activity of CC-11050 in human whole blood (HWB) TNF-α assays at expected relative plasma concentrations of M15 and CC-11050. Based on plasma levels of M15 and its in vitro pharmacodynamic properties, the metabolite will be monitored in clinical studies.

13.1.7. Clinical safety.

The safety profile of CC-11050 has been investigated in 7 clinical studies: 6 Phase 1 studies in 116 healthy male subjects and 1 Phase 2 study in 48 subjects with cutaneous lupus erythematosus (CLE). In these studies, CC-11050 has been administered at daily doses ranging from 10 to 400 mg in healthy subjects and 100 to 400 mg in subjects with CLE. The safety of CC-11050 has been primarily derived from the 6 Phase 1 clinical pharmacology studies. The Phase 2 study is complete and the data analysis is ongoing. No clinical efficacy data are available at the present time.

In the clinical pharmacology studies, no deaths or serious adverse events were reported. There were no clinically significant abnormalities in vital sign measurements, 12-lead electrocardiogram (ECG) assessments, or physical examination findings. One subject was discontinued from a study due to rash and pruritus, which occurred during the administration of ketoconazole alone and was presumed to be an allergic reaction to ketoconazole. Headache was the most frequently reported adverse event. The majority of the adverse events were mild in severity, not suspected of having a relationship to study medication, and resolved spontaneously by the end of the study.

In the clinical pharmacology studies, 6 subjects had clinically significant clinical laboratory results that were reported as adverse events: increase in serum lipase concentrations (4 subjects), decrease in absolute neutrophil count (1 subject), and increase in alanine aminotransferase (ALT) (1 subject). All of these adverse events were mild in severity and resolved spontaneously. The highest lipase concentration was approximately 4 times the upper limit of normal, occurred in a patient randomized to placebo. Two of the cases of increased lipase (the highest being 1023 U/L, which was approximately 3 times the upper limit of normal [ULN]) and an instance of increased ALT (131 U/L, which was approximately 2 times the ULN) were suspected to have a relationship to study medication. There were no clinically overt signs of pancreatitis accompanying the instances of increased serum lipase.

Preliminary safety data from the Phase 2 study of CC-11050 in subjects with cutaneous lupus erythematosis indicate that no deaths or treatment-emergent serious adverse events were reported. The most frequently reported nonserious adverse events (≥ 6 subjects) were fatigue (11 subjects) and back pain, headache, and nausea (all reported in 6 subjects for each term).

Celgene has not proceeded further with the development of CC-11050 for rheumatologic or dermatologic indications due to the marked overlap in its properties with those of apremilast. Instead, Celgene Global Health has been allowed to develop CC-11050 for illnesses that affect low and middle income settings including tuberculosis, other granulomatous diseases, and immune reconstitution inflammatory syndrome (IRIS). The study proposed here will be among the first of these trials.

The main risk posed by CC-11050 is that clinically meaningful effects may not be evident until late in TB treatment. Like all compounds in phase 2 trials, the safety profile of CC-11050 is incompletely understood. This is partially mitigated by the safety demonstrated in preclinical and clinical studies to date, and by the demonstrated safety and efficacy of other approved drugs in its class.

14. Non-investigational HDT agents and doses

These drugs have been approved by MCC for other indications.

14.1. Everolimus 0.5 mg QD (day 1 up to and including day 112)

Inhibition of phagolysosome fusion is a key pathogenic mechanism of *M. tuberculosis.* It can potentially be overcome by the induction of autophagy, which is a cellular process that delivers potentially harmful cytosolic macromolecules and organelles to lysosomes for degradation [33, 34]. Permeabilization of the phagosome by the *M. tuberculosis* ESX-1 secretion system is an Achilles heel permitting cytosolic components of the ubiquitin-mediated autophagy pathway access to bacilli that are otherwise contained in phagosomes, resulting in reduced *M. tuberculosis* survival [35]. Induction of autophagy can also have secondary effects, such as promoting antigen presentation and reducing inflammation, by sequestering and processing microbial components.

Sirolimus **Everolimus**

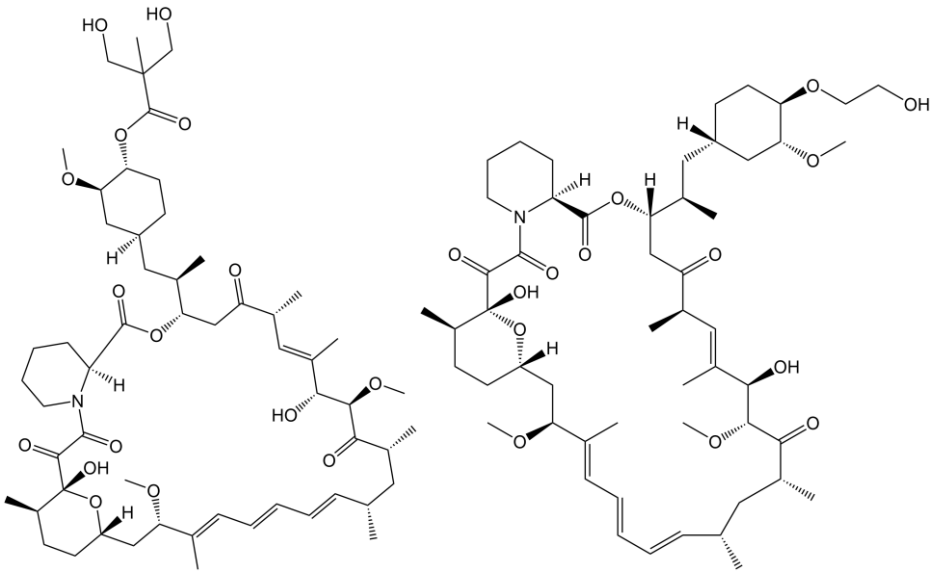


Figure 5. *Chemical structures of mTOR inhibitors.*

The best studied autophagy inducer is rapamycin (sirolimus), an immunosuppressive drug used in organ transplantation (figure 5, left). Rapamycin inhibits mTOR (mammalian target of rapamycin), which is a strong negative regulator of autophagy [36]. *In vitro* studies of *Mtb*-infected macrophages used sirolimus to show effects on autophagy resulting in antimicrobial activity [33]. Everolimus is a sirolimus analog with superior oral bioavailability (figure 5, right). The dose selected for this project (0.5 mg/d) reflects the lowest dose showing clinically meaningful immunologic effects [37]. In that study, elderly volunteers without unstable medical conditions treated with low doses of everolimus (described in the manuscript by its preclinical name, RAD001) for 6 weeks showed superior responses to influenza vaccine administered 2 weeks after everolimus had been stopped. The authors attributed the effect to enhanced T cell PD-1 expression, which persisted after treatment had stopped. PD-1 expression will be monitored in the present study to examine its relationship to possible effects on TB treatment.

Typical everolimus doses differ widely according to indications. Dosing in patients with solid tumors generally begins at 10 mg/d. In these patients, the frequency of serious adverse events is strongly related to exposure [38]. Lower doses averaging 1 mg twice daily are used in solid organ transplant recipients; however, assessing its safety profile in such patients is problematic, as they uniformly receive multiple additional immunosuppressive drugs, including cyclosporine, tacrolimus, corticosteroids, and biologic agents. Patients randomized to everolimus in the present study will receive a dose of 0.5 mg daily, without additional immunosuppressive agents. The safety profile of everolimus at this dose is best represented by that in the study of immune function in the elderly described above [37] (Appendix 2). There were no serious adverse events of any cause in 53 patients who received everolimus 0.5 mg/d in that trial. Mouth and tongue ulcers occurred numerically more frequently than in controls (table **5**); however, these were mild and did not result in discontinuation. Based on these observations, we anticipate that adverse events due to everolimus in the proposed study will most likely be limited to several cases of mild oral ulceration. This risk will be mitigated by close clinical monitoring.

Everolimus and other mTOR inhibitors are classified as category D risks to the developing fetus, causing characteristic defects in animals at therapeutic concentrations. For this reason, women of childbearing potential will be excluded from participation in this trial.

Table 5. Everolimus adverse event profile. From [37].

	Everolimus 0.5 mg/d 53 subjects N (%)	**Placebo 59 subjects N (%)**
Total treatment-related adverse events	35	21
Subjects with adverse events (%)	22 (41.5)	20 (37.7)
Mouth ulceration	6 (11.3)	3 (5.1)
Headache	0	1 (1.7)
Blood cholesterol increased	2 (3.8)	0
Diarrhea	1 (1.9)	0
Dyspepsia	0	1 (1.7)
Fatigue	0	0
Low-density lipoprotein increased	2 (3.8)	0
Tongue ulceration	3 (5.7)	1 (1.7)
Insomnia	1 (1.9)	0
Dry mouth	0	1 (1.7)
Neutropenia	0	0
Oral pain	0	0
Pruritus	0	0
Conjunctivitis	0	0
Erythema	0	0
Limb discomfort	0	0
Mucosal inflammation	0	0
Paresthesia (oral)	2 (3.8)	0
Stomatitis	0	0
Thrombocytopenia	0	0
Urinary tract infection	0	0

14.2. Auranofin 3 mg QD for 1 week, followed by 6 mg QD (day 1 up to and including day 112)

Interest in antimicrobial gold complexes originated from the work of Koch at the end of 19th century, who demonstrated that potassium dicyanidoaurate(I), $K[Au(CN)_2]$, showed activity *in vitro* against *Mtb*. The most widely used gold antimycobacterial agent during the following decades was sanochrysin, a double thiosulphate of gold(III) and sodium [39]. Sanochrysin therapy of tuberculosis was largely abandoned after a careful clinical trial in 1931 showed it provided no clinical benefit [40]. However, parenterally administered gold salts continued in clinical use through the 1980s for their anti-inflammatory effects in rheumatoid arthritis [41]. Auranofin is an orally bioavailable gold(I) complex approved in the 1980s for treatment of rheumatoid arthritis (figure 6). Although less effective than injectable gold, auranofin appears to show a superior safety profile, with lower incidence of proteinuria and rash. Auranofin is infrequently used now to treat RA, however, as its anti-inflammatory effects are delayed and reduced in comparison to methotrexate and anti-TNF agents [42, 43].

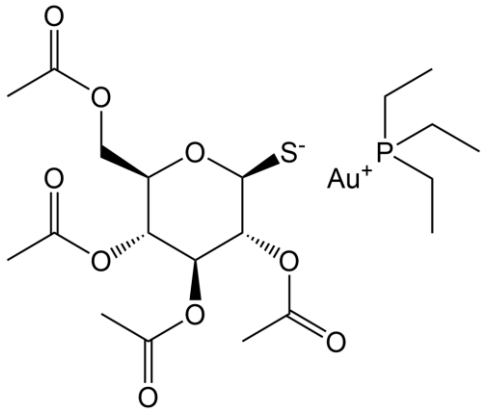


Figure 6. *Chemical structure of auranofin.*

Interest has been renewed in the use of gold in TB due both to anti-inflammatory effects and direct antimicrobial activity. An MIC against *S. aureus* of 0.1 μg/mL has been reported [44]. Several other gold(I) complexes show antimyco-

bacterial activity *in vitro*, with MICs in the 1 μM range [44]. Recent unpublished studies indicate auranofin shows mycobactericidal activity against replicating and carbon starved non-replicating *M. tuberculosis in vitro* in the range of 0.1 - 1.0 μM (0.07 - 0.7 μg/mL). For comparison, auranofin dosing at 6 mg QD results in steady-state gold concentrations of 0.3 – 1.0 μg/ml in plasma, 7 μg/ml in blood, and 5 μg/ml in liver and spleen [45]. Auranofin is thought to have direct effects on mycobacteria (inhibiting thioredoxin reductase and mycothiol reductase) as well as on host cells (promoting ROS expression). However, 40% of the gold content of auranofin in blood is associated with blood cells, and 60% with serum proteins [46]. The impact of tissue localization and protein binding of auranofin on its antimycobacterial activity is not known. When auranofin crosses the plasma membrane the acetyl groups on the thioglucose moiety are lost and some disruption of the Au-S and Au-P bonds occurs [47, 48]. These may affect antimicrobial activity. The apparent combined central and peripheral volumes of distribution of auranofin after oral administration to human subjects are very large (exceeding 100 L). For this reason, steady state concentrations are not reached for many weeks. This may delay the onset of its antimycobacterial activity.

Auranofin dosing is limited by GI tolerability. Patients randomized to auranofin in the present study will start at a dose of 3 mg QD, increasing to 6 mg QD after 1 week. Dose reductions will be permitted to maintain GI tolerability.

14.3. Vitamin D 5 mg (day 1) followed by 2.5 mg Q month x 2 (days 28 and 56) (3 doses total)

Vitamin D is to be taken on day 1 (4 tablets) and again on day 28 (2 tablets) and again on day 56 (2 tablets) i.e. a total of 3 doses.

Vitamin D is essential for human anti-mycobacterial host defences [49]. It is required for production of cathelicidin and other antimicrobial peptides following triggering of Toll-like receptors by mycobacterial ligands [50]. This process promotes autophagy and is amplified by IFNγ. Recent studies indicate induction of cathelicidin by vitamin D also results in cytotoxicity against B-cell lymphoma cells in vitro [51]. Vitamin D has anti-inflammatory properties in TB by reducing production of pro-inflammatory mediators [52]. However, multiple studies have found neither harm nor clinically meaningful benefit when vitamin D is administered together with standard TB treatment [53-57]. These studies are summarized in table **6**.

Table 6. Randomized controlled trials of vitamin D in tuberculosis.

Study	Country	N	Total dose 8 wks	Effect
Ralph [54]	Papua New Guinea	101	2.5 mg	none
Wejse [56]	Guinea Bissau	280	2.5 mg	none
Daley [53]	India	247	10 mg	none
Martineau [57]	UK	146	10 mg	2 PR cases
Nursyam [55]	Indonesia	67	10.5 mg	superior smear conversion at wk 6

Dosing schedules varied widely in these trials. For reference, 1 IU = 0.025 μg; recommended adult daily doses in many countries is 20 μg/day, with proposed maximum well tolerated doses of 100 μg/day. Three studies examined effects on serum vitamin D levels. Wejse [56] gave 2500 μg cholecalciferol or placebo on entry and at 5 and 8 months (total 7.5 mg over 8 months). No effect was observed on composite disease score, sputum smear, or mortality. There was no benefit on the composite disease score in the subsets with initial low vitamin D or in HIV-infected cases. There was no effect on vitamin D levels, which increased equally in both arms. In contrast, Martineau [57] gave 2500 μg vitamin D or placebo every 2 weeks for 4 doses (total 10 mg over 2 months). Vitamin D levels were low at baseline and were significantly augmented by supplementation. However, there was no effect on sputum smear, sputum culture, ESR, CRP, BMI, or CXR. Daley [53] similarly found that high doses led to faster normalization of vitamin D levels. Dosing was highest in the study of Nursyam [55], which gave 250 μg/day for the first 6 weeks. All 3 studies monitored patients for symptoms of hypercalcemia; none were reported. Of all the trials, only that of Martineau reported adverse events possibly related to vitamin D administration, in which two D-treated patients experienced paradoxical reactions (PR, disease worsening despite microbiologic improvement) that required therapeutic drainage of paraspinal and psoas muscle abscesses. The biologic basis of these reactions is unclear. Vitamin D dampened production of pro-inflammatory cytokines in a per protocol subset of participants in that trial [52, 57]; however, the 2 PR cases were intentionally excluded from that analysis. Paradoxical reactions are recognized to occur occasionally in patients during standard TB treatment.

Subjects randomized to the vitamin D arm of this trial will receive 5 mg initially, then 2.5 mg Q month for a total of 3 doses (*i.e.*, 10 mg over 3 months). It is not anticipated that this dose will accelerate sputum culture conversion. Instead, the arm has been included to better understand why D has consistently failed to be of benefit in multiple trials,

as previous studies did not include PET/CT scanning or gene expression profiling. The dose was selected as likely to be sufficient to increase serum D levels, but unlikely to cause PR or other adverse events.

15. Antimicrobial TB therapy

15.1. Rifabutin 300 mg QD (day 1 up to and including day 180)

Rifabutin is a semi-synthetic rifamycin approved by US FDA in 1992 for the prevention of disseminated non-tuberculous mycobacterial infection in patients with advanced AIDS. Unlike rifampin, it has minimal induction of CYP3A4, and can be safely co-administered with other drugs metabolized by CYP3A4, including efavirenz, HIV protease inhibitors, and bedaquiline [58]. Rifabutin presently is used mainly in the treatment of tuberculosis in patients requiring concomitant treatment with HIV protease inhibitors. Oral doses of rifabutin of 300 mg produce plasma concentrations of 0.5 μg/ml, approximately 10-fold above the typical MIC for *M. tuberculosis*. Levels in tissues can be 10-fold higher than in plasma.

A Cochrane review by Davies *et al* identified 5 TB treatment trials with a total of 924 participants in which rifabutin and rifampin were compared. There was no statistically significant difference between the regimens in cure rates (RR 1.00, 95% CI 0.96 to 1.04; 553 participants, 2 trials) or relapse rates (RR 1.23, 95% CI 0.45 to 3.35; 448 participants, 2 trials). The frequency of adverse events did not differ (RR 1.42, 95% CI 0.88 to 2.31; 714 participants, 3 trials), though the RR tended to increase with rifabutin dose: 150 mg (RR 0.98, 95% CI 0.45 to 2.12; 264 participants, 2 trials); and 300 mg (RR 1.78, 95% CI 0.94 to 3.34; 450 participants, 2 trials). However, the authors noted that the lack of dose adjustment by weight in the relevant trials complicates interpretation of this relationship.

Two of the studies included in the Cochrane analysis reported sputum culture findings. Gonzales [59], with 287 evaluable patients, reported rates of positivity at 12 wks of 3% in both arms. McGregor [60], with 225 evaluable patients, reported rates of culture positivity at 8 wks of 8.0% in the rifabutin arm and 12.3% in the rifampin arm.

The safety profile of rifabutin 300 mg QD is described in detail in two reports, shown in tables **7** and **8** below.

Table 7. Safety profile of rifabutin from [59].

	Rifampin 600 mg	Rifabutin 300 mg
Patients exposed	174	170
Patients (%) with AE	16 (9)	28 (17)
Patients (%) discontinuing due to AE	1 (1)	5 (3)
Patients (%) with treatment-related AE	6 (3)	9 (5)
Patients (%) with severe AE	3 (2)	3 (2)

Table 8. Safety profile of rifabutin from [60].

	Rifampin 600 mg	Rifabutin 300 mg
Patients exposed	156	142
Patients with AE	4	6
Patients discontinuing due to AE	1	1

These studies indicate that rifabutin shows similar efficacy in tuberculosis compared to rifampin, and that its safety signals are similar to those of rifampin and can be mitigated by careful clinical monitoring.

15.2. Isoniazid (day 1 up to and including 180) and pyrazinamide plus ethambutol (day 1 up to and including day 56)

Isoniazid, pyrazinamide, and ethambutol have been used in combination as essential components of antimicrobial TB therapy for many decades. All 3 drugs are generally well tolerated. Isoniazid and pyrazinamide can cause increased transaminase levels, occasionally cause symptomatic drug-induced liver injury, and rarely cause liver injury meeting Hy's criteria for serious injury [61]. These risks can be mitigated by clinical and biochemical monitoring. Isoniazid can also cause peripheral neuropathy, which can be mitigated by clinical monitoring and co-administration of vitamin B6 (pyridoxine). Pyrazinamide can cause arthralgias and GI upset. It also can cause asymptomatic increases in serum uric acid, and rarely causes symptomatic gout. These risks can be mitigated by clinical monitoring. Ethambutol can cause optic neuropathy, but rarely does so when given at standard doses limited to 2 months of treatment. These risks are all considered acceptable in routine clinical practice. There is no expectation that these risks will be affected by study participation, except in that study participants will benefit from closer clinical monitoring and early detection of adverse events. Isoniazid will be dosed at 300 mg QD. Dosing of pyrazinamide (25 mg/kg/d) and ethambutol (20 mg/kg/d) will be weight-banded (see table **9**). TB treatment will be administered daily for 26 weeks.

Table 9. Daily dosing of pyrazinamide and ethambutol.

Weight	Pyrazinamide 500 mg tablets	Ethambutol 400 mg tablets
40-50 kg	2	2
51-70 kg	3	3
71-90 kg	4	4

16. Study Endpoints

16.1. Primary Endpoint: Safety

16.1.1. For auranofin, everolimus, and vitamin D: the proportions of patients Experiencing suspected unexpected serious adverse reactions (SUSARs).

16.1.2. For CC-11050: the proportion of patients experiencing treatment emergent serious adverse events (SAEs).

16.2. Secondary Endpoints

16.2.1. Safety

16.2.1.1. TEAEs other than SAEs, categorized according to severity, drug relatedness, and leading to early withdrawal.

16.2.1.2. Quantitative and qualitative clinical safety laboratory measurements, including observed and change from baseline.

16.2.1.3. Quantitative and qualitative measurement of ECG results (heart rate, RR interval, PR interval, QRS interval, QT interval and QTc interval), including observed and change from baseline.

16.2.1.4. Proportion of patients with disease exacerbation (change from baseline) at multiple time points using IRIS criteria

The hypotheses to be tested are that TE SAEs, SUSARs and other safety endpoints will not differ between control and experimental arms.

16.2.2. Microbiology

16.2.2.1. Proportion of patients with positive sputum cultures on solid culture medium after 8 weeks of treatment

16.2.2.2. Proportion of subjects with positive cultures at other time points, using solid and liquid medium

16.2.2.3. Proportion of subjects with multiple positive cultures in liquid or solid medium after day 112 of treatment

16.2.2.4. Time to stable culture conversion in liquid medium

16.2.2.5. Change in MGIT TTP over time as examined by nonlinear regression

16.2.2.6. Whole blood bactericidal activity expressed as a cumulative effect over the dosing interval, most likely only for participants enrolled to site within the Gauteng province.

16.2.2.7. Proportion of patients with new resistance.

NB: Baseline mycobacterial isolates will be tested for susceptibility to INH, RIF EMB, and PZA in MGIT. Patients whose treatment has failed (repeated positive cultures after day 112) will have DST repeated. Those with new resistance will have deep sequencing performed on baseline and failure isolates to determine if mixed infection was present initially

Rationale: Mitchison in 1993 first proposed a role for month-2 culture status in the evaluation of new tuberculosis regimens [62]. Two independent analyses of regimen pairs of equal duration confirmed the relationship between sputum culture status and relapse [63, 64]. In 2013, meta-regression modeling of 58 diverse regimens of various durations studied in 7793 patients identified month-2 culture status and duration as independent predictors of relapse [6]. At that time, 5 phase 2 trials of 6 gatifloxacin or moxifloxacin-containing regimens had reported month-2 conversion rates of 71-92% [65-69]. The model predicted these regimens would yield unsatisfactory relapse rates (10-19%) if administered for only 4 months [6]. The predicted rates are highly consistent with those subsequently reported in the four 4-month arms of 3 unsuccessful phase 3 fluoroquinolone treatment-shortening trials (13-18%, in a per-protocol analysis of patients at risk of recurrence at end of treatment) [7-10]. Across all 8 arms in these trials, there was a high correlation between observed recurrence rates and those predicted based on month 2 cultures (R^2=0.86, figure 7A. Axes indicate logit-transformed recurrence risk, with insets indicating corresponding proportions. Red symbols indicate 4 month regimens; blue symbols indicate 6 month regimens. Error bars indicate 80% confidence intervals). Using the pre-specified threshold of 10% recurrences as the maximum likely to be judged acceptable to tuberculosis control programs, the

original model correctly predicted all 4 six-month regimens as satisfactory, and 3 of 4 four-month regimens as unsatisfactory (PPV=80%, overall accuracy=88%). A revision of the regression model based on the full dataset of 66 regimens and 11181 patients resulted in only minimal changes to its predictions (figure **Error! Reference source not found.**B. Solid and dotted lines indicate updated and original model predictions, respectively. Shading indicates 80% confidence intervals for the updated estimates). The main effect of the revision was to increase to 10% the predicted recurrence rate in the sole 4-month regimen incorrectly predicted to yield acceptable results. The criteria proposed by Chau *et al* for biomarker validation classify this measure of culture conversion as a "known valid" biomarker of relapse, based on independent replication in multiple studies [70].

Two sputum specimens will be collected at each time point in this study. If either specimen is positive, the result is scored positive. If both specimens are missing, the result is scored as missing. If both are contaminated, or one is missing and the other contaminated, the result is scored as contaminated.

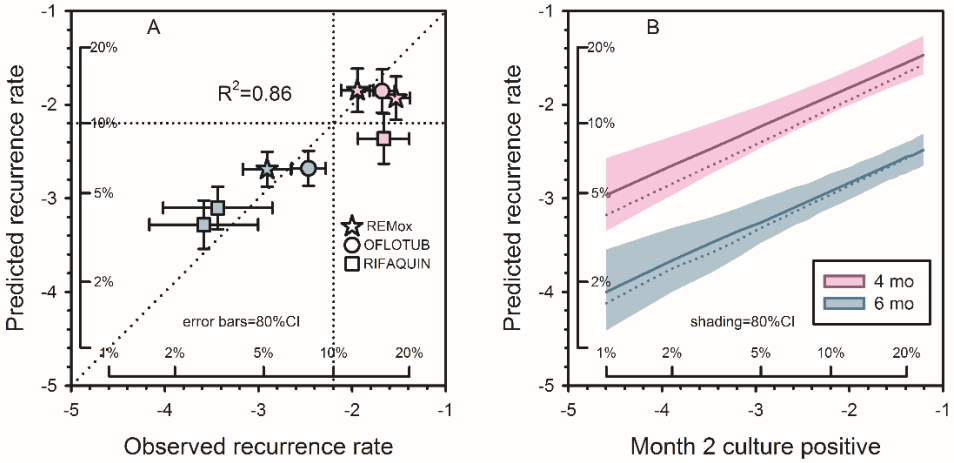


Figure 7. *Mathematical model predicting relapse risk.* ***A:*** *Observed and predicted proportions of subjects with tuberculosis recurrence in the 8 arms of 3 phase 3 fluoroquinolone treatment shortening trials [8-10].* ***B:*** *Predicted proportion of patients with recurrence based on the proportion positive after 2 months of treatment.*

The hypotheses to be tested are that sputum culture conversion and rate of change of MGIT TTP will be superior in the experimental arms *vs* control, and that the proportions of patients failing treatment will not differ. At each time point in the schedule of events at which sputum culture will be evaluated, the evaluation will consist of 2 sputum specimens, tested using both solid and liquid culture.

Rationale: WBA is a candidate biomarker for assessment of protective antimycobacterial immunity and chemotherapy. Cultures consist of equal 300 µl volumes of heparinized blood and tissue culture medium, to which *Mtb* is added. After 4 days incubation, bacilli are recovered, and the extent of growth or killing determined by inoculation into MGIT and monitoring TTP. Cell numbers and drug concentrations in whole blood cultures reflect those *in vivo* at the time of phlebotomy. Immune control of intracellular mycobacterial growth in whole blood culture is inferior in TST-negative persons and in young children, is enhanced by vitamin D and by primary but not secondary BCG vaccination, is impaired by chemokine receptor or TNF blockade, T cell depletion or HIV infection, and is restored by antiretroviral therapy [71-82]. WBA during TB treatment is superior in the intensive *vs*. continuation phase, is superior for standard *vs*. MDR regimens, and correlates with 2 month culture status, which in turn predicts relapse risk [6, 63, 83, 84]. Measurement of WBA has accelerated the development of sutezolid (figure 8), bedaquiline, and PA-824 [85-87]. WBA is uniquely suited to assess the combined effects of host-directed and antimicrobial chemotherapy [88]. WBA in this study will be examined during week 7, and will be measured using the patient's isolate, at 0, 1, 2, 4, and 8 hours post dose. The cumulative effect will be calculated as the AUC of effects at each time point. Dr. Wallis has extensive experience in the development, implementation, and evaluation of this endpoint. The hypotheses to be tested are that cumulative WBA will be superior in the experimental arms *vs* control, and will correlate with measures of bactericidal activity in sputum.

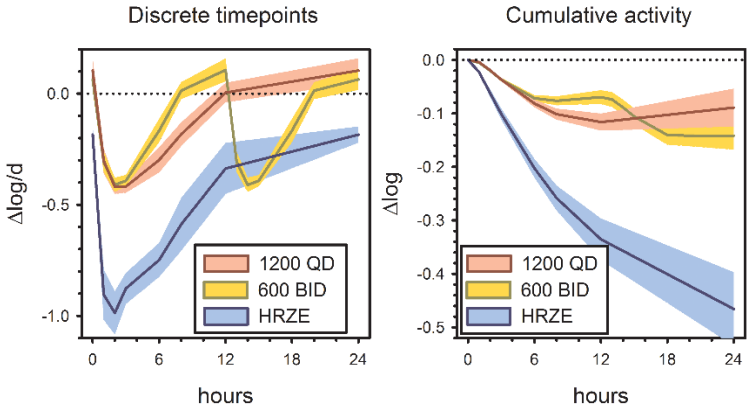


Figure 8. *WBA of sutezolid in TB patients. Shading indicates 90% CI. From [89].*

16.2.3. ^{18}F-FDG PET/CT imaging change from baseline to 2 months

16.2.3.1. Maximum standardized uptake values (SUV)
16.2.3.2. Mean SUV

Rationale: Combined imaging with ^{18}F-fluorodeoxyglucose positron emission tomography (PET) plus computed X-ray tomography (CT) is emerging as an important research tool to assess lung inflammation and lung structure integrity in tuberculosis [1, 90]. Patients in this trial will undergo PET/CT scanning twice in this study, at baseline and on week 8. PET will be assessed as change from baseline in total activity, and CT as the change in the distribution of Hounsfield units of lung voxels. Data will be analyzed according to the methods described by Chen et al [1]. The hypotheses to be tested are that resolution of PET and CT lesions will be greater in experimental *vs* control arms.

16.2.4. Serum markers of inflammation change from baseline to 2 months

16.2.4.1. Neopterin [2, 3]
16.2.4.2. CRP

Rationale: The hypothesis to be tested is that these markers of inflammation will be reduced at multiple time points in the experimental arms *vs* control.

16.2.5. Mtb-specific immune function change from baseline to 2 months

16.2.5.1. Quantiferon gold in-tube (quantitative)

Rationale: Immune activation and TNF production drive T cell apoptosis in TB [91]. The resulting loss of TB-specific T cells causes reduced Mtb-stimulated IFN-g production that persists for up to 1 year after TB treatment has ended [92]. We therefore hypothesize that TB HDT agents may reduce TB-induced IFN-g production during HDT treatment in the experimental arms *vs* control, but, by preventing T cell loss, may result in superior levels of IFN-g production at EOT.

16.2.6. Pulmonary function change from baseline to 2 and 6 months

16.2.6.1. FEV1 (% of expected value)
16.2.6.2. Forced vital capacity
16.2.6.3. 6 minute walk test (distance)
16.2.6.4. Oxygen saturation at conclusion of 6 minute walk test (%)
16.2.6.5. SGRSQ symptom score

Rationale: Spirometry is markedly abnormal at TB diagnosis. A study in Papua New Guinea, for example, found that at TB diagnosis, patients had lost nearly 1L FEV1 (expected 3L), of which only 0.2L was recovered during treatment [93]. Additional TB episodes produce additive persistent deficits [94, 95]. Willcox performed spirometry on 71 patients up to 16 years after TB cure [96]. Most respiratory parameters were reduced, with FEV1 being most strongly affected, in direct relation to radiographic extent of disease and amount of sputum produced at TB diagnosis. Other studies have examined the 6 minute walk test (6MWT) and the St. George Respiratory Symptom Questionnaire (SGRSQ) as endpoints in TB trials [54, 93]. Endpoints in this trial will include the following, measured at baseline and repeatedly during treatment. The hypothesis to be tested is that patients receiving HDT agents will show earlier and more complete recovery of lung function *vs* controls.

16.3. Exploratory Endpoints

16.3.1. Change from baseline to 2 and 6 months in gene expression, proteomic, and metabolomic profiles

Rationale: PBMC samples and whole blood will be collected for measures of transcriptomic (gene expression) profiles. Whole blood will be analysed for expression profiles of limited gene sets associated with TB risk [97]; full expression profiles will be analyzed by chip array [98]. Cryopreserved PBMCs will be sorted by immunophenotype followed by expression profile analysis by RNAseq. Serum will be analysed for changes in the proteome and metabolome. These analyses will be performed after the full set of samples has been obtained from all study participants, and will all be considered exploratory.

16.3.2. Change from baseline to 2 months in additional PET and CT parameters [1]

16.3.3. Change from baseline for PD-1 expression on CD4 and CD8 lymphocytes

Rationale: Inhibition of PD-1 expression has been implicated as a potential mechanism of immunologic effects of everolimus in the elderly [37]. PD-1 expression will be measured by flow cytometry only in everolimus and control patients.

16.3.4. Change from baseline for urine for high-sensitivity LAM assay

Rationale: Detection of *Mtb* LAM by ELISA is a highly specific but inadequately sensitive method for TB diagnosis, showing adequate sensitivity only in TB patients with advanced AIDS [99]. Ongoing BMGF-supported research is examining other, more sensitive methods of detection. Specimens will be reserved in collaboration with these projects.

16.4. Pharmacokinetics and pharmacodynamics (PK/PD)

Blood samples for PK analysis will be obtained during week 7, at 0, 1, 2, 4, and 8 hours post dose (*i.e.*, at the same time points as WBA), 1 hour time point will occur 1 hour post-dose (TB treatment and HDT treatment if applicable) and not 1 hour post 0 hour timepoint. PK profiles will be developed for CC-11050, everolimus, auranofin, rifabutin, and isoniazid. Plasma concentrations will be used to build a population PK model to evaluate the effects of patient covariates on trial drug pharmacokinetics and to confirm adequate exposures to HDT with background rifabutin. Where possible, PK samples from the proposed trial will be pooled with high resolution PK data from other trials to inform the PK model.

A population PK-PD approach will be applied to the full time course of pharmacodynamic endpoints (*e.g.*, immunologic biomarkers, transcriptomic signals, culture conversion and WBA) to better understand the relationships between drug concentrations and exposures, and HDT effects.

17. Safety monitoring

All patients will undergo routine safety monitoring that will include symptom and adverse event reviews, chemistry and hematology testing, 12 lead ECG, chest X-rays. The intervals for these tests are specified in the schedule of events.

18. Clinical management

18.1. General

Patients will be provided with contact telephone numbers for the clinic office in the event of new of symptoms. They will also be provided with mobile numbers for study physicians and nurses should new symptoms arise outside of normal clinic hours. Additional testing will be performed by study physicians to evaluate new symptoms that may arise during the conduct of the trial. Study physicians will also refer patients to local emergency medical facilities should potentially serious conditions arise outside of normal clinic hours. Site PIs will be available to study physicians on a 24 hr basis to address these concerns should they arise during the trial.

18.2. Adverse Events

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a study agent. It need not have a causal relationship with the treatment received.

18.3. Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that meets any of the following criteria:

- Results in death, or is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Other medically important conditions. This includes important medical events that may not be immediately life- threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above.

Clinical research sites must report SAEs later than 3 reporting days (Monday through Friday) after the site becomes aware of an event that meets protocol-defined criteria for reporting.

18.4. Toxicity grading

This study will use the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, November 2014 which can be found on the DAIDS RSC Web site: http://rsc.tech-res.com/Document/safetyandpharmacovigilance/DAIDS_AE_Grading_Table_v2_NOV2014.pdf. Generally, patients experiencing grades 1 and 2 toxicities may continue treatment, at the discretion of the site investigator with careful follow-up, whereas patients experiencing grades 3 and 4 toxicities will have study drugs held until signs or symptoms have resolved (or until ≤Grade 2). Study drugs may be permanently discontinued at the discretion of site investigator. Site investigators are strongly encouraged to discuss discontinuations and reintroductions of study treatment with the PI to ensure consistency.

18.5. Hepatic safety

Pyrazinamide, isoniazid, and rifabutin are all recognized to cause drug-induced liver injury (DILI). In most cases, these are recognized based on modest elevations of AST or ALT in the absence of symptoms of hepatitis, and resolve with careful monitoring, *i.e.*, do not necessarily required drug discontinuation. Events accompanied by signs or symptoms of hepatitis, marked elevations of AST or ALT, or elevations of bilirubin accompanied by those of AST or ALT meeting Hy's criteria [61] will be managed by immediate temporary discontinuation of all study drugs, including anti-tuberculous treatment. Study physicians will promptly discuss management of any such cases with site and study PIs before study drugs are restarted. Only AST will be monitored in this study.

19. Study schedule of events

19.1. Screening

The screening evaluation will occur on days -3 to -1. Patients will be asked to provide informed consent. If consent is provided, demographic and medical history questions will be examined. Inclusion and exclusion criteria will be examined. For women, a pregnancy test will be performed. Lab safety tests, HIV test, 12-lead ECG, chest X-ray, vital signs, full physical exam, TB symptoms, and concomitant medications will be reviewed. Sputum specimens will be obtained for Gene Xpert TB/Rif, and cultures on solid and liquid media. DST will be performed for first-line drugs. These activities may be spread over multiple visits within the indicated time window.

19.2. Day 0

Patients meeting all inclusion criteria and no exclusion criteria will enter the study on day 0. At that time, lab safety tests, urine tests, 12-lead ECG, vital signs, full physical exam, TB symptoms, concomitant mediations ("conmeds"), sputum cultures, and, for female patients, a pregnancy test, will be repeated. Female patients who require IUD insertion to facilitate trial participation (section 12.3.2) must have the device inserted no later than this day. Patients will also undergo randomization and will have a PET/CT performed (a window of ±1 day for only the PET/CT is allowed for day 0) . Blood will be collected for QFN, serum biomarkers, gene expression, proteomic and metabolomics, and for patients in everolimus and control arms, for PD-1 expression.

19.3. Day 1

TB and HDT treatments will begin on day 1. Vital signs, symptoms, adverse events and conmeds will be reviewed. A limited physical exam, spirometry and 6MWT will be performed.

19.4. Day 7

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. A limited physical exam will be performed. Safety labs will be obtained. Sputum cultures will be obtained.

19.5. Day 14.

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. A limited physical exam will be performed. Safety labs and urine tests will be obtained. Sputum cultures will be obtained. A 12 lead ECG will be obtained. Spirometry and 6MWT will be performed. Blood samples for PK will be obtained at 0 hrs and 2 hrs post HDT dose. Blood will be collected for QFN, serum biomarkers, gene expression, proteomic and metabolomics.

19.6. Day 21

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures will be obtained. A limited physical exam will be performed.

19.7. Day 28

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures will be obtained. A limited physical exam will be performed. Patients who had an IUD inserted to facilitate trial participation will have the IUD placement confirmed by physical examination. A urine sample will be obtained. Lab safety tests and chest X-ray will be performed. Blood will be collected for serum biomarkers.

19.8. Day 35

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures will be obtained. A limited physical exam will be performed.

19.9. Day 42

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures will be obtained. A limited physical exam will be performed. An intravenous catheter with a heparin lock will be placed. Blood specimens for PK and WBA will be obtained at 0, 1, 2, 3, 4, 6, and 8 hrs post dose. Patients will remain in clinic for the day to facilitate the collection of these samples. Treatment must be administered on-site and directly observed post 0hr phlebotomy for PK and WBA.

19.10. Day 56

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures will be obtained. A limited physical exam will be performed. Safety labs, urine, and sputum cultures will be obtained. PET/CT (a window of ±1 day only for the PET/CT over and above the overall ± 3 days window for this visit is allowed for day 56),chest X-ray, spirometry, 6MWT and for female patients, a pregnancy test will be performed. Blood will be obtained for QFN, serum biomarkers, gene expression, proteomic and metabolomics, and for patients in everolimus and control arms, for PD-1 expression.

19.11. Day 84

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures will be obtained. A limited physical exam will be performed. Chest X-ray and HIV tests will be performed, Partipcants who test HIV positive at day 84 will be withdrawn from the study and referred to their local healthcare provider or clinic for HIV management as well as TB treatment and management. Participants who have already passed the day 84 timepoint will not require additional HIV testing, but will be perfomed if requested by the participant. . Spirometry and 6MWT will be performed. Serum biomarkers, urine, and QFN will be obtained. An intravenous catheter with a heparin lock will be placed. Blood specimens for PK and WBA will be obtained at 0, 1, 2, 3, 4, 6, and 8 hrs post dose. Patients will remain in clinic for the day to facilitate the collection of these samples. Treatment must be administered on-site and directly observed post 0hr phlebotomy for PK and WBA.

19.12. Day 112

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures will be obtained. A limited physical exam will be performed. A 12 lead ECG and chest X-ray will be performed. Sputum cultures will be performed. Spirometry and 6MWT will be performed. Urine will be obtained. Blood for serum biomarkers and QFN will be obtained.

19.13. Day 140

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures and urine tests will be obtained. A limited physical exam will be performed. A chest X-ray will be obtained. Sputum cultures will be obtained. Spirometery and 6MWT will be performed. Blood for serum biomarkers and QFN will be obtained. Samples for PD-1 expression will be measured for patients in the control and everolimus arms. An intravenous catheter with a heparin lock will be placed. Blood specimens for PK and WBA will be obtained at 0, 1, 2, 3, 4, 6, and 8 hrs post dose. Patients will remain in clinic for the day to facilitate the collection of these samples. Treatment must be administered on-site and directly observed post 0hr phlebotomy for PK and WBA.

19.14. Day 180

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. Sputum cultures will be obtained. A limited physical exam will be performed. Sputum cultures will be obtained. Spirometery and 6MWT will be performed. Blood will be obtained for QFN, serum biomarkers, gene expression, proteomic and metabolomics. Urine will be obtained. Samples for PD-1 expression will be measured for patients in the control and everolimus arms. A chest X-ray will be obtained.

19.15. Day 360

Sputum cultures will be obtained for participants positive for TB Symptoms who are able to produce sputum, Efforts should be made to collect early morning sputum specimen whenever possible A limited physical exam will be performed and TB symptoms reviewed if any. Informed consent will be obtained for additional testing. Spirometry and 6MWT will be performed. The St George's Respiratory Questionnaire will be administered

19.16. Day 540

Sputum cultures will be obtained for participants positive for TB Symptoms who are able to produce sputum, Efforts should be made to collect early morning sputum specimen whenever possible. A limited physical exam will be performed and TB symptoms reviewed if any. Spirometry and 6MWT will be performed. The St George's Respiratory Symptom Questionnaire (SGRSQ) will be administered.
Note: The window of acceptable study days for this visit will be extended through day 720. Patients who have already had a day 540 visit but have not yet had spirometry, 6MWT and SGRQ will be recalled for these tests if they can be performed within the day 720 window.

19.17. Premature discontinuation

Vital signs, symptoms, adverse events, conmeds and adherence will be reviewed. For female subjects, a pregnancy test will be performed. Sputum cultures will be obtained. A full physical exam will be performed. A 12 lead ECG will be performed. Sputum cultures will be obtained. Spirometry and 6MWT will be performed. A chest X-ray will be obtained.

20. Phlebotomy volumes

Table 10. Blood volumes.

Day	scr	0	1	7	14	21	28	35	42	56	84	112	140	180	Total
HIV Test	2,0										2,0				4,0
Lab Safety Tests	9,0	5,5		5,5	5,5		5,5			5,5	5,5	5,5	5,5	5,5	58,5
WBA & PK 0, 1, 2, 3, 4, 6, 8 hrs									28,0		28,0		28,0		84,0
Lymphocyte PD-1		8,5								8,5				8,5	25,5
Serum for proteonomics and metabolomics		3,5			3,5					3,5				3,5	14,0
PBMC, WB for gene expression profiling		11,0			11,0					11,0				11,0	44,0
Serum biomarkers		3,5			3,5		3,5			3,5	3,5	3,5	3,5	3,5	28,0
QFN+ (quantitative)		6,0			6,0		6,0			6,0	6,0	6,0	6,0	6,0	48,0
Total	11,0	38,0	0,0	5,5	29,5	0,0	15,0	0,0	28,0	38,0	45,0	15,0	43,0	38,0	306,0
Total for each month						84				81	45	15	43	*38*	306

The total planned phlebotomy volume (closest approximation) will be 306 ml over 6 months, with a maximum of 84 ml during any 1 month period. This estimate is increased from the 268.5 ml total of version 3.0 and 4.0 of this protocol, but decreased from the 328 ml calculated in version 2.0. The increased blood volumes are mainly due to an additional 3 ml required for QFN+ rather than QFN.

21. Statistical and Sample Size Considerations

A sample size of 40 per arm has been proposed for this trial. As described earlier, mathematical modelling predicts that if a new 4-month regimen reduces the proportion positive after 2 months of treatment to 1%, it will reduce to 10% the risk of a relapse rate >10% in a trial with N=680 per arm [6, 7]. Poisson analysis indicates a 94% likelihood that a regimen meeting the proposed target for the proportion culture positive at month 2 (1%) would result in ≤1 subject being positive out of 40 (figure 9A, magenta curve). In contrast, the culture positive rates expected in the control arm (15%-20%) are very unlikely to yield this result (1.5%-0.3%, red and black curves), indicating the sample size will have adequate power for this key endpoint.

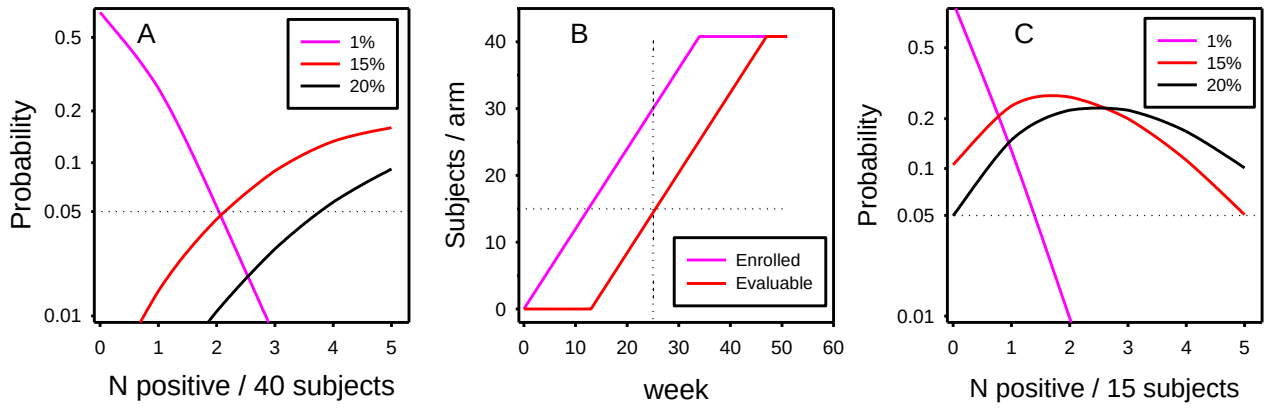


Figure 9. *Likelihood of events.*

22. Statistical Analysis

22.1. Analysis populations

22.1.1. mITT

The modified intent to treat (mITT) population will consist of all patients assigned a randomization number and who received at least 1 dose of study drug, or, in the case of the control arm, one dose of any of isoniazid, rifabutin, pyrazinamide, and ethambutol with Rif-susceptibility confirmed using Genotype MTBDR Hain.

22.1.2. PP

The per protocol (PP) population will consist of patients who received at least 90% of assigned medications during weeks 1 through 8 of the study and at least 80% of assigned medications subsequently.

22.2. Analysis methods

Each experimental arm will be compared to the control arm, after adjusting for relevant confounding baseline factors. Given the exploratory nature of this study, there will be no adjustment for multiple comparisons. The mITT population will be the primary analysis population for safety endpoints. The PP population will be the primary analysis population for efficacy endpoints. A full description of the Statistical Analysis Plan accompanies this protocol as Appendix 3.

23. Data Safety Monitoring and Trial Steering Committees

23.1. DSMC / DSMB

A Data Safety Monitoring Committee (DSMC) will be composed external experts in the field of tuberculosis clinical trials will be provided study data on a monthly basis to examine emerging differences among study arms. It will meet each month by Webex or other web-based meeting. The DSMC will make recommendations to the Trial Steering Committee (TSC) each month regarding continued recruitment into study arms and continued treatment of those already enrolled. The chair of the DSMB will have experience with serving on another DSMB. The members will be selected to not be involved with the trial in any way or have a competing interest that could impact on the trial. The DSMB will function independently of the Trial Steering Committee. All potential DSMB members will be provided with the protocol prior to joining the committee. Members will be reimbursed for travel, accommodation and a per diem only.

DSMC members' CVs and the DSMC charter accompany this protocol as an Appendices 4 and 5 respectively..

23.2. Trial Steering Committee (TSC)

The TSC will be composed of Drs. Wallis, Churchyard, Sebe, Rassool and Ahmed. It meet at least monthly to direct the conduct of the trial. TSC responsibilities include the monitoring of trial conduct and progress, review of information from external sources relevant to the design and conduct of the study, and consideration of recommendations from the DSMB.

24. Ethics Concerns

This study will be conducted according to the ethical principles set forth in the Declaration of Helsinki, ICH-GCP, and local regulatory requirements as applicable. 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 IEC of each participating clinical site prior to any protocol-specified procedures being conducted. Site investigators are responsible for ensuring that the protocol is reviewed by an IRB/IEC with the appropriate

composition (per site guidelines). The investigator will inform the IRB/IEC as to the progress of the study at applicable intervals as defined by IRB/IEC policy.

Approval for version 2.0 dated 07 January 2016 of this protocol has been received from the Research Ethics Committee of the University of Witwatersrand, South Africa. Investigators will obtain approval for version 3.0 dated 10 March 2016.

25. Pharmaceutical management

A subcontract will be established with Triclinium to manage the drug supply for the study. This will include the importation of the one investigational agent (CC-11050), and the distribution of this and other study drugs to each of the 3 clinical research sites (Tembisa, Setshaba, and Right to Care/Helen Joseph). Pharmacists at each site will maintain drug supplies under controlled access and with accounting methods in place as to GCP standards. Supplies of CC-11050 remaining after study completion will be returned to Celgene or destroyed, according to Celgene's preference. All remaining pharmaceuticals will be managed according to GCP practices.

26. Data Management

26.1. Source documentation

All clinical and laboratory information required by this protocol is to be present in the source documents and or paper case report forms (CRFs) and keyed into the database (eCRF) unless otherwise specified.

26.2. Data capture

Building on the information technology infrastructure available within the study consortium, we will use Merge eClinicalOS, an integrated data management system. Each consortium partner will be responsible for manually entering quantitative data, not already being collected routinely and required for the evaluation, into a study database at each site, or at a central location in the region if necessary. Site databases and the merged analytical datasets will be stored on password-protected, encrypted servers housed at The Aurum Institute. Scheduled backups will be performed on a daily, weekly, and monthly basis. Personal identifiers will be suppressed from the analytic dataset prior to the data analysis phase of the study. Any paper registers, forms, or records that are reviewed in order to abstract data or cross-check missing data will not be removed from the secure, programmatic area where they are stored.

26.3. Quality control

Data will be validated on entry, using range and consistency checks. Quality control procedures will include review of CRFs for completion and correctness. Logical data checks will also be performed on the data. Incomplete and incorrect data queries will be sent back to sites electronically for error resolution. Errors will be reviewed and corrected on a weekly basis. The study will be monitored by internal monitors.

26.4. Data monitoring

The study will be monitored regularly by Aurum and/or its designee throughout the study period.

26.5. Record retention

Study records (source documents, signed informed consent forms, IRB/IEC correspondence and approval letters, and screening logs) will be kept in a secure location accessible only to authorised study staff, investigators, and monitors. Secure archives are available on-site at Aurum for preliminary storage after study closure, before moving them to an off-site, secure storage facility. All records will be archived in a secure storage facility for at least fifteen (15) years after the completion of the study.

27. Pilot phase

The study will be preceded by a pilot phase, which is intended to test operational aspects of this study. In this pilot phase, up to 2 subjects per study site will undergo procedures scheduled to occur during screening and on day 0. They will also undergo spirometry and the 6MWT, both of which are ordinarily scheduled for day 1. They will not receive any study treatment, nor will they participate further in the trial, but instead will have their care transferred to the TB control program. Subjects enrolled in the pilot phase will be asked to sign separate informed consent documents describing their participation. Procedures will be reviewed during the pilot phase to ensure SOPs accurately describe study procedures and that adequate time and personnel have been allotted to conduct the trial. Data will be collected during the pilot phase and will be reviewed for consistency, but will not be included in the full study dataset.

28. Interim analysis

A formal interim analysis will be performed when month 2 culture data becomes available from 20 subjects per arm. Assuming ***1)*** a total enrolment rate of 6 subjects per week and ***2)*** a 6 week delay for culture results to become available, the interim analysis would occur when enrolment is 75% completed, *i.e.*, with 10 subjects remaining to be enrolled per arm (figure 9B). The sample size at this time point will have limited power to distinguish culture positive rates of 1% and 20%, even by Poisson analysis (figure 9C). For this reason, it will not be possible to recommend discontinuation of enrolment into experimental arms because of futility. However, this relatively small sample has the potential to identify arms with inferior performance relative to control (table **11**). Therefore, at the interim analysis, the DSMB will consider discontinuing enrolment into experimental arms with 8 or more subjects out of 15 culture positive at the 2 month time point, and discontinuing experimental treatment for subjects already in those arms. **NB**: this threshold assumes a population or "true" culture positive rate of 15-20% in control patients. In the event that the DSMB has reason to believe an unusually low or high response in the control, its recommendations may be based on performance relative to controls.

Table 11. Likelihood of inferior culture conversion occurring by chance in experimental arms in an interim analysis.

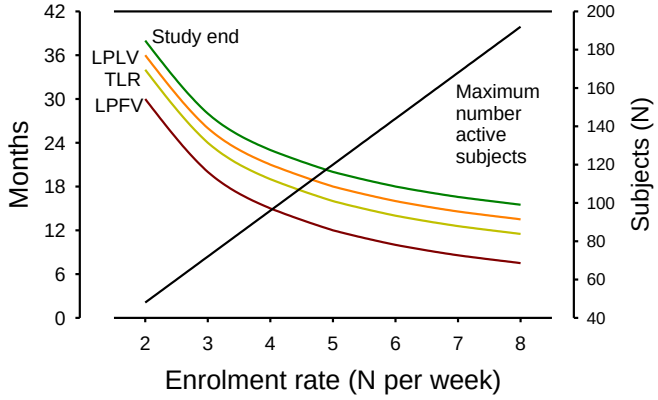
Control arm	Experimental arm	P
N pos/15		
2	6	.22
2	7	.11
2	8	.05
3	6	.43
3	7	.25
3	8	.13

29. Stopping rules

The DSMB will meet at least monthly by web-based teleconference to review the conduct of the trial. It will review summaries of enrolment and exclusion rates, demographic and baseline data, and key study endpoints, including SUSARs, TE SAEs, deaths, and discontinuations. It is not anticipated that any SUSARs will occur in the control arm. The DSMB will consider stopping enrolment into an experimental arm if it finds 3 or more similar SUSARs in that arm. It will also consider stopping enrolment for 2 excess deaths, 3 excess TE SAEs, or 4 excess discontinuations that appear related.

30. Timeline

The relationship between enrolment rate, study progression, and maximum number of active study patients is shown in figure 10. TLR refers to a top line report that includes 2 month culture and PET scan data for all patients. Enrolment is planned at 3 sites in South Africa: ***1)*** the Tembisa CRC, operated by the Aurum Institute; ***2)*** the Helen Joseph Hospital TB Clinic, operated by Right to Care; and ***3)*** the Setshaba CRC. Assuming enrolment starts in August 2016, and proceeds at a rate of 6 subjects per week, the timeline at right is anticipated.



Enrolment starts	Nov-2016
Enrolment complete	Sep-2017
Top line report	Oct 2017
Last patient last visit	Mar 2019
End	Jun-2019

Figure 10. *Study timeline.*

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32. List of Appendices

Appendix 1: Guidelines to Identifying Moderate to Far Advanced Pulmonary TB by Chest X-Ray

Appendix 2: Mannick JB, Del GG, Lattanzi M, et al. mTOR inhibition improves immune function in the elderly. Sci Transl Med 2014; 6:268ra179.

Appendix 3: Statistical Analysis Plan

Appendix 4: DSMC / DSMB Members' CVs

Appendix 5: DSMC / DSMB Charter

Appendix 6: Summary of Changes to Protocol

Addendum

Scenario 1	Scenario 2 (preferred)
AFB Sputum smear score ≥1+.	AFB Sputum smear score ≥1+.
Chest radiographic criteria for moderate or far advanced pulmonary tuberculosis met	Chest radiographic criteria for moderate or far advanced pulmonary tuberculosis met
No Gene Xpert considered (due to unavailability, conflicting results or ct values >20.)	Gene Xpert with ct value <20
A culture should be inoculated but the decision to enrol does not require waiting for the results. If culture positive, the particpant will remain in the per protocol analysis, if culture negative, the participant will be included in the mITT anlaysis.	A culture should be inoculated but the decision to enrol does not require waiting for the results. Whether culture positive or negative, the participant will remain in the per protocol analysis as the Xpert ct values <20.
All particpants will continue to receive study treatment regadless of whether they enter the mITT or PP analysis	All particpants will continue to receive study treatment.