StAT- TB (Statin Adjunctive Therapy for TB): A Phase 2b Dose-finding Study of Pravastatin in Adults with Tuberculosis

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SIGNATURE PAGE

StAT- TB (Statin Adjunctive Therapy for TB): A Phase 2b Dose-finding Study of Pravastatin in Adults with Tuberculosis, Version 3.0, Dated June 1 2019 DAIDS-ES 38558

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (eg, US National Institutes of Health, Division of AIDS) and institutional policies.

Principal Investigator: _____

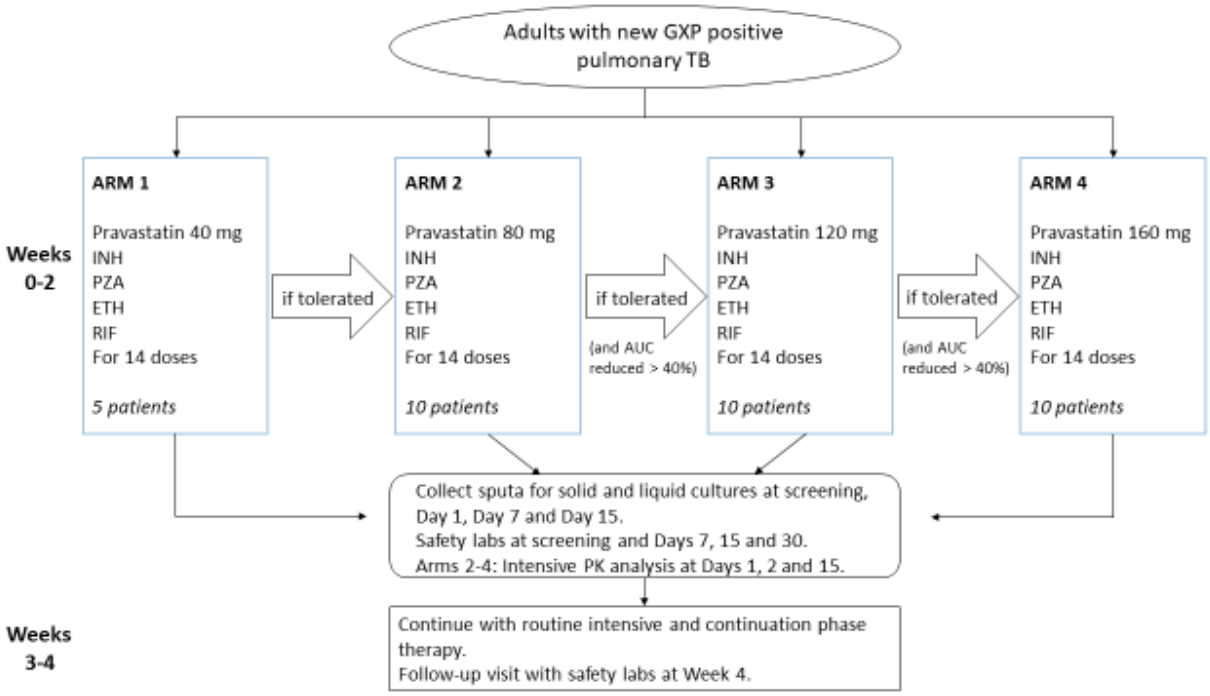
Signed: _____Date: _____

1. Abstract

Protocol Synopsis	
Protocol Title:	StAT- TB (Statin Adjunctive Therapy for TB): A Phase 2b Dose-finding Study of Pravastatin in Adults with Tuberculosis
Treatment Indication:	Pulmonary Tuberculosis (TB)
Trial Objective:	To assess the safety, tolerability, and pharmacokinetics of pravastatin adjunctive therapy when combined with the standard TB treatment regimen.
Trial Design:	Phase IIB clinical trial: A two-week safety/PK study to determine pravastatin exposures over 24 hours when given together with first-line treatment (HRZE) and ensure the combination is safe and well-tolerated.
Patient Population:	Up to 35 evaluable participants aged 18 years or older will be enrolled.
Treatment arms:	5-10 participants per arm will be sequentially recruited to receive one of the following: Arm 1: Pravastatin 40 mg and Rifafour (fixed-dose combination of isoniazid, rifampin, pyrazinamide, and ethambutol) daily for 14 days (pravastatin will be given alone on Day 1, and pravastatin + Rifafour will be given on Days 2-15) (5 participants if well tolerated) Arm 2: Pravastatin 80 mg and Rifafour (fixed-dose combination of isoniazid, rifampin, pyrazinamide, and ethambutol) daily for 14 days (pravastatin will be given alone on Day 1, and pravastatin + Rifafour will be given on Days 2-15) (10 participants) Arm 3 will only be recruited if pravastatin 80 mg is well tolerated and safe, yet drug exposures are significantly reduced due to the known interaction with rifampin. Arm 3: Pravastatin 120 mg, and Rifafour (fixed-dose combination of isoniazid, rifampin , pyrazinamide, and ethambutol) daily for 14 days (pravastatin will be given alone on Day 1, and pravastatin +, Rifafour will be given on Days 2-15) (10 participants) Arm 4 will only be recruited if pravastatin 120 mg is well tolerated and safe, yet drug exposures are significantly reduced due to the known interaction with rifampin. Arm 4: Pravastatin 160 mg, and Rifafour (fixed-dose combination of isoniazid, rifampin , pyrazinamide, and ethambutol) daily for 14 days (pravastatin will be given alone on Day 1, and pravastatin + Rifafour will be given on Days 2-15) (10 participants) Vitamin B6 will be added to each of the regimens.

Criteria for evaluation: Primary Endpoint: Safety and tolerability of pravastatin co-administered with rifampin yielding 24-hour drug exposures expected with the recommended high intensity dose for lowering cholesterol (80 mg daily).
Study site: Study participants will be recruited from clinics in Soweto, South Africa by the study team at the PHRU, Chris Hani Baragwanath Academic Hospital, Soweto, South Africa.
Study duration: It is estimated that 6 months will be required for recruitment and enrolment. The duration of participation for each study participant is 30 days; 14 days (two weeks) of experimental treatment with one follow-up visit at 30 days (four weeks).

Study Scheme



2. Objectives

Primary Objective

To assess the safety, tolerability, and pharmacokinetics of pravastatin adjunctive therapy when combined with the standard TB treatment regimen.

Our findings will inform the selection of the appropriate dose for an anticipated larger follow-up study, in which the TB treatment-shortening potential and immunomodulatory effects of pravastatin adjunctive therapy will be assessed.

3. Background

Despite more than a century of research, tuberculosis (TB) kills millions of people every year. Multidrug-resistant and extensively drug-resistant (MDR and XDR) strains of *Mycobacterium tuberculosis* (Mtb) are on the rise (1, 2), and the scientific consensus seems to be that the discovery of new antibiotics is not the only solution to this threat (3-5). A recent study (6) indicated that the number of new "druggable" targets in pathogens is relatively small and that almost all of these targets belong to evolutionarily conserved metabolic pathways that are already inhibited by current antibiotics. This could in part explain why so few new classes of antibiotics have been developed in the past decades (3) and why only one new drug against TB has been approved by the Food and Drug Administration during the last 40 years (7).

Many host factors can trigger clinical disease in a small proportion of individuals latently infected with Mtb (8). Although the immune system is responsible for clearing the invading bacilli, Mtb has evolved various

strategies to evade host defense mechanisms (9, 10), which often result in excessive inflammation, tissue destruction and morbidity (11). These observations have led to the concept that host immunity may be manipulated through host-directed therapy (HDT) (12-15) to augment bacillary killing while minimizing tissue inflammation in response to conventional anti-TB drugs, thereby improving clinical outcomes.

Statins (HMG-CoA reductase inhibitors) are among the most promising candidates as adjuvant, host-directed therapy (HDT) against TB (16). We propose to test statins in combination with first-line TB drugs as adjunctive therapy aimed at optimizing, in a graduated and safe manner, the host's treatment response to TB disease. Our study is motivated by several lines of investigation. First, several studies have shown that statins have anti-TB properties. We found that statin adjunctive therapy enhances the first-line anti-TB regimen's antimicrobial activity and shortens the time required to achieve cure in a standard mouse model of chronic TB infection ((17, 18) and unpublished data). Recently, we found that 8 weeks of adjunctive therapy with simvastatin or pravastatin significantly reduced lung bacterial burden in Mtb-infected C3Heb/FeJ mice, which develop necrotic lung granulomas histologically resembling their human counterparts(19-21). Parihar et al. showed that peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages from patients with familial hypercholesterolemia receiving statin therapy were more effective in controlling Mtb growth compared with those of healthy donors, and statin therapy protected mice against TB-induced pathology (22). In addition, statins are synergistic with the key sterilizing drug, rifampin (23). Although controversial (24), recent data from observational studies suggest that statin use among patients with type 2 diabetes mellitus may reduce the frequency of incident TB cases (25, 26). Second, statins are among the most widely used drugs worldwide, and their safety profile is well documented and highly favorable (27). Third, statins and antiretroviral drugs can be co-administered (with drug and dose selection) (28), so patients with HIV infection on antiretroviral treatment (ART) may also benefit. Fourth, statins are attractive adjunctive agents for TB treatment due to the widespread availability of less costly generics. This is particularly important for TB, which is endemic primarily in resource-limited settings.

Statins: Lipid-modulating agents with pleiotropic effects

Statins are a class of drugs used to lower cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a crucial enzyme in the cholesterol biosynthesis pathway. In addition, statins possess important immunomodulatory and inflammatory properties, which reduce mortality and multiple organ dysfunction in patients with bacteremia, the risk of lung cancer, and even virus replication (29-32). Currently, a number of statins are on the market, including atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin.

Recent reports suggest that statin effects are not limited to cardiovascular diseases, and that these drugs may confer additional benefits in patients with osteoporosis, Alzheimer disease, rheumatoid arthritis, acute lung injury, and COPD. These pleiotropic effects of statins may be mediated by:

(i) Reducing the stability of lipid raft formation with subsequent effects on immune activation and regulation, and preventing the prenylation of signaling molecules with subsequent down-regulation of gene expression (33).
(ii) Endothelial cell (EC) cytoskeletal rearrangement, NADPH oxidase, and nitric oxide activity, as well as effects on differential EC gene expression, are relevant to the pathobiology of acute lung injury (34).
(iii) Inhibition of isoprenoid synthesis, which leads to the inhibition of intracellular signaling molecules Rho, Rac and Cdc42 (35).
(iv) Altering the gene expression and function of cells of both the innate and adaptive immune systems, including endothelial cells, macrophages, dendritic cells and T cells (36)

(v) Inhibition of IκB degradation, MMP-9 activity, TNF-α production, and cell spreading, and by the upregulation of tetraspanins, especially CD9 (37)
(vi) Activation of sirtuins (38, 39), leading to induction of autophagy
(vii) Induction of HO-1-mediated processes (40)
(viii) Inhibition of TGF-β (41, 42)

Statins in the adjunctive treatment of infectious diseases

Statin treatment improves the outcome of various infections. Statin treatment is associated with a 41% reduction in 30-day mortality in patients hospitalized with laboratory-confirmed influenza (43). Statins also reduce mortality in patients with bacteremia and multiple organ dysfunction (44, 45) and kill *Chlamydia pneumoniae* and *Salmonella enterica* within macrophages and in animal models of infection (46, 47). The use of statins was strongly and independently associated with a reduction in the risk of sepsis events in patients who were receiving dialysis due to chronic kidney disease (48). Prior statin therapy reduces the incidence of community-acquired pneumonia (CAP) (49, 50) and increases the survival of patients with CAP (51).

Although the mechanism underlying the antimicrobial activity of statins remains unknown, recent reports indicate there may be multiple mechanisms of action (52), including reduced release of pro-inflammatory cytokines from mononuclear cells or monocytes (53).

Statins as adjunctive HDT for TB

Following infection by Mtb, macrophages accumulate lipid bodies, acquiring a "foamy" phenotype through an ESAT-6-dependent pathway (54). Although their precise role remains poorly characterized, these lipid bodies may serve as a food source for intracellular bacilli. In addition, their accumulation has been associated with restriction of Mtb growth, and phenotypic tolerance to front-line drugs (55).

Table 1. Statin cytotoxicity in macrophages

	CC50 (24 hrs)	
	THP1	VERO
Pravastatin	>1.25mM	>10mM
Fluvastatin	0.316mM	0.547mM
Rosuvastatin	0.154mM	>2.5mM
Atorvastatin	0.061mM	0.52mM
Simvastatin	0.054mM	>0.12mM
Lovastatin	>0.031mM	0.17mM
Pitavastatin	0.03mM	0.243mM
Mevastatin	>0.015mM	>0.125mM

Cholesterol biosynthesis and transport may play a crucial role in the adaptation of Mtb within host tissues (56, 57) and its inhibition by statins could potentially alter protective immunity, thus altering disease outcome in the infected host (22). In *ex vivo* studies, statins reduced the accumulation of lipid droplets (22, 58), as well as the Mtb burden in human macrophages and in mice by enhancing phagosome maturation and autophagy (22). The process of autophagy is important for control of Mtb growth *in vivo*, as well as for preventing excessive inflammation in the host (59). Previous reports have highlighted the importance of autophagy for the full activity of isoniazid and pyrazinamide against intracellular Mtb through a mechanism based on release of reactive oxygen species (ROS) by host cells (mitochondria and NADPH oxidase) (60, 61). A recent study demonstrated the anti-TB activity of statins in the lungs of Mtb-infected mice (54). We recently showed simvastatin increases the bactericidal activity of the first line regimen against chronic TB in mice (62) and reduces the duration of curative treatment in this model (18).

Screening of statins for in vivo testing based on cytotoxicity in macrophages

We showed that, although statins lack direct antimicrobial activity, they promote intracellular Mtb killing *in vitro* (17). Others have shown that statins enhance macrophage killing of Mtb by promoting phagosomal maturation and autophagy (22). Moreover, simvastatin and atorvastatin increase rifampin-mediated killing of Mtb in macrophages (23).

For seven marketed statins and mevastatin (one of the original statins, which was never marketed), we first studied the cytotoxicity in THP-1 and Vero cells. Next, we characterized: (1) *in vitro* potency against intracellular Mtb in THP-1 cells and in murine macrophages, with and without companion anti-TB drugs, and (2) *in vivo* PK, drug-drug interactions and tolerability in mice.

Table 2: Statin EC50 values	
Simvastatin	0.2 μM
Pravastatin	7.8 μM
Fluvastatin	0.032 μM

To determine the 50% cytotoxic concentration (CC50) of each statin, human monocytic THP-1 (ATCC TIB-202) and Vero cells (ATCC CCL-81) were treated with a range of doses of each statin (with lowest dose corresponding to the inhibitory dose (IC50) reported in the literature) and toxicity was measured at 24 hours post-treatment. The results are summarized in **Table 1** (drugs are listed according to decreasing toxicity in THP-1 cells). Pravastatin was fpund to be the least toxic statin in this assay.

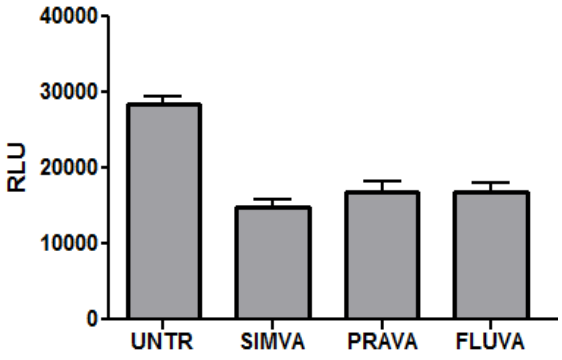


Figure 1: *M. tuberculosis* H37Rv-*lux* growth in THP-1 cells following 6 days of exposure to statins at the EC50 concentration.

Statin efficacy testing in macrophages

In order to determine the activity of statins against intracellular bacilli, we used a bioluminescent Mtb strain expressing luciferase to infect THP-1 cells. The luciferase strain was used to monitor growth of mycobacteria in real-time using a luminometer by measuring the relative light units (RLU), which serves as a reliable surrogate for colony-forming units (CFU) (16). The concentration of statin required to reduce bacterial colony counts by 50% after 6 days of exposure was defined as the effective concentration 50% (EC50). At non-toxic doses, atorvastatin and mevastatin were found to have no Mtb growth inhibition (not shown), while rosuvastatin, pitavastatin and lovastatin gave <50% inhibition. EC50 values could be obtained only for simvastatin, pravastatin and fluvastatin (**Figure 1** and **Table 2**).

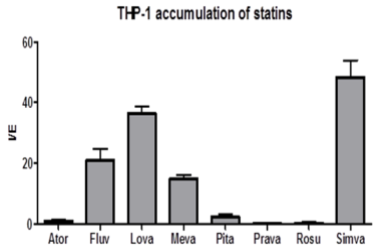


Figure 2. Ratio of intracellular to extracellular concentration (I/E) of statins.

Intra-macrophage concentrations of statins

In order to determine whether cytotoxicity or anti-TB activity of individual statins was associated with their intracellular accumulation, we studied their concentration inside THP-1 cells by liquid chromatography/tandem mass spectroscopy (LC-MS/MS) (18) following 30-minute exposure to the following concentrations of statins:

5x EC50 for the three statins in **Table 2**; for the remaining compounds we used 5-fold the concentration giving ~30-35% inhibition (atorvastatin, 24 µM; lovastatin, 4.35 µM; mevastatin, 0.47 µM; pitavastatin, 0.3 µM); rosuvastatin, 5.75 µM). Our data suggest that the relatively low CC50 of lovastatin and mevastatin may be related to their relatively high intracellular accumulation, while pravastatin, which showed a highly favorable CC50/EC50 ratio (161.3) had the lowest intracellular accumulation of the 8 statins tested (**Figure 2**).

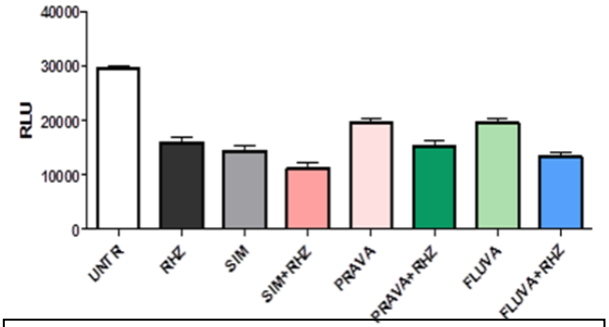


Figure 3: *In vitro* synergy of statin with the combination treatment of rifampin (0.0055 µM), isoniazid (0.006 µM) and pyrazinamide (81.23 µM) (RHZ). SIM (simvastatin), PRAVA (pravastatin) and FLUVA (fluvastatin). The statin concentrations are shown in Table 2.

Tolerability, pharmacokinetics (PK), and dose proportionality studies

In vitro synergy

In order to determine whether statins can enhance the activity of anti-TB drugs against intracellular bacilli, we used the RLU assay described above. We first determined the EC50 of each of the three first-line drugs separately and then in combination. When we tested the 3-drug combination (each drug dosed at the EC50—see **Figure 3** legend) alone or with simvastatin, pravastatin or fluvastatin (each tested at the respective EC50—see **Table 2**), we observed an additive effect of each of these three statins with the 3-drug combination (**Figure 3**).

Tolerability and PK of statins in uninfected C3HeB/FeJ mice

Mtb residing in necrotic mouse lung lesions may be more akin to persisters in human lesions with reduced response to direct-acting anti-TB drugs; further these areas represent relative pharmacological sanctuaries. Because of these favorable features, we and other groups have begun to use C3HeB/FeJ mice to test the efficacy of various antitubercular regimens and novel anti-inflammatory therapies (21, 63-66). In steady-state studies, separate groups of mice were given one of the 7 statins (mevastatin was not tested due to cytotoxicity) orally at a dose of 90 mg/kg together with isoniazid 10 mg/kg, rifampin 10 mg/kg, pyrazinamide 150 mg/kg and ethambutol 100mg/kg (HRZE refers to the 4-drug combination) for 5 successive days. Rifampin doses were separated from the accompanying drugs by 1 hour to minimize drug–drug interactions at the absorption stage (67, 68). In single-dose studies, mice were given one dose of simvastatin alone (20 mg/kg, 60 mg/kg and 90 mg/kg) or pravastatin (90 mg/kg and 180 mg/kg). Plasma drug concentrations were measured by LC-MS/MS.

Each statin given at 90 mg/kg was well tolerated for 5 successive treatments. To select a mouse dose for efficacy studies, we aimed to reproduce the human AUC at the highest clinically licensed dose, and twice that dose for selected compounds exhibiting the most attractive cytotoxicity profile and anti-TB activity in

macrophages (simvastatin and pravastatin). In the case of inactive prodrugs for which the acid conversion product is the active molecule (simvastatin and lovastatin), we matched the human AUC of the acid. Note that for most statins, matching the AUC resulted in C_{max} significantly higher than in humans (**Table 3**).

Table 3. Comparison of statin pharmacokinetics in mice and humans

	MOUSE			HUMAN			
Active drug	**dose**	**$AUC_{[0-2]}$**	**C_{max} (SD)**	**Highest licensed dose**	**$AUC_{[0-24]}$ at highest licensed dose**	**C_{max} at highest licensed dose**	**Liabilities/ characteristics**
	mg/kg	ng*h/ml	ng/ml	mg	ng*h/mL (SD)	ng/mL (SD)	
Atorvastatin	90	1,464	2,131 (3,610)	80	630-650	81-84	
Fluvastatin	90	6,028	4,201 (1,090)	80	1400	1000	CYP2C9 mainly
Lovastatin -OH	90	1,081	967 (896)	80	75-150	18-25	Prodrug
Pitavastatin	90	3,398	2,110 (449)	4	269		
Pravastatin	90	1,391	1,959 (2,498)	80	96 (40 mg)	45-55 (40 mg)	
Rosuvastatin	90	7,704	13,993 (8,383)	40	300	40	
Simvastatin	90	SIM BLQ	SIM BLQ	80	SIM 94	SIM 18	CYP3A substrate, prodrug
Simvastatin-OH		SIM-OH 240	SIM-OH 310		SIM-OH 63 (63)	SIM-OH 6 (7)	

Drug-drug interaction (DDI) and PK studies in mice

Significant effects of rifampin co-administration on simvastatin or pravastatin exposures were not observed in mice (data not shown), indicating that the standard mouse model does not recapitulate the known drug-drug interactions observed between rifampin and simvastatin and pravastatin seen in humans (69, 70). Therefore, the mouse PK interaction data were not used directly as a criterion for selecting pravastatin doses for the Stage I study in the UH3 phase.

On the basis of their favorable profiles (EC50, CC50, PK parameters), simvastatin and pravastatin were the 2 statins selected to progress to dose-response efficacy studies in mice.

Anti-TB activity of statin adjunctive therapy in mice

We showed previously that statins lack direct antimicrobial activity but promote intracellular Mtb killing in vitro (17). Others have suggested that statins enhance macrophage killing of Mtb by promoting phagosomal maturation and autophagy (22). Moreover, simvastatin and atorvastatin increase rifampin-mediated killing of Mtb in macrophages (23). This potential synergy between statins and the key sterilizing anti-TB drug may help explain our in vivo findings.

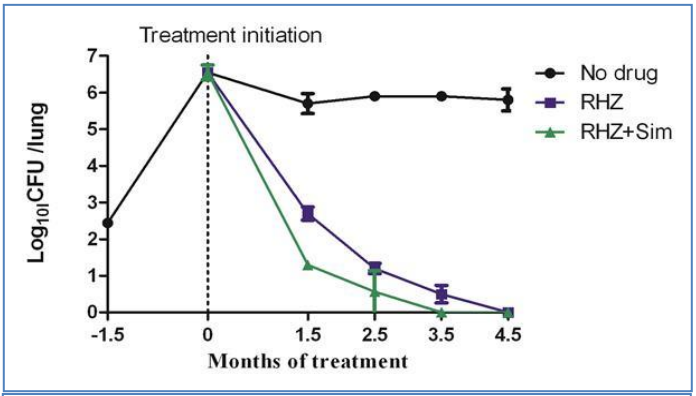


Figure 4: Adjunctive treatment with simvastatin reduces the time required to achieve culture-negative lungs in *Mtb*-infected BALB/c mice. RHZ= rifampin 10 mg/kg+ isoniazid 10 mg/kg + pyrazinamide 150 mg/kg. Sim= simvastatin 60 mg/kg. CFU= colony-forming units.

We studied the role of adjunctive treatment with simvastatin 60 mg/kg on the time required to achieve stable cure in Mtb-infected BALB mice. After 6 weeks of treatment, simvastatin adjunctive therapy led to a 1.4 $\log_{10}$ greater reduction in lung bacillary counts relative to the standard regimen alone (rifampin/isoniazid/pyrazinamide, RHZ) given at human-equivalent doses (**Figure 4**). The addition of simvastatin shortened the time required to achieve lung culture negativity from 4.5 months to 3.5 months. After 3.5 months of treatment relapse rates were 50% (5/10 mice) and 20% (2/10 mice) in the RHZ and RHZ + simvastatin groups, respectively. No relapses were observed in either group after 4.5 months of treatment. These encouraging results suggest that adjunctive statin treatment may help to reduce the duration of treatment required to cure TB.

Next, we conducted a preclinical study aimed at comparing the bactericidal activities of the standard TB regimen (rifampin, isoniazid, pyrazinamide and ethambutol; RHZE) with or without escalating doses of pravastatin against chronic TB in BALB/c mice. Antibiotics were given five times weekly for 8 weeks (continuous phase) beginning 6 weeks after infection.

Treatment with RHZE plus pravastatin at doses ranging from 30 to 180 mg/kg demonstrated a dose-dependent increase in bactericidal activity, reducing lung bacillary counts by 0.2–0.6 $\log_{10}$, 0.3–0.6 $\log_{10}$ and 0.3–0.8 $\log_{10}$ compared to RHZE alone at weeks 2, 4 and 8, respectively (**Figure 5**).

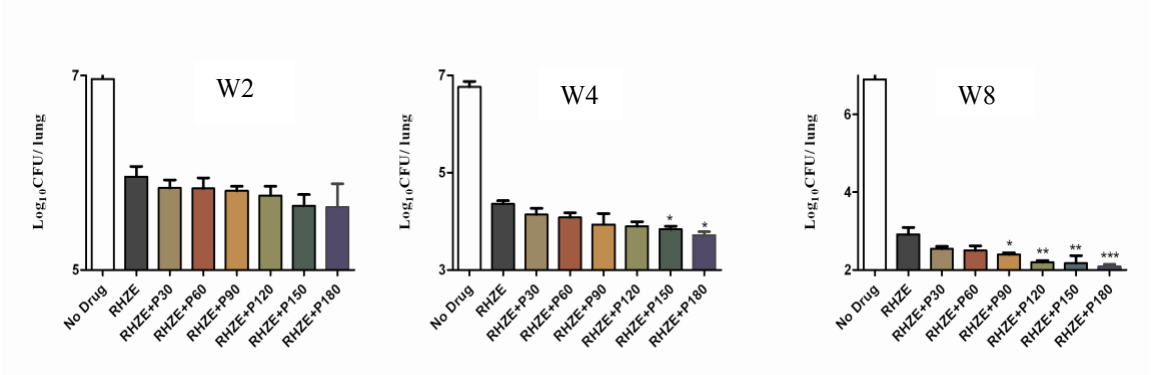


Figure 5: Adjunctive treatment with pravastatin augments bactericidal activity of the first-line TB regimen in BALB/c mice. RHZE= rifampin 10 mg/kg+ isoniazid 10 mg/kg + pyrazinamide 150 mg/kg+ Ethambutol 100 mg/kg. P=Prav CFU= colony-forming units. . *p<0.05, **p<0.01, ***p<0.001

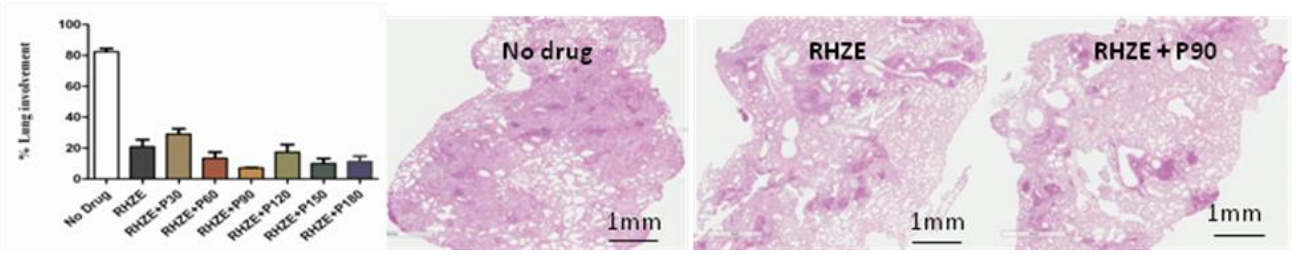


Figure 6: Lung inflammation 8 weeks after initiation of treatment. At least one entire H&E-stained cross section per animal lung (5animals/group) was analyzed for degree of inflammation. The surface area occupied by granulomatous inflammation was determined by ImageJ software-based morphometry of digitized images of lung sections and results are represented as percentage of lung surface area involved.

After 8 weeks of treatment, the degree of lung inflammation correlated with the bactericidal activity of each drug regmen (**Figure 6**)

Statin adjunctive therapy for TB in a mouse necrotic granuloma model of TB

Mtb residing in necrotic mouse lung lesions may be more akin to persisters in human lesions with reduced response to direct-acting anti-TB drugs; further these areas represent relative pharmacological sanctuaries. Because of these favorable features, we and other groups have begun to use C3HeB/FeJ mice to test the efficacy of various antitubercular regimens and novel anti-inflammatory therapies (21, 63-66). C3HeB/FeJ mice were infected with ~50 bacilli of *Mtb* H37Rv and 6 weeks later were treated with one of the following regimens: 1) No treatment (negative control); 2) Human-equivalent doses of the first-line regimen (RHZE) (positive control); 3) RHZE + simvastatin 90 mg/kg; 4) RHZE + pravastatin 50 mg/kg; 5) RHZE + pravastatin 90 mg/kg. The pravastatin 50 mg/kg dose was selected in

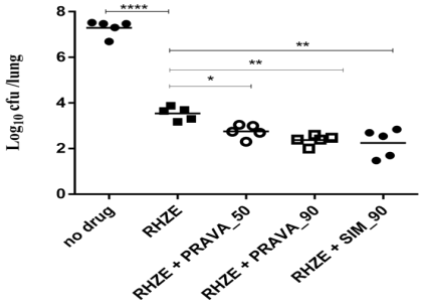


Figure. 7. Adjunctive anti-TB activity of pravastatin and simvastatin in C3HeB/FeJ mice. Data represent log10 colony-forming units (cfu)/lung on week 8 after initiation of treatment. Prava= pravastatin; SIM= simvastatin.

this study to match the 24-hr AUC achieved with a human dose of pravastatin 80 mg. After 8 weeks of treatment, mice receiving statin adjunctive therapy had significantly reduced lung bacillary burdens relative to control mice receiving RHZE alone. Thus, relative to the control regimen, adjunctive therapy with simvastatin 90 mg/kg, pravastatin 90 mg/kg, and pravastatin 50 mg/kg further reduced lung cfu by 1.28 $\log_{10}$ ($p< 0.0001$), 1.16 $\log_{10}$ ($p< 0.01$), and 0.78 $\log_{10}$ ($p< 0.05$), respectively (**Figure 7**).

Statin adjunctive therapy for TB in mice with cell-mediated immune deficiency

In order to determine the contribution of cellular immunity to the anti-TB effect of statins, we tested the activity of pravastatin in combination with the first-line anti-TB regimen in Mtb-infected athymic NU/NU (nude) mice (71).

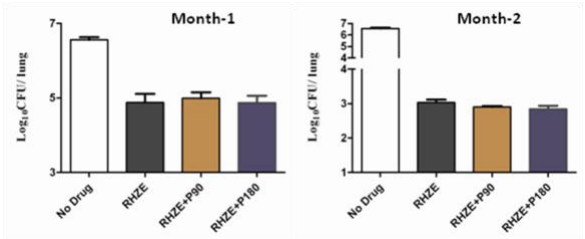


Figure 8. Adjunctive treatment with pravastatin (P) in Mtb-infected NU/NU mice.

Nude mice were aerosol-infected with Mtb H37Rv and treatment was initiated 4 weeks later with RHZE or RHZE + pravastatin 90 mg/kg (P90) or pravastatin 180 mg/kg (P180) administered once daily 5 days a week (5/7) for a total of 8 weeks. As expected, nude mice treated with RHZE showed significantly reduced lung CFU counts compared to the untreated control group. There is a trend toward reduced lung CFU in the RHZE+ P90 or P180 groups to RHZE alone, but this is not statistically significant (**Figure 8**). Thus, one may speculate the lack of bactericidal effect of statins in nude mice is probably due to deficient adaptive immunity (72), which may be required for proper activation of macrophages.

Potential modes of action of statins as adjunctive HDT against TB

The target enzyme of statins is HMG-CoA reductase, which catalyzes the conversion of HMG-CoA into mevalonate, a rate-limiting step in the cholesterol biosynthesis pathway (73). The mevalonate pathway is a multi-branched pathway leading to the biosynthesis of cholesterol, lipid hormones, including vitamin D, isoprenoids, and isoprenoid intermediates, such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP), which are required for protein prenylation. The prenylation state of proteins, including the Rho, Rac and Ras protein families (74), results in their subcellular redistribution and membrane anchoring. In particular, Rac transduction signaling modulates generation of reactive oxygen species (ROS), which is an important mechanism by which macrophages control Mtb infection (75). Given the multiple functions of the mevalonate pathway, statins possess anti-inflammatory activities in addition to their well-known lipid-lowering properties. For example, statins reduce expression of pro-inflammatory chemokines and cytokines in animal models of atherosclerosis and in patients with rheumatic diseases (76-81). Indeed, the cardioprotective effect of statins is likely related to their immunomodulatory, rather than lipid-lowering, properties (74). In addition, statins appear to activate peroxisome proliferator-activated receptors (PPAR) - α and –γ in inflammatory cells and other cell types (82). PPAR-γ regulates lipid uptake, adipogenesis, and glucose metabolism, and has been implicated in the pathology of obesity, diabetes, atherosclerosis, and cancer (83).

Table 4. Genes differentially regulated after treatment with pravastatin with and without RHZE

	Induced genes				Reduced genes			
	Fold change				Fold change			
Treatment	>10	>2	>1.5	Total	>10	>2	>1.5	Total
P30	1	37	90	128	3	18	98	119
P90	1	103	180	284	0	32	193	225
P180	1	44	190	235	0	54	223	277
RHZE	8	751	1469	2228	12	1080	1454	2546
RHZE+P90	1	235	1834	2070	4	923	1518	2445
RHZE+P180	4	678	1600	2282	19	816	1571	2406

<u>Cholesterol-lowering effect</u>

It has been postulated that the lipid-lowering properties of statins may play a role in their anti-TB activity. Mtb infection induces intracellular lipid accumulation in macrophages in the form of lipid droplets (LD), quasi-organelles consisting of cholesterol esters and triglycerides surrounded by a phospholipid monolayer (84). Lipid-laden macrophages acquire a "foamy" phenotype, which is associated with

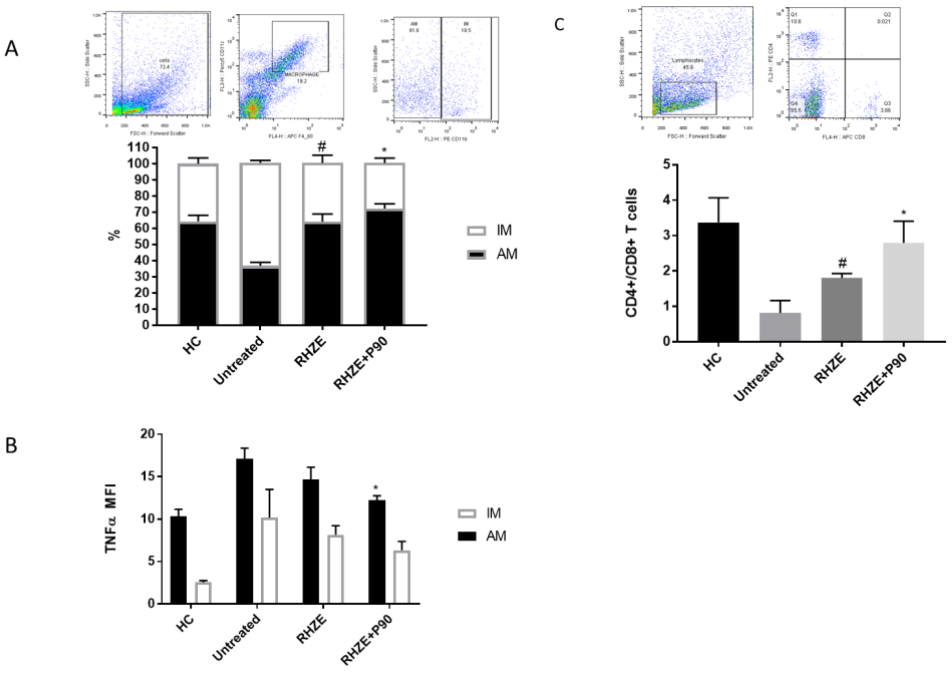


Figure 10. Changes in immune cell profiles in the lungs following TB treatment and adjunctive statin therapy. RHZE plus pravastatin 90 mg/kg increased the % of lung alveolar macrophages (A), decreased TNFα in alveolar macrophages (B), and increased the ratio of $CD4^+/CD8^+$ T cells in Mtb-infected lungs (C).
# $p<0.05$ compared to no Tx (untreated). * $p<0.05$ compared to RHZE.

delayed phagolysosomal maturation, enhanced IL-10 induction, alternative macrophage polarization, and blunting of innate immunity (85). Although their precise role remains poorly characterized, LD may serve as a food source for intracellular bacilli (85). In addition, their accumulation has been associated with slowed Mtb division and phenotypic tolerance to front-line drugs (55). Therefore, foamy macrophages appear to represent a stable reservoir for Mtb, facilitating persistence of the organism in the host.

We have shown that there is a correlation between the antimycobacterial and lipid-lowering activities of statins, since statin treatment of Mtb-infected macrophages reduces bacterial burden, LD formation, and intracellular cholesterol levels (17). We found that the antimicrobial effect of statins is reproduced by treatment with inhibitors of 7-dehydrocholesterol reductase (DHCR7, an enzyme required for cholesterol biosynthesis), but not with inhibitors of prenylation enzymes (FPP transferase and type I and II GGPP-transferases). However, prenylation-associated effects of statins cannot be excluded, since the inhibitory effects of statins are partially reduced by addition of GGPP (22).

In order to determine if the anti-TB activity of statins correlated with their cholesterol-lowering properties in vivo, we measured plasma total cholesterol and low-density lipoprotein (LDL), as well as the content of cholesterol and cholesterol esters in lung lesions using MALDI-MSI, following adjunctive therapy of Mtb-infected mice with simvastatin 60 mg/kg. No significant differences were observed between the control group and simvastatin arm with respect to cholesterol levels in plasma or lung lesions (data not shown). These results suggest that cholesterol depletion is unlikely to be the major mechanism by which simvastatin potentiates anti-TB drugs, although an effect on intracellular cholesterol levels cannot be excluded.

Statin effects on cytokine expression

In order to gain insight into the anti-TB mechanism of action of statins in vivo, the lungs of mice treated with pravastatin adjunctive therapy and those treated with RHZE alone were analyzed by whole-genome microarrays and RT-PCR. Mouse sera were studied by multiplex enzyme-linked immunosorbent assays.

Treatment with pravastatin had a profound effect on the global transcription in mouse lungs (**Table 4**).

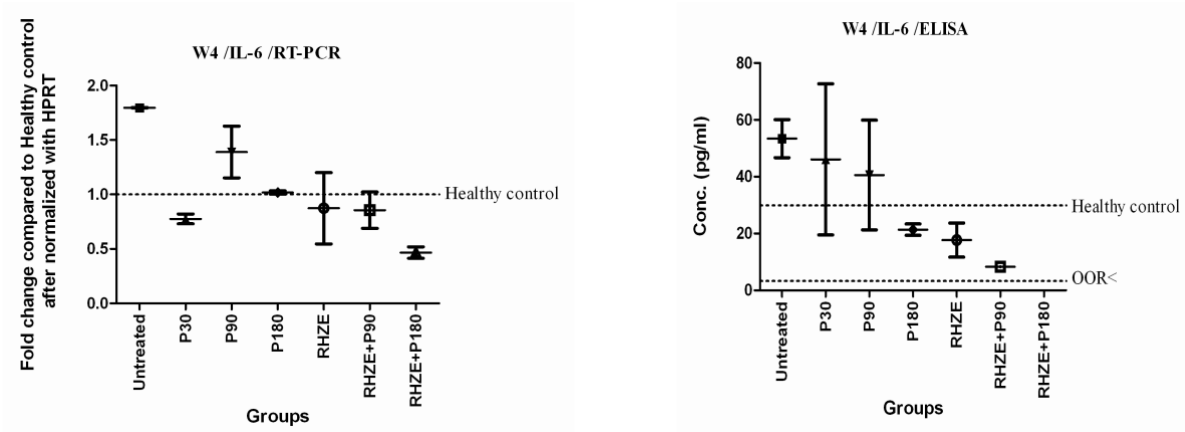


Figure 9. Pravastatin adjunctive therapy for tuberculosis reduces IL-6 in the lungs and serum of mice

Major pathways that were altered after statin treatment included the following: differential regulation of cytokine production in macrophages and T helper cells by IL-17A and IL-17F, the role of JAK family kinases in IL-6-type cytokine signaling pathway, and hematopoiesis from pluripotent stem cells.
Interestingly, global gene expression profiling of the lungs following Mtb infection revealed increased expression of IL-6, and increased IL-6 levels were detected by ELISA in the serum of Mtb-infected mice when compared with uninfected controls. RHZE reduced IL-6 gene expression in mouse lungs and

circulating IL-6 (by ELISA) in the serum relative to infected untreated controls (**Figure 9**). Pravastatin adjunctive therapy further reduced IL-6 expression in the mouse lungs and serum relative to RHZE controls.

Pravastatin adjunctive therapy favorably alters immune cell profiles in mouse lungs

Female BALB/c mice were aerosol-infected with Mtb H37Rv and, 6 weeks after infection, were randomized to receive not treatment, human-equivalent doses of the standard firs-line regimen (RHZE), or RHZE + pravastatin 90 mg/kg once daily for 28 days. After harvesting the lungs, the intrapulmonary lymphocyte and macrophage populations were measured by FACS. RHZE treatment was found to restore the ratio of alveolar macrophage (AM) to interstitial macrophage (IM) relative to uninfected (naïve) mice. RHZE plus pravastatin 90mg/kg further increased the ratio of AM to IM (**Fig. 10A**). Using intracellular cytokine staining, we found that TNFα levels were significantly lower in AM of mice treated with RHZE and pravastatin 90 mg/kg compared to those of control mice or those treated with the control regimen (**Fig. 10B**). Similar to the findings with AM, RHZE treatment restored the ratio of $CD4^+$ to $CD8^+$ T cells in mouse lungs and adjunctive therapy with pravastatin 90 mg/kg further increased the ratio of $CD4^+$ to $CD8^+$ T cells (**Fig. 10C**).

Effects of statins on anti-TB functions of macrophages

Treatment of macrophages with 100 nM simvastatin inhibits Mtb growth and supplementation with mevalonate was able to partially overcome the anti-TB effect, suggesting that statin treatment was not exerting an off-target effect (**Figure 11A**). Moreover, we found that exposure of primary murine macrophages to increasing concentrations of simvastatin resulted in phagosome maturation in a dose-dependent manner. Two parameters of phagosome maturation were tested: proteolytic capacity (**Figure 11B**) and acidification (**Figure 11C**). Recently, we found that pravastatin 125 μM similarly promotes phagosome-lysosome fusion in THP-1 cells (data not shown). The mechanism underlying the statins' antimycobacterial effect and how it links with the observed phagosome/lysosome fusion is being actively investigated. One step is to determine whether the antibacterial activity of the statin can be assigned to a branch of the mevalonate pathway. Our preliminary results rule out a role for farnesylation and type I geranylgeranylation (data not shown). Under current investigation is the role of cholesterol biosynthesis and of the geranylgeranylation type II. The latter is promising because Rab GTPases (which are prenylated by GGT type II) are involved in phagosome maturation and vesicle trafficking. The effects of statins on other immune mediators affecting phagosome/lysosome fusion are also being investigated.

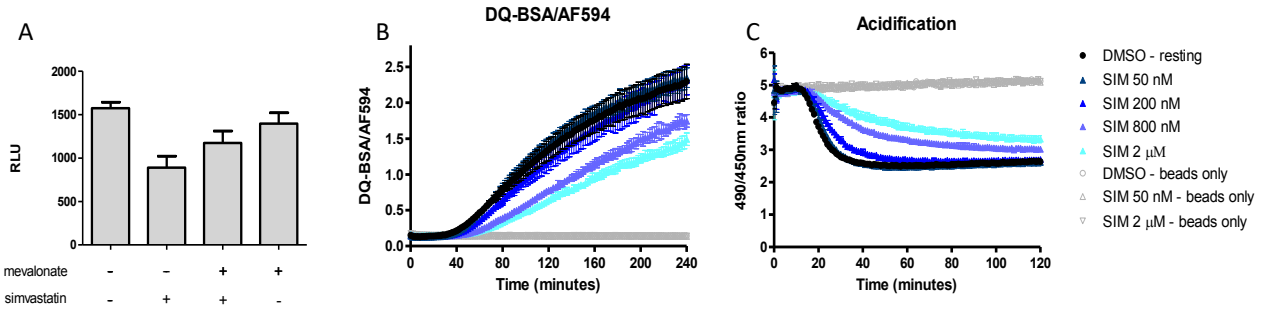


Figure 11. Reversal of the anti-Mtb effect of simvastatin in THP-1 cells by addition of mevalonate (A). Proteolysis bead assay (B): Beads were coated with a fluorescently tagged protein (DQ-BSA) and added to cultured macrophages. Substrate hydrolysis in phagocytosed beads results in loss of fluorescence signal (for each dose tested, the increase of signal with time is a function of bead uptake). Acidification bead assay (C): beads were coated with pH-sensitive carboxyfluorescein and added to cultured macrophages. Emission of fluorescence by the phagocytosed beads following excitation at two wavelengths (490nm and 450 nm) varies with phagosomal pH (for each dose tested, ratio decrease with time is a function of bead uptake). Beads only = beads in assay buffer without macrophages.

Rationale for conducting the StAT-TB clinical trial

Our clinical study to test the adjunctive anti-TB activity of statins is motivated by several lines of investigation. First, several studies have shown that statins have anti-TB properties. We found that statin adjunctive therapy enhances the first-line anti-TB regimen's antimicrobial activity and shortens the time required to achieve cure in a standard mouse model of chronic TB infection ((17, 18) and unpublished data). Recently, we found that 8 weeks of adjunctive therapy with simvastatin or pravastatin significantly reduced lung bacterial burden in the highly clinically relevant C3Heb/FeJ mouse model of chronic TB, which develops human-like, necrotic TB lung granulomas(19-21). Parihar et al. showed that peripheral blood mononuclear cells and monocyte-derived macrophages from patients with familial hypercholesterolemia receiving statin therapy were more effective in controlling Mtb growth compared with those of healthy donors, through induction of autophagy and promotion of phagosome maturation (22). In addition, statins are synergistic with the key sterilizing drug, rifampin (23). Recent data from observational studies suggest that statin use among patients with type 2 diabetes mellitus may reduce the frequency of incident TB cases (25, 26). Second, statins have immune-modulatory properties and statin use improves mortality in patients with sepsis (44, 45) or with community-acquired pneumonia (51). Several studies have shown that statins reduce lung inflammation in the mouse model of chronic TB (22) (and unpublished data). Our data suggest that these effects may be mediated by modulation of lung cytokine expression, including reduction of levels of IL-6 and TNFα, and an increase in the ratio of $CD4^+/CD8^+$ T cells in the lungs of Mtb-infected mice. Third, statins are among the most widely used drugs worldwide, and their safety profile is well documented and highly favorable (27). Fourth, statins and antiretroviral drugs can be co-administered (with drug and dose selection) (28), so patients with HIV infection on antiretroviral treatment (ART) may also benefit. Fifth, statins are attractive adjunctive agents for TB treatment due to the widespread availability of less costly generics. This is particularly important for TB, which is endemic primarily in resource-limited settings.

Of 8 statins tested, pravastatin was selected for testing in the current study based on its favorable CC50/EC50 values and demonstration of adjunctive activity against chronic TB in the standard mouse model. Specifically, pravastatin demonstrated the least toxicity of all statins in our assays (CC50> 1.25 mM and > 10 mM in THP-1 and Vero cells, respectively) and was one of 3 statins for which an EC50

could be obtained within the tested range in THP-1 cells (7.8 μM), yielding a highly favorable CC50/EC50 >160.3. Importantly, pravastatin showed dose-dependent activity against chronic TB in the standard mouse model, and improved the percent surface area of lung involved by inflammation. Adjunctive therapy with pravastatin 50 mg/kg, which yields human-like drug exposures following an 80-mg dose, significantly reduced the lung bacillary burden in a clinically relevant mouse model of necrotic TB granulomas (C3Heb/FeJ mice) relative to controls receiving the first-line regimen alone. Although simvastatin also showed a highly favorable CC50/EC50 ration in macrophages and adjunctive activity in both mouse models of chronic TB, several factors dampened our enthusiasm for this agent relative to pravastatin. Based on our PK data, simvastatin is almost entirely converted to its acid metabolite in mice, whereas in humans metabolism to simvastatin acid is ~1:1. Since we do not know if the parent drug or acid metabolite is responsible for the anti-TB effect, it is possible that the mouse data overestimate the adjunctive activity of simvastatin. Perhaps more importantly, peak concentrations of both simvastatin and simvastatin acid are greatly reduced (by 90%) by rifampin (69). On the other hand, pravastatin exposures are reduced by only 30-50% during co-administration with rifampin (70). Our PK data suggest that drug clearance and exposures differ markedly between mice and humans. Given that we observed a dose-responsive anti-TB effect with pravastatin adjunctive therapy in mice and the known drug interactions between pravastatin and rifampin in humans, we will study the safety/tolerability and PK of the highest clinically recommended dose of pravastatin (80 mg daily) in this clinical trial. Due to the anticipated ~50% reduction in 24-hour pravastatin exposures when the drug is co-administered with rifampin, dose escalation (if tolerated) will continue up to 160 mg daily. The anti-TB effect of adjunctive statin therapy observed in mice was accompanied by a reduction in serum IL-6 (**Figure 9**) to levels seen in uninfected mice. If safety, tolerability, and PK of pravastatin adjunctive therapy are deemed acceptable following completion of this study, we will proceed to a larger follow-up study in which we will also explore the potential treatment-shortening and immunomodulatory effects of pravastatin adjunctive therapy for TB.

4. Study Procedures

Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol informed consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) *WILL* be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment), sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. Site-specific ICF(s) *WILL* NOT be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification from the DAIDS PRO that approves the site specific ICFs and indicates successful completion of the amendment protocol registration process. A copy of the final amendment Registration Notification issued by the DAIDS PRO should be retained in the site's regulatory files.

Screening

Participants with a presumptive diagnosis of sputum GeneXpert positive pulmonary TB will be invited to participate. Because culture confirmation is rarely available when TB treatment is initiated, patients will be recruited based on having a sputum specimen that is positive for TB by GeneXpert MTB/RIF.

Informed written consent, using IRB-approved consent forms, will be obtained by trained study personnel prior to performing any study-specific procedures. Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation.

The following will be performed after obtaining written informed consent:

- Eligibility criteria will be confirmed
- All participants will undergo HIV testing and counseling regardless of whether they have had a previous HIV test or not. HIV testing will be carried out with two rapid tests (the second test will be to confirm the result of the first); only one test need be carried out if the participant is known to have HIV, the single test will be confirmatory. Should there be a discrepancy between the two rapid tests, blood will be sent to the laboratory for enzyme-linked immunosorbent assay ELISA and Western Blot test in order to confirm the diagnosis.
- For HIV-positive participants, a CD4 count should be performed unless written results are available from a test done within the preceding 3 months or less.
- Participants will be asked questions related to demographics, current and past medical history, concomitant medications, alcohol and drug use and TB symptoms.
- Participants will have heart rate, blood pressure, respiratory rate, temperature and weight measured.
- An expectorated sputum sample will be obtained for GeneXpert MTB/RIF (as per inclusion criterion d), smear, culture, and drug susceptibility testing at the study laboratory (or documentation of results).
- Blood will be drawn for liver function tests (AST, ALT, total bilirubin), Hepatitis B and C serology, CK, serum creatinine, CBC (WBC, hemoglobin, and platelets).
- A posterior anterior chest radiograph will also be taken, unless a posterior anterior chest radiograph done within the previous 14 days or less is available for review.
- Urine will be collected for pregnancy testing if a woman is of child-bearing potential.
- Complete physical exam and vital signs
-
- Targeted symptom assessment

The Screening evaluation process itself may occur over a few days with more than one visit, however, all Screening evaluations will be obtained within 3 days of study entry.

Day 1 (Baseline)

- Treatment Assignment
- DOT of pravastatin
- Adverse event assessment
- Chemistry (Comprehensive metabolic panel: total cholesterol, LDL, , C-reactive protein, uric acid)
- Intensive PK for pravastatin
- Lung inflammation assessment (PFT, St. George's respiratory questionnaire, 6-minute walk test); +/- 3 days.
- Targeted symptom assessment
- Sputum

Day 2

- Intensive PK for pravastatin
- DOT of pravastatin and TB treatment
- Adverse event assessment

Days 3-6

- DOT
- Adverse event assessment

Day 7 (Week 1)

- Targeted symptom assessment
- Concomitant medications
- DOT
- Adverse event assessment
- Sputum
- Liver function tests
- Chemistry (Comprehensive metabolic panel:, total cholesterol, LDL, , C-reactive protein, uric acid)
- CK, serum creatinine

Days 8-14

- DOT
- Adverse event assessment

Day 15 (Week 2)

- Targeted symptom assessment
- Concomitant medications
- DOT
- Adverse event assessment
- Sputum
- Liver function tests
- Chemistry (Comprehensive metabolic panel:, total cholesterol, LDL, , C-reactive protein, uric acid)

- Urine pregnancy test
- Intensive PK for pravastatin
- Lung inflammation assessment (PFT, St. George's respiratory questionnaire, 6-minute walk test); +/- 3 days.
- CK, serum creatinine

Day 21

- Chemistry,(Comprehensive metabolic panel: total cholesterol, LDL, C-reactive protein, uric acid)
- Liver function tests
- Targeted symptom assessment
- Adverse event assessment
- CK, serum creatinine

Day 30 (Month 1)

- Targeted symptom assessment
- Liver function tests
- Chemistry (Comprehensive metabolic panel: total cholesterol, LDL, C-reactive protein, uric acid)
- Lung inflammation assessment (PFT, St. George's respiratory questionnaire, 6-minute walk test); +/- 3 days
- Urine pregnancy test
- Adverse event assessment
- CK, serum creatinine
-

Unscheduled visit

- Exam and vital signs
- Targeted symptom assessment
- Adverse event assessment
- Concomitant medications
- Liver function tests
- Chemistry

TB treatment continuation

All study participants will be referred appropriately to continue standard TB treatment at study completion.

Targeted symptom assessment

Participants will be asked whether they have experienced any of the following since the last study visit: fevers, sweats, cough, rash, itching, jaundice, nausea, vomiting, diarrhea, loss of appetite, vision problems, numbness/tingling of extremities, headache, joint pain, eye pain. In addition, patients will be asked whether they have had other symptoms (not listed above); if yes, then these symptoms will be recorded and graded. In addition, targeted diagnostic evaluations may be indicated to determine the underlying cause of symptoms and their association with study drug use.

Concomitant medications

The use of all non-study drugs (including over-the-counter medications) from 14 days before starting study therapy through the end of experimental treatment will be monitored and recorded. In addition, if a grade 3 or higher AE or SAE occurs within 28 or fewer days after completion of experimental phase therapy, then the use of all non-study drugs (including over-the-counter medications) during the interval from study drug completion to AE/SAE onset will be recorded. The use of all non-study drugs (including over-the-counter medications) will be recorded at this study visit.

Concomitant antimicrobials with known antituberculous activity (amoxicillin/clavulanic acid, amikacin, kanamycin, streptomycin, moxifloxacin, ofloxacin, ciprofloxacin, levofloxacin, sparfloxacin, gatifloxacin, gemifloxacin, linezolid, capreomycin, cycloserine, ethionamide, para-aminosalicylate sodium) should not be used during the study phase therapy. A second statin should not be used during study phase therapy. Any patient who receives more than 2 doses of any of the medications listed above will be classified as being on a non-study regimen. Antimicrobials with no known antituberculous activity may be prescribed for intercurrent infections at the discretion of the investigator.

Ethanol can exacerbate the potential hepatotoxicity of isoniazid, rifampin, and pyrazinamide. Participants will be counseled to abstain from alcohol while on TB therapy.

Administration of study drug

Patients should start on assigned study drug (pravastatin)_as soon as possible after recruitment. Doses of study drug will be given as directly observed therapy (DOT) by study personnel, or by a health care worker or lay treatment supervisor who is aware of the study protocol and trained regarding the study protocol. Alternatively, doses of study drug can be given via DOT by a family member or employer who has been trained by the study team. Adherence will be confirmed by review of the participant's treatment card, and retraining of the DOT provider will be provided if needed. Adherence will be defined as the number of prescribed doses taken. DOT may be administered at the TB clinic or other health care facility, or, with the participant's permission, at the participant's residence, workplace, or other mutually agreed upon location convenient for the participant.
HRZE will not be provided by the study team.

Obtaining sputum specimens

Sputa should be obtained by spontaneous expectoration whenever feasible. Sputa should be refrigerated at approximately 4 degrees Celsius after collection, and transported to the laboratory as soon as possible but within 2 working days of collection (i.e. received in the laboratory within 2 working days of collection).

Laboratory aspects of sputum analysis

Laboratory evaluation of sputa will be performed at a qualified mycobacteriology reference laboratory and will be done according to detailed written lab SOPs. Briefly, sputa will be homogenized and decontaminated using the NALC-NaOH method. Fluorescence smear microscopy will be performed on an aliquot of the concentrated processed specimen. Aliquots will be added to Lowenstein Jensen (solid) media and MGIT (liquid) media. Nucleic acid and/or biochemical methods will be used for mycobacterial species determination. Quality monitoring procedures will be documented, and laboratory activities will be participant to regular study monitoring. Sputum collection will not occur after Friday of each week, as the protocol requires transport of specimens to the laboratory within 2 working days of sputum collection.

Steady State Pharmacokinetic Assessments

Intensive PK sampling: Each participant will have initial PK assessments for pravastatin at Day 1 (immediately prior to dose administration and at 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours post-dose) as well as single point PK assessment on Day 2 (trough level immediately before the 2nd dose and 4-6 hours after the 2nd dose of pravastatin). The post-dose PK assessment on Day 2 will be completed prior to the administration of the first dose of TB medications. Each participant will also have steady-state PK assessment at Day 15 immediately prior to administration of study medications and then at 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours post-dose. Samples will consistent of 4cc of blood collected via an indwelling catheter or by direct venipuncture.

For participants who miss or vomit the first dose of pravastatin on Day 1, that visit should be rescheduled >= 1 day later and study procedures recommenced. For participants who miss or vomit pravastatin dose on Day 2, that visit should be rescheduled within 2 days if possible, holding the first dose of TB medications until after these Day 2 PK draws. For participants who miss doses on one of the two days preceding the PK collection on Day 15 (steady state) or who vomit that day's doses, the Day 15 visit should be rescheduled for >=2 days later.

Specimens will be obtained, processed, and assayed according to the PK standard operating procedures manual.

Procedures to maximize compliance with study visits

Several methods will be used to maximize the proportion of participants who routinely keep their study visit appointments. First, only individuals with an expressed interest in participating and keeping appointments will be enrolled. Second, at enrollment, information will be collected including the participant's name, address, phone number, and, with the permission of the participant, names and telephone numbers of friends/relatives who would normally know the participant's whereabouts. Each participant will be contacted on the day prior to the scheduled clinic visit. If the participant cannot be reached at the primary phone number listed, every effort will be made to contact him/her at the secondary numbers listed for friends/relative. Third, participants will be given written appointment cards. Fourth, participants with telephones may be called the day before the scheduled visit to remind them. Fifth, participants who miss appointments will be contacted either by phone or by domiciliary visit or through friends/family whose names were provided by the participant. Finally, study participants will be compensated for their time and travel to and from study visit. The form and amount of compensation will be in accordance with local guidelines.

Informed Consent

Participants enrolled in the study will be approached and invited to participate in the study when they present for TB evaluation and care at the CHBH, or local clinics in Soweto. Individual informed consent will be obtained by trained study personnel using Ethics Committee/IRB-approved consent forms. The study will be explained to potential participants using an information sheet available in the common local languages (e.g., Sesotho, isiZulu). If a participant is unable to read or write, witnessed verbal consent will be obtained. Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation. Discussion of risks and possible benefits of study participation will be provided by designated study staff to potential participants. Potential participants will receive counseling about study objectives and procedures, potential risks and benefits, and the informed consent process. The participants will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. A copy of the signed and

dated informed consent document will be offered to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to participants that the quality of their medical care will not be adversely affected if they decline to participate in this study, and that they may withdraw consent at any time. The investigator will retain a copy of the signed consent forms, which may be inspected at the monitor's/auditor's request. The investigator will promptly report to the Ethics Committee/IRB of all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make changes in the research without Ethics Committee/IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

In South Africa, patients are provided free access to TB care, and HIV testing and treatment through the public sector. According to current South African National Department of Heath guidelines, HIV-seropositive patients with a CD4+ count <500 cells/μL should be advised and encouraged to start ART without delay. The current first line ART regimen in South Africa is a fixed-dose combination containing tenofovir, emtricitabine, and efavirenz.

Diagnosis and treatment of TB is provided for free through public sector TB Clinics and hospitals in South Africa. GeneXpert testing, which can rapidly and accurately detect *Mycobacterium tuberculosis* and rifampin resistance, is available at public health facilities in South Africa, including at PHRU and SoMCHAT. GeneXpert will be used at study sites to diagnose pulmonary TB and exclude patients with rifampin resistance, which is a surrogate for multi-drug resistance (MDR). Phenotypic drug susceptibility testing will be routinely performed on all isolates from participants enrolled in the study to confirm susceptibility to all first-line drugs.

Contraception

Females may participate if they are: 1) of non-childbearing potential (have had a bilateral oophorectomy, tubal ligation and/or hysterectomy or have been postmenopausal for at least 12 consecutive months), 2) if they are using effective barrier birth control methods and are willing to continue practicing birth control methods throughout treatment or 3) have a vasectomized male partner (confirmed sterile), or have a female partner. Therefore, to be eligible for this study women of childbearing potential should either: 1) use a double barrier method to prevent pregnancy (i.e. use a condom with either diaphragm or cervical cap) or 2) use an intrauterine device in combination with a barrier contraceptive. They must also be willing to continue these contraceptive measures until one week after the last dose of study medication or one week after discontinuation from study medication in case of premature discontinuation. (Note: Hormone-based contraception alone may not be reliable when taking rifampin-containing TB regimens; therefore, hormone-based contraceptives alone cannot be used by female participants to prevent pregnancy).

Safety and Tolerability Endpoints

The primary endpoint for analysis of safety and tolerability is Grade 3 or higher adverse events. Secondary endpoints include permanent discontinuation of assigned study regimen for any reason (other than new recognition of participant ineligibility based on absence of *M. tuberculosis* growth in baseline sputum cultures, or growth of *M. tuberculosis* resistant to rifampin by GeneXpert. Other aspects of safety and tolerability that will be assessed include mortality and the rates and types of toxicity thought related to study drugs by the Investigator.

Safety Evaluation

Participants will be carefully monitored to assure safety and detect and manage adverse events. Since treatment will be directly observed, a trained member of the team will assess participants daily for adverse

events. Specific tests to identify statin activity and potential toxicity will be performed at baseline (screening or Day 1) and Days 7, 15, and 30, and will include a serum chemistry panel and CK. A Data and Safety Monitoring Board (DSMB) will review the study protocol and oversee progress of the trial. The DSMB will review safety data after the recruitment of each dosing cohort. Since this is an open-label study, the DSMB will have access to treatment group assignment. The DSMB will be comprised of at least the following: an expert in statistics, clinical trials, clinical TB and statins. No early stopping rules will be formally adopted.

5. Adverse Events

The Investigators are responsible for eliciting adverse events by observing study participants and recording all adverse events observed by him/her or reported by study participants during the trial.

Definitions

Adverse event (AE): Any untoward medical occurrence in a study participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Serious adverse event (SAE): Any untoward medical occurrence that at any dose:

- results in death;
- is life threatening (any event in which the subject was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect; or
- is a medically important event.
  Note:
  Medical and scientific judgment should be exercised in deciding events are "medically important." These events are those that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the study participant or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

Unexpected Adverse Event (UAE): An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

Relationship of AE to Study

The relationship of each adverse event (AE) to the study procedures will be characterized. The PI will make this determination and will classify AEs as related or unrelated. "Related" will mean that there is a reasonable possibility that the AE may be related to the study agent(s). "Not Related" will mean that there is not a reasonable possibility that the AE is related to the study agent(s). When a SAE is assessed as "not related" to study agent(s), an alternative etiology, diagnosis, or explanation for the SAE should be provided. If new information becomes available, the relationship assessment of any AE should be reviewed again and

updated, as required. The study drug (pravastatin) and standard-of-care regimen, Rifafour (isoniazid, rifampin, pyrazinamide, ethambutol) will be assessed for relationship with the AE.

Reporting of Serious Adverse Events, and Unanticipated Problems

Requirements, definitions and methods for expedited reporting of adverse events are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the DAIDS RSC website at: https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids. This study will use the SAE form of expedited adverse event reporting, as defined in Version 2.0 of the DAIDS EAE Manual. Expedited reporting is required for the study drug (pravastatin) and the standard-of-care regimen, Rifafour (isoniazid, rifampin, pyrazinamide, ethambutol). The EAE reporting period for this study will be 30 days, which is the day of the final study visit. During these 30 days, all SAEs will be reported to DAIDS in an expedited fashion, within 3 days of occurrence, in accordance with DAIDS EAE Manual. Any of the study and non-study drugs which a participant has taken will be assessed for relationship with the SAE.

After the protocol-defined EAE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the DAIDS EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, EAEs will be submitted using the DAIDS EAE Form. This form is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting

For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact the DAIDS RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com.

All AEs will be graded using the Division of AIDS table for Grading Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study intervention or participation is not the cause. Serious AEs that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious AE which we become aware of through a passive basis (participant reporting back to the study team, or through publicly available information), which occurs after the study period (after day 30) and is considered to be possibly related to the study intervention (suspected, unexpected serious adverse reactions, or SUSARs) or study participation will be recorded and reported immediately.

Association and Attribution/causality

An adverse event is considered associated with the use of the drug (Adverse Drug Reaction) if the attribution is possible, probable or definite.

The following definitions for rating attribution/causality will be:

Relatedness Rating	Definition
Not Related	There is not a reasonable possibility that the AE is related to the study agent (pravastatin) or Rifafour (isoniazid, rifampin, pyrazinamide, ethambutol)

Related	There is a reasonable possibility that the AE may be related to the study agent (pravastatin).or one or more components of Rifafour (isoniazid, rifampin, pyrazinamide and ethambutol)

Severity*

With the exception of creatinine kinase elevation, all symptoms and laboratory findings will be graded according to severity using the Division of AIDS Table for Grading Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, which, in our view is the most comprehensive grading scale for patients with infectious diseases, including TB or HIV.

The protocol-specific criteria for grading elevated CK are as follows:

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Creatinine kinase, high	3 to < 6 x ULN	6 to <10 x ULN	10 to <20 x ULN	>20 x ULN

The DAIDS toxicity table criteria for grading liver function tests are as follows:

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
AST or SGOT, High	1.25 to < 2.5 x ULN	2.5to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥10.0 x ULN
ALT or SGPT, High	1.25 to < 2.5 x ULN	2.5to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥10.0 x ULN
Total bilirubin	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN

*The DAIDS AE Grading Table is available at: https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

Reporting

AE: Adverse events (AE) will be collected by the Investigators from the time a study participant receives the first dose of study drug through the Day 30 study visit. Any AE (serious or non-serious) observed by the Investigator or reported by the study participant that meets the AE reporting requirements (see below) will be recorded on an Adverse Event Case Report Form. The Investigator will review each AE and assess its relationship to drug treatment based on all available information at the time of the completion of the case report form. The following information will be recorded for each AE reported:

- Diagnosis of the AE, if possible. In the case where an overall diagnosis cannot be made, each specific sign and/or symptom will be recorded as individual AEs;
- Date of onset;
- Stop Date (duration) if applicable;
- Severity;

- Action taken with study drugs;
- Other action taken;
- Outcome;
- Relationship to study drugs;
- Seriousness.

The following adverse events must be reported on an Adverse Event Report Form:

- New medical diagnosis (at the time of enrollment, if the patient already has a medical diagnosis whose signs or symptoms worsen during the study to a Grade 3 or 4, this is an adverse event that must be reported)
- Any grade 3 or higher adverse event
- Study drug discontinuation due to an adverse event
- Pregnancy
- Lab values that are considered clinically significant by the investigator

The standard-of-care drugs are Rifafour (Ethambutol, Isoniazid, Rifampin, and Pyrazinamide) and the study drug is pravastatin. The most common adverse effects associated with the standard-of-care drugs and the study drug are specified in the "Study Drugs" section. The Investigator is responsible for monitoring all adverse events that are observed or reported during the study, regardless of whether they are related to the study drug. Participants presenting with AEs to other facilities during the study period will be referred back to the study site in order to complete all study visits and data collection. The participant will be given instructions to notify the study team if any AEs develop following the study period.
In accordance with the FDA's Code of Federal Regulations, the study Sponsor and the participating Investigators are responsible for reviewing all information relevant to the safety of the study drug and standard-of-care therapy. Reporting and monitoring of SAEs is required to alert the FDA, Sponsor, institutional review boards, and the clinical Investigators of real and potential safety issues. The Investigators will carefully review the AE Reports and use this information to monitor the investigational drug's toxicity profile and patient safety. Any AE associated with the use of the study drug and/or the standard of care regimen that is both serious and unexpected will be reported to the FDA in the form of a written Safety Report.
The responsible institutional review boards, FDA and South African Regulatory body South African Health Products Regulatory Authority will be notified of all SAEs and AEs according to their individual guidelines.

Pregnancy

Since HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly synthesis of other biologically active substances derived from cholesterol, they are contraindicated during pregnancy and in nursing mothers.

The Investigator will immediately notify the Sponsor of any pregnancy that is discovered during study drug administration or which started during study drug administration. Pregnancy forms will be completed for all pregnancies reported during the clinical trial. In addition, with the permission of the study participant, or female partner of a male participant, the investigator will report follow-up information regarding the outcome of the s, including perinatal and neonatal outcome. Infants will be followed for 6 months.

All women of childbearing potential will be instructed to contact the study team immediately if they suspect they might have become pregnant (for example, missed or late menses) during study treatment or within one month of receiving their last dose of study medication.

If pregnancy is suspected for a participant in the experimental arm while the participant is receiving pravastatin, the pravastatin will be withheld immediately until the result of the pregnancy test is known. If pregnancy is confirmed, the pravastatin will be permanently discontinued in an appropriate manner and the participant withdrawn from the trial. Protocol-required procedures for trial discontinuation and follow-up will be performed unless contraindicated by the pregnancy. Patients will be referred to prenatal care and to the local TB program for continuation of their TB treatment. Women will be asked questions about pregnancy history as well as information about the current pregnancy and birth. Permission will also be requested to follow the infant for six months.

Should the female partner of a male participant enrolled in the pravastatin arm become pregnant during the study or in the 4 weeks after the completion of pravastatin (Week 16 after study entry) and the Investigator becomes aware that this situation has occurred, consent will be requested from the female partner for collection of information on her pregnancy history and for information on the current pregnancy and birth. Permission will also be requested to follow the infant for six months.

Pregnancy reporting will follow the same time lines and reporting structures as for a SAE (see above), but by using Pregnancy forms to be sent to the data management center. SAE reporting will also occur if the pregnancy outcome is a congenital anomaly. This will follow the reporting procedures described above for SAE reporting plus an additional clinical report.

Clinical Management of Adverse Events

Liver toxicity

Study treatment will be permanently discontinued if any of the following criteria are met:

- ALT > 5x the upper limit of normal (ULN) without symptoms
- ALT > 3x ULN with signs or symptoms suggestive of clinical hepatitis including one or more of the following: nausea, vomiting, abdominal pain, dark colored urine or clay colored stools, unexplained fever, jaundice, liver tenderness, or hepatomegaly
- ALT > 3x ULN and Total Bilirubin ≥ 2x ULN

If a patient develops hepatic toxicity meeting the above stopping criteria, the following evaluation will be undertaken: assessment for history of injection or non-injection drug use, alcohol ingestion, use of other hepatotoxic drugs, and performance of serologic tests for viral hepatitis (IgM antibody for Hepatitis A, Hepatitis B surface antigen, IgM antibody to Hepatitis B core antigen, antibody to Hepatitis C). In addition, an AST and INR should also be checked, if not already done.
Participants will be followed until resolution (return to baseline) or stabilization of the ALT elevation.

Creatinine Kinase Elevation

For Grade 1 (3 to < 6 x ULN) or Grade 2 (6 to < 10 x ULN) creatinine kinase elevation, participants may continue study drug treatment and should be carefully evaluated and followed closely. For Grade 3 (10 to < 20 x ULN) or higher creatinine kinase elevation, participants will permanently discontinue statin but will be requested to attend follow-up visits until study end.

Myalgias and Myopathy

Significant myalgias (Grade ≥3, i.e., muscle pain causing inability to perform usual social and functional activities) should be evaluated with a clinical assessment that includes an evaluation of CK, serum

creatinine, potassium, and urinalysis. Myopathy is defined as muscle aches, soreness, tenderness, or weakness with creatinine kinase (CK) >10 x ULN not related to exercise or other causes, including trauma. If the symptoms are associated with Grade ≥3 elevation in CK (10 x ULN) that is not related to exercise or other cause, study medications should be permanently discontinued. Participants will be followed on study, off study drug treatment through the study termination visit per protocol.

Other toxicities

Grade 1 toxicities: In general, for grade 1 toxicities, the patient will be followed carefully, and the study drugs will be continued.

Grade 2 toxicities: For grade 2 toxicities, the patient will be followed more carefully, with additional laboratory and/or clinic visits as necessary, and the study drugs temporarily held at the investigator's discretion.

Grade 3 toxicities: For any grade 3 toxicity involving creatinine kinase elevation, with or without myopathy, myalgias, hepatitis, nausea/vomiting, and/or diarrhea, that, in the principal investigator's judgment is due to study drug, the study drug will be discontinued. The clinician should rule out other possible causes of the symptoms before discontinuing study medication. When possible, concomitant medications should be held first at the discretion of the principal investigator if he/she suspects they are contributing to the toxicity. In the event of study product discontinuation, management of the standard of care TB drugs will revert to the site clinician as per local practice.

Grade 4 toxicities: Any patient with grade 4 renal, hepatic, cardiac or hematological toxicity will be immediately discontinued from study therapy. The laboratory test or clinical finding in question will be reassessed as soon as possible. The repeat test will guide management of the event as follows:

- If the repeat assessment shows toxicity of grade 3 or lower, and if the patient has continued to receive study drugs between the two testing dates, then the patient will be managed according to the appropriate toxicity level of the repeat test.
- If the repeat test shows toxicity of grade 3 or lower, and if the participant has not received study drugs between the two testing dates, then the participant will be managed at the discretion of the investigator with regard to the re-administration of study drugs, and otherwise according to the toxicity level of the repeat test.
- If the repeat test shows grade 4 toxicity, then the participant will be permanently discontinued from study medications. Further treatment of TB will be directed by the investigator on an individualized basis. The participant will continue to be followed for study monitoring purposes (as are other participants who make a permanent departure).

For other grade 4 toxicities, the study drugs will be permanently discontinued. Toxicities graded 3 or 4 and occurring during study therapy will be documented on the AE Form, according to the criteria described in the following sections. The maximum level of toxicity reached will be clearly indicated.
For all toxicities that are treatment-emergent and that require permanent discontinuation of study treatment, relevant clinical and laboratory tests will be obtained as clinically indicated and repeated as needed until final resolution or stabilization of the toxicity.

Permanent discontinuation of study drugs: Certain events or conditions may necessitate permanent discontinuation of the statin study drug. Participants who experience such events or conditions, however, will still be "on study" and will be followed until study completion. Study drug will be discontinued with continued study follow-up for participants in whom treatment-emergent drug toxicity warrants discontinuation of study therapy and for participants who become pregnant. If study drug is permanently

discontinued, continued standard-of-care therapy for tuberculosis may be administered at the investigator's discretion. If Rifafour is discontinued, the individual components (isoniazid, rifampin, pyrazinamide, and ethambutol) may be gradually re-introduced, as per national guidelines.

6. Blinding, including justification for blinding or not blinding the trial, if applicable.

Not applicable

7. Justification of why participants will not receive routine care or will have current therapy stopped.

Not applicable

8. Justification for inclusion of a placebo or non-treatment group.

Not applicable

9. Definition of treatment failure or participant removal criteria.

Participants who prematurely discontinue study treatment after having received one or more doses of pravastatin will be replaced.

Study treatment must be immediately discontinued as a result of the following:

- Withdrawal of informed consent.
- Investigator considers it for safety reasons in the best interest of the participant that he/she be withdrawn.
- If a participant develops a new onset stage 4 illness after enrollment, they would meet the criteria for withdrawal from the study and, if HIV-positive, for fast track initiation of ART per the South African National Department of Health ART guidelines. The Interim WHO Clinical Staging of HIV/AIDS and HIV/AIDS Case Definitions for Surveillance, African Region (2005) will be used for guidance on definitions of stage 4 illnesses.
- Resistance to rifampin, as determined by GeneXpert.
- Participant experiences specific toxicities (including liver toxicity, CK elevation and myalgias and myopathy, as outlined in the "Clinical Management of Adverse Events" section)
- Participant becomes pregnant.
- Termination of the study by the Sponsor (US FDA, OHRP, or other relevant regulatory agencies)
- Non-compliance with the study protocol (missing more than 2 doses of study drugs or not completing one of the PK sampling visits)

All participants discontinuing study drug due to severe AE will remain in the study and continue to be monitored until the final scheduled clinic visit/laboratory evaluation. Their treatment will be referred to the local community TB clinics for standard anti-tuberculosis chemotherapy according to National Guidelines. The participants will be provided with a referral letter to take with them to the TB Clinic. Participants will be transported by the study staff to the clinic, or a follow-up call will be made by the study site staff to the clinic to determine if the participant attended the clinic on the date as arranged. Every effort will be made to continue to follow participants who discontinue study medicine prematurely until the final study visit, if they are agreeable. Any participant who received at least one dose of pravastatin and is withdrawn or withdraws early from study treatment will undergo an early treatment discontinuation visit and will be requested to return for the applicable follow up visits. Evaluations at these visits will not include PK sampling.

Patients will be encouraged at each visit to report new symptoms or illness experienced using a standardised checklist of questions which will be recorded in the source document that will also include participant attending other non-study health care workers or facilities since their last study visit.
All participants are provided with a 24 hour study phone number and are requested to report any adverse events to the site first before consulting any other health care facilities.
In the event that there has been a clinical consultation with a non-study health care worker or facility, the site will request a copy of the clinical records including results of investigations done and treatment prescribed. As soon as it is clinically appropriate, and in conjunction with the attending physician, the management of patients will be transferred to the study site. Any missed visit will trigger an assessment of a possible adverse event.
The most likely referral site for an admission to hospital is the adult wards at the Chris Hani Baragwanath Academic Hospital on the same campus as the research site. There is a good relationships with staff there and the site is frequently notified of study participant admissions. As soon as the site is notified of an admission either by the participant, their family or the attending doctor, they visit the patient in the ward at least daily.

Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Participants who withdraw from the trial after having received one or more doses of pravastatin or who are discontinued from the trial because of new recognition of participant ineligibility based on absence of *M. tuberculosis* growth in baseline sputum cultures, or growth of *M. tuberculosis* resistant to rifampin will be replaced. A participant may be prematurely withdrawn from the trial as a result of the following:

- At their own request or at the request of their legally acceptable representative.
- If, in the investigator's opinion, continuation in the trial would be detrimental to the well-being of the participant.
- At the specific request of the Sponsor (FDA, OHRP, or other regulatory agencies).

Every effort will be made to reduce loss to follow-up. Participants will be contacted by phone if they do not return for study visits.
To ascertain the reason why patients have not returned for a scheduled study visit we will do the following**:**

Participants will be contacted telephonically as soon as it becomes apparent, on the day of the scheduled visit, that they will not be attending the study visit, usually by noon of the day of the visit. The site's usual practice is to identify at least three contact numbers which are tested at least once at each face-to face visit. The site will routinely call or text participants a day prior to their scheduled visit to remind them thereof. If they are able to contact the patient on any of the three numbers, they will ascertain the reason for the visit non-attendance, and if it is an issue with transport they will attempt to resolve this by making an offer for the study site to collect the participant. The site will also ask if there are any other reasons for non-attendance. A script for telephonic contacts of this nature will be drafted to ensure that questions are asked about possible adverse events that may have occurred and prevented a participant from attending the scheduled study visit.

In the event that the telephonic contact, that day is unsuccessful, study staff will call the next day, on at least two occasions following the same process as above.

If the patient is uncontactable a day after the visit, a home visit will be made later that day to contact the patient.

All participants withdrawn will be referred to the local community TB clinics for standard anti-tuberculosis chemotherapy according to National Guidelines. The participants will be provided with a referral letter to take with them to the TB Clinic. The participants will either be transported to the clinic by the study staff, or else a follow-up call will be made by the study site staff to the clinic to determine if the participant attended the clinic on the date as arranged.

10. Monitoring and data handling

Site Monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet Sponsor, JHU/PHRU, and regulatory guidelines, and that the study is conducted in accordance with the protocol and site SOPs. Site monitoring will performed according to details in a written monitoring plan.

Data handling and record keeping will be performed by the site, under the supervision of the site PI in accordance with procedures that are documented in the site's detailed Data Management Operations Manual. Each participant will be assigned a unique study ID number. This number will be recorded on each data collection form and clinical specimen. Personally identifiable information including names will not be used on data collection forms. All study records will be stored in locked files in a secure area and access will be limited to study personnel and designated regulatory personnel. All forms will be reviewed prior to data entry for accuracy, consistency, and completeness by designated study staff. Statistical analysis will be done as a joint effort between JHU Investigators and the site PI, using JHU statisticians.

Source Documents and Access to Source Documents

Appropriate records will be maintained for this trial, in compliance with ICH E6 GCP, and regulatory and institutional requirements for the protection of confidentiality of participants. The following will have access to study records: members of the study team; IRBs that review the study, the Office of Human Research Protections, the US FDA, EMEA, the NIAID, and other local, US, and international regulatory entities. Authorized representatives of the Sponsor and regulatory agencies will be permitted to examine study records for QA reviews, audits, and evaluation of the study safety and progress.

Data Management Responsibilities

The on-site Principal Investigator and data manager will be responsible for accuracy, completeness, and storage of source records and data collection forms according to site quality procedures. The study team and data entry staff will review source documents to ensure accuracy and completeness.

Data Capture Methods

Data capture will be accomplished via a set of study-specific paper source documents which have been developed for use in direct recording of data and observations during the patient visits. These records will be included in the patient chart as source records. Data captured during the patient visits will be reviewed to assure accuracy and completeness according to site quality procedures. Once reviewed, these data will be entered into the study database by manual data entry. The study database will be maintained on JHU servers. Data in the study database will be reviewed by site personnel and monitored according to site procedures. All data management for this study will be performed manually, without the aid of a computerized data management system, for which computer system validation would be required.

Reports. The timing of reports will be detailed in the Data Management Operations Manual. Briefly, safety data will be reviewed by the DSMB after 50% of the participants have been enrolled and annually thereafter; reports for the DSMB will be prepared for the DSMB according to a schedule determined at the first convened DSMB meeting.

Study Records Retention

Study records will be maintained by the investigator following study discontinuation in accordance with all applicable NIH and local South African regulatory requirements. The NIH will be notified prior to destruction of study records.

Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or Manual of Procedures. As a result of deviations, corrective actions are to be developed and implemented promptly. The site will identify and report deviations according to the guidelines of the Sponsor and IRBs.

Quality Management

Procedures for quality management will be detailed in a separate Quality Management Plan. The study site will provide direct access to all trial-related facilities, source documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by regulatory authorities. Following written procedures, study monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol and the applicable regulatory requirements.

11. Inclusion/Exclusion Criteria

Inclusion criteria:

a) 18 years of age or older
b) Clinical signs and symptoms of pulmonary tuberculosis
c) Abnormal chest radiograph consistent with pulmonary tuberculosis
d) At least one sputum positive for M. tuberculosis by Xpert MTB/RIF with a cycle threshold (Ct) <28.
e) Documentation of HIV status
f) Weight ≥45 kg
g) Karnofsky score of at least 60
h) Ability to provide informed consent
i) Ability to adhere to study follow-up visits
j) Negative pregnancy test in women of child-bearing age
k) Ability to adhere to contraceptive requirements and willing to use two forms of contraception: 1) a double barrier method to prevent pregnancy (i.e. use of a condom with either diaphragm or cervical cap) or 2) use of an intrauterine device in combination with a barrier contraceptive. The participant must be willing to continue these contraceptive measures throughout the duration of the study and until one week after the last dose of study medication or one week after discontinuation from study medication in case of premature discontinuation.
l) Five days or fewer of anti-tuberculosis treatment within the previous 3 months

Exclusion criteria:

a) A history of severe adverse reactions to any statin or any other study agent or contraindications to use of statins.
b) Current use of statins or other lipid-lower agents;
c) Clinical indication for statin therapy based on cardiovascular risk:
    1) Familial hypercholesterolemia
    2) Previous history of myocardial infarction or stroke
d) For HIV-positive individuals, a CD4+ T-cell count <350/mm3
e) Use of antiretroviral drugs
f) Hemoglobin concentration less than 8 g/dL;
g) Baseline creatinine kinase elevation more than three times the upper limit of normal
h) Abnormal baseline laboratory values
    1) Baseline alanine aminotransferase (ALT) concentration more than 2.5 times the upper limit of normal (Grade 1)
    2) Serum creatinine concentration more than twice the upper limit of normal;
    3) Serum total bilirubin level greater than twice the upper limit of normal
    4) Platelet count < 100,000/mm3
    5) ANC <1,000/mm^3
i) Pregnant or breastfeeding;
j) Silico-tuberculosis.
k) Currently receiving TB treatment
l) Serologies or PCR positive for viral hepatitis (Hepatitis, B, C)
m) Concomitant disorders or conditions for which isoniazid, rifampin, pyrazinamide, or ethambutol is contraindicated. These include cirrhosis, acute liver disease of any cause, acute uncontrolled gouty arthritis and peripheral neuropathy.
n) Any medical or psychological condition which, in the view of the study investigator, makes study participation inadvisable.
o) Infection with an isolate determined to be resistant to rifampin by GeneXpert.
p) More than five days of anti-tuberculosis treatment within the previous 3 months
q) Planned or current use of cyclosporine, tacrolimus, erythromycin or colchicine
r) CNS TB
s) Extra-pulmonary TB only, not in combination with pulmonary TB
t) History of TB

12. Special Populations

There will be no exclusion based on race, gender or ethnicity.

Children, Pregnant Women, and Breast-Feeding Women

Pregnant or breast-feeding women will be excluded from this study because statins are contraindicated during pregnancy based on their potential teratogenic effects. According to the NIH definition, a child is "any individual under the age of 21". We will enroll participants ≥18 years of age with pulmonary tuberculosis, and, therefore, children are eligible for enrollment. It is anticipated that approximately 40% of study participants will be women, since, historically, approximately 60% of patients with tuberculosis at the clinical sites in our study are males. The gender, ethnicity, and socioeconomic background of study participants are expected to mirror that of the population of the hospital and clinics served, and that of the population most affected by tuberculosis. It is anticipated that the majority of study participants will be black South Africans.

Prisoners

This study will not enroll prisoners. However, it is possible that a participant will be incarcerated after enrollment. If an enrolled individual is incarcerated, then study medications will be stopped and the participant will be treated for active TB according to the standards of the institution in which s/he is incarcerated. While incarcerated, individuals will not be followed in the study. When the individual is no longer incarcerated, study follow-up may continue, at the discretion of the Investigator.

HIV-Infected Individuals

HIV-infected individuals and HIV-uninfected individuals will be included in this study. HIV-infected individuals with CD4 <350 cells/mm^3 will be excluded in this study, as these patients are at increased risk of death and may be better served by individualized (non-study) TB and HIV therapy. If one or both of the proposed experimental regimens is demonstrated to be effective and safe in this study, then subsequent future studies including individuals with advanced AIDS may be warranted. This study will not enroll participants with HIV infection who are taking antiretroviral therapy. For patients with active TB and CD4 cell counts >50/mm^3 who are not already taking ART, initiation of ART is recommended after 2 months of TB treatment. HIV-infected individuals in South Africa are routinely treated with fixed-dose combination pills containing efavirenz, tenofovir, and emtricitabine. Efavirenz is known to lower the mean AUC of pravastatin by ~40%. Therefore, HIV-infected individuals receiving antiretroviral drugs will be excluded from the study. If a participant is already enrolled in the study, and they require antiretotroviral therapy, they will be taken off of study medicines and referred to the clinics for treatment with TB and HIV therapy.

Ethical Issues in Doing This Trial in South Africa

The vast majority of the global TB burden occurs in developing countries. South Africa has an exceedingly high TB case rate of approximately 600/100,000 population/year, and ranks #7 in the world based on number of incident cases. New treatment regimens that substantially improve TB treatment (shorten and/or simplify therapy) are critically needed in this setting. Therefore, it is appropriate to evaluate a new host-directed treatment for TB in South Africa.

Management of participants deemed ineligible

Because of the relatively slow growth of *M. tuberculosis* in culture, the results of sputum mycobacterial cultures and drug susceptibility testing are rarely available at the time of initiation of TB treatment. Therefore, for this study we plan to enroll patients on the basis of a positive sputum test for TB on GeneXpert testing. We anticipate that approximately 10% of enrollees subsequently will be deemed protocol ineligible based on culture results.

Management of individuals having either a) NO baseline sputum culture that is positive for *M. tuberculosis* OR b) a baseline *M. tuberculosis* isolate that is determined to be resistant to rifampin by GeneXpert: these individuals will be taken off of their assigned regimen, and treated with an individualized regimen at the discretion of the treating clinician.

13. Drugs/ Substances/ Devices

Study drug descriptions

Pravastatin 40 mg tablets

Pravastatin

Pravastatin (marketed as Sandoz Pravastatin) is a member of the drug class of statins, used in combination with diet, exercise, and weight loss for the treatment of dyslipidemia and the prevention of cardiovascular disease.

Mechanism of action: Pravastatin acts as a lipoprotein-lowering drug through two pathways. In the major pathway, pravastatin inhibits the function of hydroxymethylglutaryl-CoA (HMG-CoA) reductase. As a reversible competitive inhibitor, pravastatin sterically hinders the action of HMG-CoA reductase by occupying the active site of the enzyme. Taking place primarily in the liver, this enzyme is responsible for the conversion of HMG-CoA to mevalonate in the rate-limiting step of the biosynthetic pathway for cholesterol. Pravastatin also inhibits the synthesis of very-low-density lipoproteins, which are the precursor to low-density lipoproteins (LDL). These reductions increase the number of cellular LDL receptors, thus LDL uptake increases, removing it from the bloodstream. Overall, the result is a reduction in circulating cholesterol and LDL. A minor reduction in triglycerides and an increase in high-density lipoproteins (HDL) are common.

Pharmacokinetics

Pravastatin is administered orally in the active form. In studies in man, peak plasma pravastatin concentrations occurred 1 to 1.5 hours upon oral administration. Based on urinary recovery of total radiolabeled drug, the average oral absorption of pravastatin is 34% and absolute bioavailability is 17%. While the presence of food in the gastrointestinal tract reduces systemic bioavailability, the lipid-lowering effects of the drug are similar whether taken with or 1 hour prior to meals. Pravastatin plasma concentrations, including area under the concentration-time curve (AUC), C_{max}, and steady-state minimum (C_{min}), are directly proportional to administered dose. Systemic bioavailability of pravastatin administered following a bedtime dose was decreased 60% compared to that following an AM dose. Despite this decrease in systemic bioavailability, the efficacy of pravastatin administered once daily in the evening, although not statistically significant, was marginally more effective than that after a morning dose. The coefficient of variation (CV), based on between-subject variability, was 50% to 60% for AUC. The geometric means of pravastatin C_{max} and AUC following a 20 mg dose in the fasted state were 26.5 ng/mL and 59.8 ng*hr/mL, respectively. Steady-state AUCs, C_{max}, and C_{min} plasma concentrations showed no evidence of pravastatin accumulation following once or twice daily administration of PRAVACHOL tablets.

Distribution

Approximately 50% of the circulating drug is bound to plasma proteins.

Metabolism

Importantly, pravastatin is not metabolized by the CYP450 system in the liver (86, 87). Instead, the major metabolites are produced by chemical degradation in the stomach and the intact drug and its metabolites are cleared through both hepatic and renal routes, reducing the need for dosage adjustment if the function of either the liver or kidney is impaired, and also reducing the possibility of drug interactions compared with other statins (87). Therefore, increased toxicity due to potential reduced clearance of pravastatin by TB meds is not expected.
The major biotransformation pathways for pravastatin are: (a) isomerization to 6-epi pravastatin and the 3α-hydroxyisomer of pravastatin (SQ 31,906) and (b) enzymatic ring hydroxylation to SQ 31,945. The 3α-hydroxyisomeric metabolite (SQ 31,906) has 1/10 to 1/40 the HMG-CoA reductase inhibitory activity of

the parent compound. Pravastatin undergoes extensive first-pass extraction in the liver (extraction ratio 0.66).

Excretion

Approximately 20% of a radiolabeled oral dose is excreted in urine and 70% in the feces. After intravenous administration of radiolabeled pravastatin to normal volunteers, approximately 47% of total body clearance was via renal excretion and 53% by non-renal routes (i.e., biliary excretion and biotransformation).

Drug-drug interactions

Medications that may cause drug interactions with pravastatin include fibrates, erythromycin, niacin, and many others. These drug interactions may result in side effects or complications such as increased levels of medicine in the blood and an increased risk of developing serious muscle problems. The metabolism of pravastatin is not significantly mediated by CYP enzymes. However, pravastatin is a substrate of MRP2 and the uptake transporter OATP2. Recent studies have illustrated differences in the interaction potential of the different statin drugs. Thus, itraconazole increased the AUC of simvastatin acid at least tenfold but had no significant effect on that of pravastatin. Furthermore, combination therapy with the HIV protease inhibitors ritonavir and saquinavir increased the median AUC(0–24) of simvastatin 30-fold, but decreased that of pravastatin by 50%. Pravastatin is a substrate of both influx and efflux transporters, and although significant interactions mediated by induction of pravastatin metabolism are not likely, the *in vivo* effects of induction of transporters are largely unknown.

Rifampin caused a statistically significant decrease in the plasma concentration of pravastatin given as a single oral dose to healthy subjects. However, the effect of rifampin varied greatly between subjects. The mean rifampin-induced decrease in pravastatin concentration was considerably smaller than that observed previously for simvastatin. Unlike simvastatin and its metabolite (simvastatin acid), in which steady-state AUC is reduced by ~90% by rifampin (69), requiring co-administration with rifabutin to obtain adequate drug exposures, pravastatin AUC is reduced by only 30-50% during co-administration with rifampin (70).

Tolerability

Recent prospective (Prospective Pravastatin Pooling (PPP) Project) analysis indicates that during prolonged exposure, 40 mg of pravastatin is well tolerated, with no excess of noncardiovascular serious adverse events, including liver function abnormalities and laboratory and clinical evidence for myositis.

The safety and tolerability of pravastatin at a dose of 80 mg in 2 controlled trials with a mean exposure of 8.6 months was similar to that of pravastatin at lower doses except that 4 out of 464 patients taking 80 mg of pravastatin had a single elevation of CK >10 times ULN compared to 0 out of 115 patients taking 40 mg of pravastatin.

In the PRAVACHOL placebo-controlled clinical trials database of 21,483 patients (age range 24-75 years, 10.3% women, 52.3% Caucasians, 0.8% Blacks, 0.5% Hispanics, 0.1% Asians, 0.1% Others, 46.1% Not Recorded) with a median treatment duration of 261 weeks, 8.1% of patients on PRAVACHOL and 9.3% patients on placebo discontinued due to adverse events regardless of causality.

Adverse event data were pooled from 7 double-blind, placebo-controlled trials (West of Scotland Coronary Prevention Study [WOS]; Cholesterol and Recurrent Events study [CARE]; Long-term Intervention with Pravastatin in Ischemic Disease study [LIPID]; Pravastatin Limitation of Atherosclerosis in the Coronary Arteries study [PLAC I]; Pravastatin, Lipids and Atherosclerosis in the Carotids study [PLAC II]; Regression Growth Evaluation Statin Study [REGRESS]; and Kuopio Atherosclerosis Prevention Study

[KAPS]) involving a total of 10,764 patients treated with pravastatin 40 mg and 10,719 patients treated with placebo. The safety and tolerability profile in the pravastatin group was comparable to that of the placebo group. Patients were exposed to pravastatin for a mean of 4.0 to 5.1 years in WOS, CARE, and LIPID and 1.9 to 2.9 years in PLAC I, PLAC II, KAPS, and REGRESS. In these long-term trials, the most common reasons for discontinuation were mild, non-specific gastrointestinal complaints. Collectively, these 7 trials represent 47,613 patient-years of exposure to pravastatin. All clinical adverse events (regardless of causality) occurring in ≥2% of patients treated with pravastatin in these studies are identified in the package insert for Pravastatin.

Dosage

The recommended starting dose of pravastatin for cholesterol-lowering is 40 mg once daily. If a daily dose of 40 mg does not achieve desired cholesterol levels, 80 mg once daily is recommended. In patients with significant renal impairment, a starting dose of 10 mg daily is recommended. Pravastatin sodium tablets can be administered orally as a single dose at any time of the day, with or without food.

Rifampin

Rifampin is a semi-synthetic rifamycin derivative that is highly active against mycobacteria, most gram-positive bacteria, and some gram-negative bacteria. It is bactericidal for both intracellular and extracellular microorganisms. By inhibiting prokaryotic DNA-dependent RNA polymerase, it suppresses the early elongation of the nucleotide chain in RNA synthesis.

Pharmacokinetics

Rifampin is normally absorbed completely when taken orally, but food delays absorption. After 1.5 to 2 hours, a 600-mg dose yields a peak blood level of 8-20 μg/mL. The half-life of rifampin varies from 2 to 5 hours, and it is shortened by approximately 20-40% after the first week of daily treatment because of the induction of hepatic microsomal enzymes. The half-life is unaffected by renal impairment but is increased by liver disease or biliary obstruction. Rifampin is deacetylated to an enterohepatically recirculated active metabolite, and 50% to 60% is excreted in the feces. Up to 30% of a dose is excreted in the urine. Approximately 85% of circulating rifampin is bound to plasma proteins, and is widely distributed throughout the body.

Drug-drug interactions

Rifampin is an inducer of a number of hepatic enzymes involved in the metabolism of drugs and some hormones. This enzyme induction causes more rapid elimination (and potential loss of efficacy) of many drugs. Medications for which concomitant rifampin is contraindicated include: HIV-1 protease inhibitors (other than ritonavir), delavirdine, cyclosporine, tacrolimus, itraconazole, and ketoconazole. For many other medications, the dose can be increased to compensate for the effect of rifampin. Rifampin significantly lowers the peak levels of most statins. Specifically, pravastatin exposures are reduced by 30-50% during co-administration with rifampin (70).

Tolerability

In the usual daily dose of 600 mg, rifampin is well tolerated. It often causes harmless but disconcerting red-orange discoloration of tears, sweat, saliva, feces, and urine. Less than 4% of TB patients experience significant adverse reactions to rifampin. Gastrointestinal AEs are the most common, and they include epigastric distress, anorexia, nausea, vomiting, cramps, and diarrhea. Hepatitis rarely occurs in persons who

have normal baseline hepatic function. The incidence of hepatitis may be increased for older persons and those who have chronic liver disease or alcoholism, but remains substantially lower than that for pyrazinamide or isoniazid. Rifampin can cause a flu-like syndrome of fever, chills, and myalgia, although this is uncommon using the 600-mg dose given daily or thrice-weekly. In a very small proportion of patients the flu-like syndrome is associated with interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia, and shock. There may be changes in menstruation.

Dosage

According to South African guidelines the recommended dose of rifampin for the treatment of TB is 450 mg (weight < 50 kg) or 600 mg (weight>50 kg).

Isoniazid

Isoniazid is the hydrazide of isonicotinic acid and is one of the primary drugs for TB treatment. The activity of isoniazid is limited to the mycobacteria of the *M. tuberculosis* complex; it is bactericidal for rapidly dividing organisms and bacteriostatic for "resting" bacilli. Its mechanism of action is the inhibition of the biosynthesis of mycolic acids, a component of the mycobacterial cell wall.

Pharmacokinetics

Isoniazid is generally well absorbed; food and antacids decrease the rate, but not the extent of absorption. The peak blood levels of isoniazid, 3 to 5 μg/ml, are obtained 30 minutes to 2 hours after ingestion of routine doses. It diffuses into all body fluids and cells and penetrates into the caseous material of a tuberculoma or pulmonary cavity. In the liver, it is acetylated to inactive metabolites, and 75% to 95% of the dose is excreted as inactive metabolites in the urine within 24 hours. Isoniazid clearance rates depend on 2 metabolic phenotypes, slow and fast acetylation. The isoniazid AUC among persons who have fast acetylation is 30% to 50% of that among persons who have slow acetylation. Because isoniazid is well tolerated over a wide range of therapeutic doses, a single dose per body mass is recommended. Persons who have rapid acetylation achieve effective concentrations, while persons who have slow acetylation do not experience increased toxicity. Half-life ($t_{1/2}$) may vary from 1 hour in fast acetylators ($t_{1/2}$ < 90min) to 3 hours in slow acetylators ($t_{1/2}$ >90min).

Drug-drug interactions

Isoniazid decreases the clearance of some medications that are metabolized in the liver (particularly carbamazepine, phenytoin, diazepam).

Toxicity

The total incidence of all adverse effects from isoniazid is approximately 5%, many of which do not require discontinuation of the drug. Peripheral neurotoxicity is dose dependent and it is uncommon (<0.2%) at conventional doses. The risk of peripheral neuritis increases for persons who are malnourished or predisposed to neuritis by other illnesses. Concomitant administration of pyridoxine (vitamin B_6) is recommended for HIV positive person in South Africa. It will be given to every participant in this trial. Other nervous system reactions are rare at normal doses, and they include convulsions, encephalopathy, optic neuritis, memory impairment, and psychosis. Gastrointestinal adverse effects include nausea, vomiting, and epigastric distress. Asymptomatic elevation of aminotransferases is common and occurs in 10-20% of persons receiving isoniazid. However, idiosyncratic severe hepatic reactions are uncommon but are more likely in older persons (up to 2.3% hepatitis incidence in persons more than 50 years old), and

may be life threatening. Daily consumption of alcohol increases the risk of isoniazid-associated hepatotoxicity by approximately 4-fold. The risk of isoniazid-induced hepatotoxicity may also be increased in the postpartum period. The prodromal symptoms of hepatotoxicity are anorexia, nausea, vomiting, fatigue, malaise, and weakness; persons who take isoniazid and have these symptoms should stop therapy and be evaluated immediately.

Dosage

According to South African guidelines the recommended dose of isoniazid for the treatment of TB is 300 mg.

Pyrazinamide

Pyrazinamide is an analog of nicotinamide and has unique activity against *M. tuberculosis*, allowing the duration of treatment to be decreased from 9 months to 6 months (assuming rifampin is used throughout). The mechanism of action of pyrazinamide remains unknown.

Pharmacokinetics

Pyrazinamide is well-absorbed from the gastrointestinal tract and widely distributed into all tissues. Usual doses are 15-30 mg/kg/d, up to 2 gm/d. Peak serum concentrations of about 45 μg/ml are achieved approximately 2-3 hours after a dose. Food and antacids do not significantly affect the absorption of pyrazinamide. The half-life of pyrazinamide is approximately 9-10 hours, and is prolonged in the presence of hepatic insufficiency. Pyrazinamide is metabolized to pyrazinoic acid by the hepatic microsomal enzyme pyrazinamide deamidase. Approximately 40% of a dose is recovered in the urine as pyrazinoic acid and an additional 4% is excreted in the urine as the unchanged parent drug. The remaining drug is thought to be excreted in the bile.

Drug-drug interactions

There are no known clinically significant drug-drug interactions involving pyrazinamide.

Toxicity

The most frequent side effects are skin rash, gastrointestinal intolerance, hepatotoxicity (1.3%), arthralgias (1-7%), hyperuricemia due to blockade of urate excretion (up to 66%), and rarely acute gouty arthritis. These side effects are seldom dose-limiting. Asymptomatic elevations in serum uric acid are frequent, usually occur during the first or second month of treatment, and are self-limited and require no specific treatment. Minor arthralgias also may occur during pyrazinamide treatment and can usually be treated with salicylates or non-steroidal inflammatory agents such as indomethacin while continuing the drug. The most common serious side effect of pyrazinamide is hepatotoxicity.

Dosage

According to South African guidelines the recommended dose of pyrazinamide for the treatment of TB is 20-25 mg/kg.

Ethambutol

Ethambutol is an ethylene derivative of butane that interferes with cell wall synthesis in mycobacteria; other bacteria are uniformly resistant to ethambutol. In the treatment of human TB, ethambutol is effective in preventing the emergence of drug resistant strains, although it has no sterilizing activity at clinically-tolerated doses.

Pharmacokinetics

Ethambutol is well absorbed from the gastrointestinal tract, reaching peak serum concentrations of 3-5 mcg/ml in normal volunteers 2-4 hours after a dose. Food slows absorption and decreases the peak serum concentration by 10-20%, but has no effect on the total systemic exposure (AUC). Antacids decrease both the peak serum concentration and AUC, and so should not be administered at the same time. Ethambutol is primarily eliminated by the kidneys as unchanged drug; the serum half-life averages 4 hours. Patients with renal insufficiency are prone to accumulation of the drug and the resultant toxicity.

Drug-drug interactions

There are no known drug–drug interactions involving ethambutol.

Toxicity

Ethambutol is usually well-tolerated with low rates of skin rash, nausea, vomiting, or diarrhea. Fever, allergic reactions, abdominal pain, mental status changes, peripheral neuropathy, and increased liver function tests have rarely been associated with ethambutol. Adverse events occur in less than 2% of patients receiving ethambutol at the 15 mg/kg dose and include decreased visual acuity (0.8%), rash (0.5%) and asymptomatic hyperuricemia. The most common serious side effect of ethambutol is retinal toxicity, often first perceived as a decrease in color perception. Patients receiving ethambutol should be instructed about symptoms of ocular toxicity. If stopped promptly, permanent visual loss is rare among patients with ethambutol-related retinal toxicity. Rates of retinal toxicity are very low when the drug is given for relatively short periods, as is the case in this study.

Dosage

According to South African guidelines the recommended dose of ethambutol for the treatment of TB is 15-20 mg/kg. This is the dosage that will be used for this study.

Pharmacy: Product Supply, Distribution, and Accountability

Pravastatin, Rifafour, and vitamin B6 will be supplied through the study. An FDA-approved preparation of Pravastatin 40 mg tablets will be made available through the National Institute of Allergy and Infectious Diseases (NIAID) Clinical Research Products Management Center (CRPMC). The site pharmacist can obtain the study product for this protocol by following the instructions in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks in the section Study Product Management Responsibilities. Rifafour and vitamin B6 will be obtained in South Africa; Rifafour will be obtained from the National TB Program (purchased from Sanofi-Aventis) and B6 from a commercial distributor. Rifafour will be given in doses consistent with South African guidelines.
Rifafour dosage by pre-treatment body weight
Rifafour = Rifampin/isoniazid/pyrazinamide/ethambutol (150 mg/75 mg/400 mg/275mg)

30-37 kg 2 tabs

38-54 kg 3 tabs

55-70 kg 4 tabs

>70 kg 5 tabs

Study Product Accountability

The site pharmacist is required to maintain complete records of all study products received from the NIAID CRPMC and subsequently dispensed. All unused study products must be returned to the NIAID CRPMC (or as otherwise directed) after the study is completed or terminated. The procedures to be followed are provided in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks in the section Study Product Management Responsibilities.

14. The rationale for choice of statin and dose

Pravastatin was selected for testing in this safety/PK study based on its favorable CC50/EC50 values and demonstration of adjunctive activity against chronic TB in the standard mouse model. Specifically, pravastatin demonstrated the least toxicity of all statins in our assays (CC50> 1.25 mM and > 10 mM in THP-1 and Vero cells, respectively) and was one of 3 statins for which an EC50 could be obtained within the tested range in THP-1 cells (7.8 μM), yielding a highly favorable CC50/EC50 >160.3. Importantly, pravastatin showed dose-dependent activity against chronic TB in the mouse model, and improved the percent surface area of lung involved by inflammation. Adjunctive therapy with pravastatin 50 mg/kg, which yields human-like drug exposures following an 80-mg dose, significantly reduced the lung bacillary burden in a clinically relevant mouse model of necrotic TB granulomas (C3Heb/FeJ mice) relative to controls receiving the first-line regimen alone. Although simvastatin also showed a highly favorable CC50/EC50 ratio in macrophages and adjunctive activity in the two different mouse models of chronic TB, several factors dampened our enthusiasm for this agent relative to pravastatin. Based on our PK data, simvastatin is almost entirely converted to its acid metabolite in mice, whereas in humans metabolism to simvastatin acid is ~1:1. Since we do not know if the parent drug or acid metabolite is responsible for the anti-TB effect, it is possible that the mouse data overestimate the adjunctive action of simvastatin. Perhaps more importantly, peak concentrations of both simvastatin and simvastatin acid are greatly reduced (by 90%) by rifampin (69). On the other hand, pravastatin exposures are reduced by only 30-50% during co-administration with rifampin. Our PK data suggest that drug clearance and exposures differ markedly between mice and humans. Given that we observed a dose-responsive anti-TB effect with pravastatin adjunctive therapy in mice and the known drug interactions between pravastatin and rifampin in humans, we will study the highest clinically recommended dose of pravastatin in this clinical trial. The anti-TB effect of adjunctive statin therapy observed in mice was accompanied by a reduction in serum IL-6 (**Figure 9**) to levels seen in uninfected mice. In addition to IL-6, a recent thesis project at JHU investigating predictors of respiratory impairment in TB identified levels of the cytokines IL-1β and TGF-β as being directly proportionate and levels of the cytokines IL-12 and TIMP-2 as being inversely proportionate to changes in St. George's respiratory questionnaire scores during TB treatment (Akshay Gupte, unpublished).

15. Justification and safety information if FDA-approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

The goal of this study is to evaluate pravastatin as host-directed therapy for TB, a non-approved indication. Pravastatin has favorable, dose-dependent activity against chronic TB in two different mouse models. Pravastatin exposures are reduced by 30-50% during co-administration with rifampin (70). Therefore, we anticipate that a dose of pravastatin 160 mg daily when co-administered with rifampin will give similar pravastatin exposures as monotherapy with pravastatin 80 mg daily. However, to address safety concerns regarding the unknown effect of co-administering pravastatin with the other anti-TB drugs (isoniazid, ethambutol, and pyrazinamide) and to minimize study risk to participants, we will initially recruit a cohort

of 5 participants to assess safety/tolerability of pravastatin 40 mg daily in combination with standard of care (Rifafour) prior to further study drug dose escalation. If this dose is well tolerated when given with Rifafour, we will recruit 10 participants to receive pravastatin 80 mg daily and Rifafour. If it is determined that this dose, when co-administered with Rifafour is well-tolerated, yet the mean pravastatin exposures during adjunctive therapy are reduced by $\geq$ 40% relative to pravastatin monotherapy in the same patients (as assessed prior to initiating anti-TB drugs), we will increase the dose of pravastatin incrementally up to a maximum of 160 mg/d, in order to achieve drug exposures equivalent to those of pravastatin 80 mg/d given as monotherapy.

16. Justification and safety information if non-FDA approved drugs without an IND will be administered.

Not applicable

17. Study statistics

Primary outcome variable.

Safety and tolerability of pravastatin adjunctive therapy relative to standard treatment

Secondary outcome variables

Steady-state PK of pravastatin among patients with pulmonary TB receiving co-administered Rifafour (isoniazid, rifampin, pyrazinamide and ethambutol)

18. Statistical Analysis Plan

Hypothesis

Pravastatin given at the standard clinically recommended dose will be safe and well tolerated, and pravastatin drug exposures will not be significantly altered following co-administration with rifampin in the 14-day study.

Sample size

The sample size will be between 5and 35 evaluable participants, who will be recruited into the following arms: 1) Standard-of-care regimen (Rifafour) + pravastatin 40 mg daily (5 evaluable participants); 2) Standard-of-care regimen (Rifafour) + pravastatin 80 mg daily (10 evaluable participants); 3) Standard-of-care regimen (Rifafour) + pravastatin 120 mg daily (10 evaluable participants); 3) Standard-of-care regimen (Rifafour) + pravastatin 160 mg daily (10 evaluable participants). Participants in Arms 2, 3, and 4 (10 participants each) will only be recruited if necessary, i.e., if pravastatin 40 mg is well tolerated and safe, and if escalated doses are also tolerated and safe, yet drug exposures are reduced by $\geq$ 40% in participants due to the known interaction with rifampin.

A sample size of 10 evaluable participants for pravastatin PK analysis was chosen based on within-subject variability (CV%) of statin AUC in interaction studies ranging from 6-60% and a within-subject coefficient of variation (CVw) of 33%. With 10 evaluable participants per arm, the precision (i.e., half width of the 90% confidence interval on the log scale) for the treatment difference will be within 26% of the point estimate for AUC. If the point estimate of the ratio of geometric mean is 1, then 90% confidence interval will be approximately (0.74, 1.35).

Data Analysis

The current study is a 2-week, 4-arm dose-escalation trial to determine the safety/tolerability and PK of pravastatin when co-administered with the standard drug regimen for drug-susceptible TB (Rifafour). Participants will receive standard anti-TB therapy and pravastatin beginning at a dose of 40 mg once daily. Each subsequent arm (pravastatin 80 mg, 120 mg, and 160 mg) of this study will continue to recruit participants until at least 10 participants in each group are evaluable for full PK analysis.

The primary endpoints in this study are safety/tolerability and pravastatin exposures. A regimen will be deemed safe and well tolerated if none of the participants require treatment discontinuation for a pravastatin-related adverse reaction and $\leq$3 participants experiences a pravastatin-related grade 3 (or higher) adverse event during the 14-day study period and follow up. PK parameters (e.g., area under the concentration-time curve over 24 hours (AUC_{0-24}), minimum concentration (C_{min}), maximum concentration (C_{max}), and oral clearance (Cl/F)), will be estimated for pravastatin from intensive sampling data after a single dose of pravastatin (baseline PK, prior to initiation of TB treatment) and after 14 days of co-administration with the first-line anti-TB regimen. Based on PK data of pravastatin when co-administered with rifampin (T_{max} = 1 hour, $T_{1/2}$ = 1.5 hours), plasma samples will be collected for pravastatin measurements by LC-MS/MS at the following time points: pre-dose, 0.5 hrs, 1 hr, 1.5 hrs, 2 hrs, 3 hrs, 4 hrs, 6 hrs, and 8 hrs. Pravastatin exposures will be calculated using a noncompartmental approach using standard PK software, e.g., Phoenix WinNonLin. Using all PK data, a PK/PD model will be developed to evaluate the relationship between drug exposures (e.g., C_{max}, T>MIC or AUC_{0-24}) and outcomes (e.g., safety/tolerability). C_{max} and t_{max} will be taken directly from the original data. The terminal log-linear phase of the plasma concentration-time curve will be identified visually for each curve.

The elimination rate constant (k_e) will be determined by a linear regression analysis using the last 3–8 points on the plot of the natural logarithm of the plasma concentration-time curve. The $t_{1/2}$ value will be calculated from the equation $t_{1/2} = \ln 2/k_e$. AUC values will be calculated by the linear trapezoidal rule for the rising phase and the log-linear trapezoidal rule for the descending phase, with extrapolation to infinity, when appropriate, by dividing the last measured concentration by k_e. Results will be expressed as mean values (± s.d.). 95% confidence intervals will be calculated for the mean differences between the baseline pravastatin and rifampin co-administration phases for $AUC(0–\infty)$, C_{max} and $t_{1/2}$. Statistical comparisons of the pharmacokinetic variables (except t_{max}) will be carried out by the Student t-test for paired values (two-tailed), and the Wilcoxon signed-rank test will be used for analysis of t_{max}. Logarithmic transformation of C_{max} and AUC values will be done prior to statistical analysis. The effect of rifampin (and other anti-TB drugs) on the $AUC(0–\infty)$ of pravastatin will be assessed with a t-test of log-transformed values. The analysis will be performed with Systat for Windows, V6.0.1 (SPSS Inc., Chicago, Ill). Statistical significance will be taken as $P < 0.05$.

Decision-making algorithm for pravastatin safety/PK study:

Scenario/Pravastatin Dose	Safe/tolerable?	AUC0-24 reduction	Decision
1 40 mg daily	Y	-	Increase dose to prava 80 mg and enroll additional participants
2 40 mg daily	N	-	Enroll 5 additional participants (10 total) and discontinue study

3 80 mg daily	N	-	Discontinue study
4 80 mg daily	Y	< 40%	Discontinue study
5 80 mg daily	Y	≥ 40%	Increase dose to prava 120 mg and enroll additional participants
6 120 mg daily	N	-	Discontinue study
7 120 mg daily	Y	<40%	Discontinue study
8 120 mg daily	Y	≥ 40%	Increase dose to prava 160 mg and enroll additional participants
9 160 mg daily	N	-	Discontinue study
10 160 mg daily	Y	-	Study complete

Safety/tolerability: Y= Yes; <3 participants with ≥ one grade 3 (or higher) event of musculoskeletal symptoms, creatine kinase elevation, or liver function test elevation attributed to study drug (probable or definite)

N= No; For Arm 1 (pravastatin 40 mg daily): ≥ 1 participant with ≥ one grade 3 (or higher) event of musculoskeletal symptoms, creatine kinase elevation, or liver function test elevation attributed to study statin medication (probable or definite).
For Arms 2, 3, and 4: ≥ 3 participants with ≥ one grade 3 (or higher) event of musculoskeletal symptoms, creatine kinase elevation, or liver function test elevation attributed to study statin medication (probable or definite)

$AUC_{0\text{-}24}$= Area-under-the-plasma-concentration-time curve for 24-hour exposure

Decision-making algorithm for initiating new dosing cohorts

The study will be monitored by a DSMB, which will review the safety data for each cohort and will give approval before initiation of each possible dose escalation. Specifically, study drug dose will not be escalated and the study if any three participants develop grade 3 or higher musculoskeletal/CK-related adverse events (AEs), LFT-related AEs (> 5 x UNL without symptoms, but > 3 x UNL with symptoms or bilirubin > 2 x ULN) or any type of grade 4 event regardless of causal attribution. Study drug dosage will be escalated only if the dose is deemed safe/tolerable based on the above rules and the mean pravastatin AUC is reduced by ≥ 40% relative to the pre-Rifafour value. Study participants in whom the study drug has been discontinued will remain in the study until the final clinical visit and laboratory evaluation. In the event of study product discontinuation, management of the standard-of-care TB drugs will revert to the site clinician as per national guidelines.

Early stopping rules
Early stopping of the study will be considered if adverse events occur in a great proportion or with greater severity than would be expected at the dosage of the study drug being used based on the safety profile in patients with cardiovascular disease. Upon reviewing the AE data, the DSMB will make a formal determination about early stopping of the study.

Recruitment strategy

Eligible patients (who meet all of the inclusion criteria and none of the exclusion criteria) will be recruited in a step-wise fashion to the study arms.

Although rifampin is known to reduce pravastatin plasma levels by 30-50% in healthy volunteers, the PK of pravastatin has not been studied during co-administration with the combination regimen Rifafour in patients with TB. Therefore, out of caution, we will begin to study pravastatin dosed at half the highest clinically recommended dose of pravastatin (40 mg/day). Drug exposures are expected to be lower following standard-of-care TB treatment (Rifafour) in the experimental group receiving pravastatin relative to pre-Rifafour pravastatin exposures.

Participants meeting entry criteria will be recruited sequentially into each study arm for 10 -30 evaluable participants.

This will be an open-label trial in which neither the participants nor the study staff will be blinded as to treatment assignment.

19. Risks

Institutional Review Board Involvement

This research will be conducted in compliance with the requirements of the US DHHS regulations to protect human subjects from research risk (45 CFR Part 46), in compliance with South African regulations protecting human subjects, and with oversight by the IRBs of Johns Hopkins University School of Medicine (FWA #00005752) and the University of Witwatersrand (FWA #00000715). All study personnel will complete a course in protection of human subjects prior to participating in the study.

The Investigator will ensure that the purpose of the study is explained to the participant and that written consent is obtained prior to participation in the study. The participant, Investigator or designee, and others as required by local regulatory guidelines will sign the consent prior to entry into the study. The participant will receive a copy of his/her signed consent form. The Investigator will retain a copy of the signed consent forms, which may be inspected at the monitor's or auditor's request.

The investigator will promptly report to the Ethics Committee/IRB and regulatory body of all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make changes in the research without Ethics Committee/IRB and regulatory body approval, except where necessary to eliminate apparent immediate hazards to human subjects.

Written informed consent will be obtained by trained study personnel prior to screening for inclusion and exclusion criteria. Informed consent is a process that will be initiated prior to the individual's agreeing to participate in the study and will continue throughout the individual's study participation.

Medical risks, listing all procedures, their major and minor risks and expected frequency.

Risks associated with blood specimen collection include those related to venipuncture and blood loss. The risks of drawing blood from a vein include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, faintness from the procedure.

Steps taken to minimize the risks

Appropriate clinical practices will be performed by trained clinical and study staff to minimize complications from the risk mentioned above.

Plan for reporting unanticipated problems or study deviations.

The Investigator will promptly report to the Ethics Committee/IRB and regulatory body of all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make changes in the research without Ethics Committee/IRB and regulatory body approval, except where necessary to eliminate apparent immediate hazards to human subjects.

Legal risks such as the risks that would be associated with breach of confidentiality.

Risks to confidentiality will be minimized by the following procedures: study records will be stored in locked cabinets in secure locations, study records will be accessible only by authorized study staff, and databases will be password-protected and accessible only to authorized study staff. Participant names will not be stored in the study database. All electronic communications involving study data will be encrypted. Laboratory results will be maintained in a password-protected file accessible only to study personnel. The study monitor(s) or other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the investigator.

Financial risks to the participants.

There are no financial risks to the participants. Treatment for TB is routine and free in South Africa. Participants will receive reimbursement for study visits in accordance with SAHPRA guidelines. There will be no other costs to the participants.

20. Benefits

The proposed research may not have direct benefit to individual study participants. The South African government provides HIV medicines, TB medicines, and prenatal care to its citizens free of charge. For individuals in this trial, follow-up visits and counseling may enhance these services. In addition, study-related tests, such as viral load and resistance testing, may provide information regarding appropriate treatment that may benefit study participants directly. A better knowledge of the impact of statin adjunctive therapy for pulmonary TB is anticipated to inform public health practices and individual health activities to better treat drug-susceptible TB in HIV-infected and HIV-uninfected individuals. This will be especially pertinent in South Africa, a country that bears a disproportionate burden of HIV/TB co-infection.

21. Payment and Re-numeration

Participants will receive reimbursement in line with South African guidelines and approved by the local Ethics Committee or SAHPRA.

22. Costs

There are no costs to the participants.

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Appendix 1

	Screening[9]	(Visit Window Days 1-15)																	Unscheduled Visit
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	21	30	
Informed consent	x																		
Confirm eligibility	x																		
HIV testing/documentation	x																		
CD4 count (if applicable)	x																		
Treatment Assignment		x																	
Demographics	x																		
Complete Physical Exam	x																		x
Vital signs (heart rate, blood pressure, temperature, respiratory rate	x																		
Targeted symptom assessment	x	**x**						x								x	x	x	x
Weight	x																		
Adverse event assessment		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant Medications	x							x								x			x
Liver Function Tests[1]	x							x								x	x	x	x
Viral hepatitis serologies[10]	x																		

Appendix 1

	Screening[9]	(Visit Window Days 1-15)																Unscheduled Visit
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	21	30	
CK and serum creatinine	x							x								x	x	x	
Chemistry[2]		**x**						x								x	x	x	x
Sputum Xpert MTB/RIF	**x**																		
Sputum AFB Smear and Culture In Liquid/Solid Media[3] (with DST)	x	**x**						x								x			
PK Sampling (intensive)		X[4]	X[8]													X[4]			
Urine pregnancy test	x															x		x	
Chest X-ray	x																		
CBC	x																		
DOT		X[5]	X[6]	x	x	x	x	x	x	x	x	x	x	x	x	x			
Lung Inflammation Assessment[7]		x														x		x	

[1]Liver function tests (AST/ALT, T. bilirubin)
[2] Chemistry (Comprehensive metabolic panel, total cholesterol, LDL, C-reactive protein, uric acid)
[3]Two sputum samples for AFB smear microscopy and culture in liquid and solid media will be collected at each time point; sputum specimens and culture isolates will be stored

for culture-based drug-susceptibility testing and targeted deep sequencing in the event of TB treatment failure or TB recurrence.
[4] PK (intensive sampling)
[5] DOT of statin only
[6] DOT of statin and first dose of TB treatment
[7]Pulmonary function test, St George's Respiratory Questionnaire, 6-minute walk test; +/- 3 days
[8] Sparse PK
[9] The Screening evaluation process itself may occur over a few days; all Screening evaluations will be obtained within 3 days of study entry.

[10] Viral hepatitis serologies; IgM antibody for Hepatitis B surface antigen, IgM antibody to Hepatitis B core antigen, antibody to Hepatitis C