

PROTOCOL

CAAE 58751622.8.0000.0021

NCT 05413551

PHASE 1 STUDY: Point-of-care pharmacogenomic trial to optimize isoniazid dosing in the treatment of latent tuberculosis.

POCPOI (Point-of-Care Pharmacogenomics Optimize Isoniazid).

Version 6.0, dated September 4, 2024

CONFIDENTIAL

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Declaration of conformity

This clinical trial will be conducted in accordance with all provisions of this protocol, the approval conditions of the Ethics Committees and the Integrated Addendum to ICH E6 (R1): Good Clinical Practice Guideline E6 (R2), dated November 9, 2016.

Furthermore, this study will follow the guidelines of Resolution No. ⁴⁶⁶ of the National Health Council, dated December 12, 2012 (and other legal instruments that guide the CEP/CONEP System) and RDC/ANVISA 09 of February 20, 2015 (and other legal provisions that guide the conduct of clinical research in Brazil).

This clinical trial is not sponsored by any pharmaceutical company or other commercial entity.

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Karina Marques	Study coordinator. Responsible for study logistics, drafting SOPs, team training, report writing, adverse event assessment, and data management.
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SYNOPSIS OF THE PROTOCOL

TITLE	Phase 1 study: Point-of-care pharmacogenomic trial to optimize isoniazid dosing in the treatment of latent tuberculosis.
STUDY DESCRIPTION	<p>This is a diagnostic, observational, cross-sectional evaluation study to assess the accuracy of a rapid cartridge-based molecular assay for detecting single nucleotide polymorphisms in the <i>NAT2 gene</i>, associated with a multicenter, phase I/II, randomized, non-blinded clinical trial involving healthcare and prison system workers, household contacts of confirmed cases of pulmonary tuberculosis, incarcerated population, and individuals living with HIV, to evaluate the pharmacogenomics-adjusted dosage pharmacokinetics of isoniazid (INH), administered in combination with rifapentine, for tuberculosis chemoprophylaxis.</p> <p>Participants will be allocated into three groups according to <i>NAT2 acetylation patterns</i> (control, group 1, and group 2). Groups 1 and 2 will only receive the second dose of the genetically adjusted 3HP regimen, completing the treatment according to the dose recommended by the Ministry of Health. Participants will be monitored in two ways: a) via clinical visits for 3 weeks and b) through telephone contact. Blood samples will be collected before allocation for complete blood count, liver function tests, and <i>NAT2</i> acetylation profile. At the physician's discretion, blood samples will be collected to assess the occurrence of hepatotoxicity.</p>
OBJECTIVES	<p>Primary objectives</p> <ol style="list-style-type: none"> 1. <i>NAT2</i> genotyping. 2. To evaluate the pharmacokinetics of pharmacogenomics-adjusted isoniazid dosage, administered concomitantly with rifapentine, for tuberculosis chemoprophylaxis. <p>Secondary objectives</p> <ol style="list-style-type: none"> 1. To determine the diagnostic accuracy among slow acetylators. 2. To determine the diagnostic accuracy among rapid acetylators. 3. To evaluate the relationship between the variability of the <i>NAT2</i>, <i>AADAC</i>, <i>CYP2E1</i>, and <i>SLCO1B1</i> genes with gene expression and drug metabolism. 4. To evaluate gene expression signatures associated with the occurrence of systemic adverse drug reactions. 5. To evaluate the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification (25 mg/kg, maximum 1500 mg) among slow <i>NAT2</i> acetylators and compare it with the standard dosage (15 mg/kg, maximum 900 mg) among intermediate acetylators. 6. To evaluate the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification (5 mg/kg, maximum 300 mg) among slow <i>NAT2 acetylators</i> and compare it with the

	<p>standard dose (15 mg/kg, maximum 900 mg) among intermediate acetylators.</p> <ol style="list-style-type: none"> 7. To compare the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification with standard weekly dosing among <i>NAT2 rapid acetylators</i>. 8. To compare the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification with standard weekly dosing among slow <i>NAT2 acetylators</i>. 9. To compare the pharmacokinetics of standard weekly isoniazid dosing between rapid and intermediate <i>NAT2 acetylators</i>. 10. To compare the pharmacokinetics of standard weekly isoniazid dosing between slow and intermediate <i>NAT2 acetylators</i>. <p>Exploratory objectives</p> <ul style="list-style-type: none"> ● To evaluate the safety and tolerability of pharmacogenomics-guided isoniazid dose modification among <i>NAT2 rapid acetylators</i>. ● To evaluate the safety and tolerability of pharmacogenomics-guided isoniazid dose modification among slow <i>NAT2 acetylators</i>.
OUTCOMES AND MEASURES OF OUTCOMES	<p>Primary outcome</p> <ol style="list-style-type: none"> 1. Area under the curve of isoniazid over 24 hours after dosing (AUC₀₋₂₄). <p>Secondary outcomes</p> <ol style="list-style-type: none"> 1. Maximum concentration of isoniazid (C_{max}). 2. Isoniazid concentration 24 hours after dosing (C₂₄). <p>Exploratory outcomes</p> <ol style="list-style-type: none"> 1. Frequency of adverse events and serious adverse events according to standardized classification criteria.
STUDY POPULATION	<p>The study will recruit approximately 300 people for the diagnostic evaluation study and 100 for the RCT, from five groups of individuals eligible for tuberculosis preventive therapy: 1) healthcare professionals enrolled in a BCG revaccination study (BRACE) with latent tuberculosis evidenced by a positive QuantiFERON test; 2) prison system workers; 3) household contacts of confirmed cases of pulmonary tuberculosis residing in the cities of Campo Grande/MS and Manaus/AM; 4) incarcerated population; and 5) people living with HIV, eligible for preventive therapy based on CD4 count, tuberculin skin test, or QuantiFERON® test.</p>
DESCRIPTION OF RECRUITMENT LOCATIONS	<p>The study will be conducted in two centers in Brazil, in Campo Grande/MS and in Manaus/AM.</p> <p>In Campo Grande/MS, the study will be conducted at the Faculty of Medicine (FAMED/UFMS); at the Esterina Corsini Day Hospital</p>

	<p>of the Federal University of Mato Grosso do Sul; at the Center for Infectious and Parasitic Diseases (CEDIP); at the Testing and Counseling Center (CTA) (both from the Municipal Health Department of Campo Grande/MS) and at the Jair Ferreira de Carvalho and Instituto Penal de Campo Grande prison units.</p> <p>In Manaus, the study will be conducted at the Dr. Helio Vieira Dourado Tropical Medicine Foundation.</p>
DESCRIPTION OF INTERVENTIONS	<p>1) Intermediate acetylator group (control group) will receive a standard dosage of 15mg/kg (maximum of 900mg) of isoniazid and rifapentine orally weekly for 12 doses.</p> <p>2) The rapid acetylator group (group 1) will receive a standard dose of isoniazid (15 mg/kg, maximum 900 mg) and, one week later, will receive an increased dose (25 mg/kg, maximum 1,500 mg). Then, one week later, they will complete the remaining therapy with the standard dosage (total of 12 doses).</p> <p>3) Slow acetylators (group 2) will receive a standard dose of isoniazid (15mg/kg) and, one week later, will receive a lower dose (5mg/kg, maximum of 300mg). Then, one week after that, they will complete the remaining therapy with the standard dosage (total of 12 doses).</p>
DURATION OF THE STUDY	36 months
DURATION OF PARTICIPANT FOLLOW-UP	12 weeks from randomization to the end of follow-up.

LIST OF ABBREVIATIONS

MG/ML	Micrograms per milliliter
μ L	microliter
3HP	Isoniazid and rifapentine for 3 months, one dose weekly.
AIDS	Acquired immunodeficiency syndrome
ALT/TGP	Alanate aminotransferase
ANVISA	National Health Surveillance Agency
AST/TGO	Aspartate aminotransferase
AUC	Area of concentration under the curve
C ₂₄	Concentration after 24 hours
CDC	US Centers for Disease Control and Prevention
ZIP CODE	Research Ethics Committee
C _{max}	Maximum concentration
CONEP	National Research Ethics Committee
CPNP	1-cyclopentyl-4-nitrosopiperazine
CRF	Case Report Form
DNA	Deoxyribonucleic acid
DR-TB	Drug-resistant tuberculosis
DSMB	Data and Security Monitoring Committee
EA/EAG	Adverse event / Serious adverse event
ECR	Randomized clinical trial
USA	United States of America
FDA	(US) Food and Drug Administration, equivalent to ANVISA in Brazil
FIOCRUZ	Oswaldo Cruz Foundation
FMT/HVD	Dr. Heitor Vieira Dourado Tropical Medicine Foundation
H/INH	Isoniazid
HIV	Human immunodeficiency virus
ICH	International Consortium for Harmonisation
ILTB	Latent tuberculosis infection
IRB	Institutional Review Board
LAB DIP	Laboratory of Infectious and Parasitic Diseases
MDR-TB	Multidrug-resistant tuberculosis
MG/KG	Milligrams per kilogram
M _{IC}	Minimum inhibitory concentration
<i>M tb</i>	<i>Mycobacterium tuberculosis</i>
NAT2	N-acetyltransferase 2
NIH	National Institutes of Health
P/RPT	Rifapentine
PI	Principal Investigator
PK	pharmacokinetics
POP	Standard operating procedure
PPL	Prisoner population
ppm	Parts per million

RA/RAG	Adverse reaction/serious adverse reaction
RDC	Resolution of the Board of Directors
REDCAP	Research Electronic Data Capture
RHS	Rifampicin hypersensitivity syndrome
RIF	Rifampicin
SINAN	Information system on notifiable diseases
CNS	Central nervous system
SNP	Single nucleotide polymorphism
T1/2	Half life
ALSO	Tuberculosis
INFORMED CONSENT FORM	Free and informed consent form
TDO	Directly observed treatment
UFMS	Federal University of Mato Grosso do Sul
ULN	Upper limit of normal
WHO	World Health Organization

INVESTIGATOR AGREEMENT

I read the protocol entitled *"Phase 1 study: Point-of-care pharmacogenomic testing to optimize isoniazid dosage in the treatment of latent tuberculosis"*.

By signing this protocol, I agree to participate in the clinical trial, after approval by a Human Research Ethics Committee, in accordance with the protocol, the principles of the Declaration of Helsinki and the guidelines for good clinical practice [Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated November 9, 2016].

Changes to the protocol will only be implemented after written approval from the Research Ethics Committee involving human subjects, except in cases of medical emergencies.

I will ensure that the study team fully understands and follows the protocol, and that evidence from their training is documented.

Name	Function	Signature and date
Julio Croda	Principal Investigator	

1. ADMINISTRATIVE INFORMATION

1.1 Study Record

This study It is registered on [ClinicalTrials.gov](https://clinicaltrials.gov) under number **NCT 05413551**.

1.2 Sponsor

Study sponsor	United States National Institutes of Health
Name of principal investigator	Julio Croda
Address	Federal University of Mato Grosso do Sul

1.3 Expected duration of the study

The recruitment and administration period for the investigational product is scheduled to take place between August 2022 and March 2023. 2025.

Participants will be followed for 12 weeks, starting from the date of randomization.

1.4 Involvement of third parties

Stakeholders
Oswaldo Cruz Foundation – Mato Grosso do Sul
State Health Department of Mato Grosso do Sul
Maria Aparecida Pedrossian University Hospital – HUMAP/UFMS
Stanford University
University of San Francisco
Municipal Health Department of Campo Grande/MS - SESAU
State Agency for Prison System Administration - AGEPE/MS
Dr. Heitor Vieira Dourado Tropical Medicine Foundation (FMT/HVD)

2. INTRODUCTION AND BACKGROUND

2.1 Justification and objective of the study

The discovery and development of effective drug regimens for the treatment and prevention of tuberculosis are among the most impactful scientific and public health advances of the last century, leading to substantial reductions in morbidity and mortality. However, it is increasingly evident that a standardized dosing approach, which has enabled the global increase in treatment and preventive therapy, results in excess toxicity and suboptimal efficacy for a considerable portion of the millions of individuals who receive them each year. Up to one-third of patients receiving standard 4-drug therapy develop hepatotoxicity, 5% fail therapy, and 6-10% relapse within 24 months of treatment completion (1–5). Growing data demonstrate that varying drug levels are a major determinant of these toxicities and poor outcomes (6).

Along with rifamycins, isoniazid is the most important drug for the treatment of active and latent TB. The efficacy of isoniazid in preventive therapy was demonstrated in the 1960s and, more recently, short-course preventive therapy in the form of isoniazid and rifapentine once a week for three months (3HP) has proven highly effective and is associated with higher treatment completion rates (7). Isoniazid is metabolized via acetylation by arylamine N-acetyltransferase 2 (*NAT2*) in the liver. Polymorphisms in the *NAT2* gene account for more than 80% of pharmacokinetic variability (8). Individuals are commonly classified as “rapid”, “intermediate” or “slow” metabolizers depending on whether they possess 0, 1 or 2 *NAT2* genes containing mutations that reduce *NAT2* activity. Approximately half of the global population are “slow” or “rapid” acetylators. Slow acetylation is associated with a 3-4 risk of hepatotoxicity with active TB treatment (9,10) and increases the risk of systemic drug reactions with 3HP (11) by 7 times, while the fast acetylator genotype is associated with an increased risk of microbiological failure, relapse and acquired drug resistance (12). Despite these well-established risks of standardized dosing, *NAT2*-guided isoniazid dosing is still not conventional.

A major obstacle to this is that testing is limited to reference laboratories and is not accessible in locations where the tuberculosis burden is higher. Recently, we developed and tested a rapid cartridge-based molecular test for the 5 most important single nucleotide polymorphisms (SNPs) that determine the acetylator genotype, which can be performed using the GeneXpert® platform, widely used globally for tuberculosis diagnosis. We developed an algorithm using over 8,500 globally representative genomes that allowed 100% accuracy in predicting the acetylator type of these 5 SNPs and demonstrated that the assay could accurately predict isoniazid clearance in a cohort of TB patients (13). We also found that existing *NAT2* classification algorithms had substantial classification errors, omitting a key SNP prevalent in African populations and not taking into account the differential functional impairment of the enzyme of various haplotypes.

Pharmacogenomic dosing of isoniazid has the potential to reduce toxicities and improve treatment outcomes for up to half of the population with slow or fast acetylator genotypes. A randomized study in Japan showed that *NAT2*-guided dosing significantly reduced hepatotoxicity and early treatment failure (14), providing a strong premise for this approach, but requiring replication. Previous dose optimization studies used imprecise acetylator classifications, and no *NAT2*-based dosing studies have been conducted for weekly isoniazid dosing (e.g., 3HP), which involves higher isoniazid doses (standard 15 mg/kg versus 5 mg/kg for daily dosing).

We propose to validate our prototype rapid molecular test using non-invasive or minimally invasive samples to test the feasibility of *NAT2* genotyping in clinical settings and to conduct pharmacokinetic studies to determine whether *NAT2*-guided isoniazid dose modification can reduce variation in drug levels in a diverse population of HIV+ and HIV- individuals receiving TB preventive therapy.

2.2 Background

We compiled genomic data from 8,561 individuals from the published literature and classified acetylator status using the international consensus database on arylamine N-acetyltransferases (15). In this globally representative database, 40% of individuals were intermediate acetylators, 42% were slow acetylators and 18% were fast acetylators, as shown in Table 1 below.

Table 1 - Global distribution of NAT2 acetylator types (13).

Region	Acetylation Genotype, n (%)			
	Number of Individuals	Low	Intermediate	Rapid
Americas	1,112	432 (39%)	463 (42%)	217 (20%)
Central and Southern Asia	588	355 (60%)	198 (34%)	35 (6%)
East Asia	2,308	340 (15%)	1,049 (45%)	919 (40%)
Europe	3,458	1,966 (57%)	1,249 (36%)	243 (7%)
South Africa	44	30 (68%)	10 (23%)	4 (9%)
Sub-Saharan Africa	1,051	450 (43%)	459 (44%)	142 (14%)
Total	8,561	3,573 (42%)	3,428 (40%)	1,560 (18%)

In all regions, more than half of the individuals are either fast or slow acetylators. We tested random forest models on a geographically stratified 2/3 random sample of the data and assessed out-of-sample prediction accuracy on the remaining 1/3. With 5 SNPs, the accuracy in predicting the acetylator genotype was 100%. On the other hand, the more widely used previous tool (*NAT2Pred*) had error rates of 5% overall and 14% among samples from Sub-Saharan Africa, due to the omission of the G191A SNP common in Sub-Saharan Africa. To clinically validate the *NAT2 algorithm*, we conducted a pharmacokinetic study among 48 individuals initiating treatment for active TB in Brazil. We found that predicted fast acetylators had a much higher INH clearance than slow acetylators (median 53.0 versus 14.5 L/h, $p<0.0001$; Figure 1).

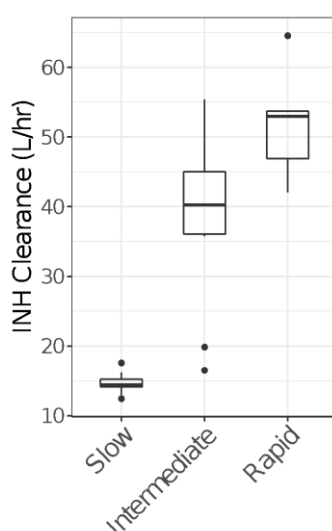


Figure 1 - INH clearance by the predicted acetylator phenotype (13).

NAT2 genotyping in clinical practice, we developed a molecular multiplex qPCR assay based on molecular beacon probes that detects the 5 most important SNPs (A191G, C282T, T341C, G590A, G857A), reporting whether the individual has 0, 1, or 2 polymorphisms at each position through high-resolution fusion curve analysis. We then developed the assay for the GeneXpert® platform using Cepheid® flexible cartridges, which allow for customized assays (Figure 2).

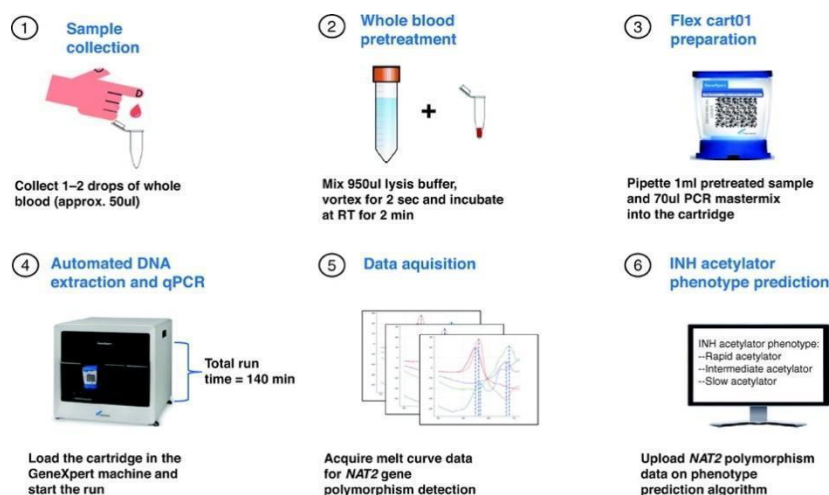


Figure 2 - Steps in performing the cartridge-based *NAT2* assay for the GeneXpert® platform

We adapted the program to identify the peaks of the fusion curve and report the SNP patterns. We validated the assay using whole blood samples with various *NAT2* genotypes and found 100% accuracy compared to sequencing (13). We also found accuracy in SNP identification in an input volume range of 12.5 µl to 500 µl of whole blood.

An important issue regarding the implementation of pharmacogenetically guided treatment is cost-effectiveness in low- and middle-income countries where the TB burden is higher. We developed a *NAT2 pharmacogenetically guided TB treatment model*, incorporating toxicities and treatment response data from the published literature. Using *NAT2 acetylator distributions* and healthcare cost data from Brazil, South Africa, and India, we estimated that pharmacogenetically guided therapy would be highly cost-effective in these three diverse settings if the diagnostic cost were similar to (or even several times higher than) Xpert MTB/RIF (16).

Standardized, weight-based dosing of TB drugs for treatment and chemoprophylaxis has been the basis in the US and worldwide for over 35 years. However, interindividual differences in metabolism lead to marked variation in drug levels, which has been increasingly considered a predictor of poor treatment response (17–19). A recent study showed that having a drug below the target AUC threshold was associated with a 14-fold increased chance of microbiological failure, disease relapse, or death (6).

The WHO recently expanded its recommendation for TB preventive therapy, now advising that it be offered to all household contacts of TB patients and HIV-positive individuals or those with unknown tuberculin test results. Modeling has shown that increasing preventive

therapy could prevent millions of TB cases and deaths in the coming decades, proving highly cost-effective (20–23). A 3-month regimen of weekly isoniazid and rifapentine (3HP), widely used in the US, is now recommended by the WHO as it is associated with higher completion rates and no inferior efficacy compared to 6–9 months of INH (7). However, in randomized studies, 3HP was associated with a higher risk of adverse events and a 9-fold increase in the chance of systemic drug reactions. (24). In the largest 3HP study, 35.7% of participants experienced at least one adverse event and 21% of these individuals discontinued treatment (25). It has recently been found that 24-hour INH concentration predicts systemic drug reactions (11). Dosage optimization is crucial to maximize efficacy and avoid toxicity since 3HP is scaled to tens of millions of people.

INH remains one of the most important drugs in the treatment and prevention of TB. INH is metabolized by arylamine N-acetyltransferase 2 (*NAT2*); single nucleotide polymorphisms (SNPs) in this gene strongly predict INH metabolism. Individuals with 2, 1, or no copies of *NAT2* with canonical SNPs are considered slow, intermediate, or fast acetylators. The *NAT2* *genotype* strongly predicts INH clearance, explaining >80% of interindividual variation (8). Meta-analyses of more than 25 studies found a 3-4 times higher risk of drug-induced liver injury in slow acetylators (9,10). The slow acetylator type is also associated with INH-induced peripheral neuropathy (26). Meta-analyses of 13 studies examining disease outcomes found that the fast acetylator state was associated with an increased risk of microbiological treatment failure, relapse, and acquired drug resistance (12). A recent study found that the *NAT2* *fast acetylator state* was associated with an increased risk of death in TB meningitis (27). Weekly trials of INH and rifapentine for the continuation phase of TB treatment failed to establish non-inferiority due to increased failure rates among fast acetylators (28). For preventive therapy with 3HP, *NAT2* *polymorphisms* were associated with a 7-fold increased risk of systemic drug reactions (11).

Early pharmacokinetic studies found that increasing the daily INH dosage among rapid acetylators and decreasing the dosage among slow acetylators could achieve pharmacokinetic targets consistent with standard doses among intermediate acetylators (8,29,30). A small, non-blinded study of increased doses for rapid metabolizers and reduced doses for slow acetylators showed that INH concentrations for rapid metabolizers were equivalent to the standard dosage among intermediate acetylators, while adverse drug reactions were reduced. Pharmacogenomics-guided dosing reduced early treatment failure by more than 50%, while eliminating drug-induced liver injury (14); however, this study requires replication in other populations. Pharmacogenomics-guided dosing has not been tested or established for weekly INH dosing as administered in preventive therapy with the 3HP regimen.

A major obstacle to scaling up individualized treatment or preventive therapy for TB is the lack of diagnostics. Therapeutic drug monitoring is sometimes used to guide dosing in resource-rich settings, but requires post-dose venous blood sampling and equipment (e.g., HPLC/GC) that are not widely available, hindering rapid or point-of-care testing. *NAT2* *genotyping* is also not routinely performed in clinical laboratories and has been largely relegated to a few reference laboratories. Recently, we developed a rapid cartridge-based molecular assay for *NAT2* *genotyping* directly from whole blood that can be performed on the

GeneXpert® platform, which is widely used for TB diagnostics worldwide. We now propose to further validate this assay in a diverse population in Brazil. We will test the use of alternative sample types, including fingertip capillary blood, filter paper dried blood, and oral swabs, evaluating accuracy, reproducibility, and robustness to sample variation. Validation of this assay may allow its use in TB prevention and treatment programs globally.

The relationship between *NAT2* and isoniazid acetylation has long been recognized, but one of the challenges with applying SNP data to predict haplotypes is that SNP data are not phased: two SNPs occurring on the same chromosome (resulting in one defective enzyme and one intact enzyme, resulting in an intermediate acetylation phenotype) cannot be distinguished from two SNPs occurring on different chromosomes (causing two deficient enzymes, a slow acetylation phenotype). This problem is overcome by haplotype phase, leveraging population genomic data. However, the most widely used tool for predicting the *NAT2* phenotype (*NAT2Pred*): 1) is over 12 years old and predates the massive expansion in human genome sequencing (31); 2) omitted a common and functionally important *NAT2* SNP (G191A) prevalent in African populations, resulting in high classification error rates(32); 3) is now offline and unavailable. We recently trained a random forest model on over 8,500 globally representative genomes that achieved 100% out-of-sample genotype prediction using 5 SNPs (including G191A) (13). We will perform further validation of this tool and make it publicly available via an internet interface.

The 3HP is now a WHO-recommended regimen and is increasingly being implemented in TB control programs worldwide. However, while *NAT2* -related dose modifications have been studied for daily INH (standard: 5 mg/kg), no study has evaluated alternative dosing for weekly INH dosing (standard: 15 mg/kg) as used in 3HP. One study examined higher daily INH doses for *Mtb* carrying *inhA* mutations that confer resistance to low-level INH, finding that acetylator status was critical for achieving pharmacokinetic (PK) targets; however, they administered INH as monotherapy for 7 days, did not test doses higher than 15 mg/kg, and concluded that this dose was too low for rapid acetylators (33). Trial of higher doses of isoniazid with rifapentine is needed to optimize preventive therapy and will have implications for high-dose INH, as used in INH-resistant TB containing *inhA*. We filled this knowledge gap by conducting a prospective dose-modification study guided by *NAT2* in a diverse population of HIV-infected and non-infected individuals in Brazil.

2.3 Risk/benefit assessment

2.3.1 Known potential risks

Risks associated with the use of rifapentine

Rifapentine, like other drugs in the rifamycin class, causes orange-red discoloration of body fluids and may stain contact lenses. In clinical trials where rifapentine was combined with isoniazid and other antituberculosis drugs and administered once or twice weekly, adverse reaction rates were similar with rifampicin and rifapentine, with increased hepatic aminotransferase activity in approximately 5% of patients.

The only adverse effect that occurred more frequently with rifapentine than with rifampicin was hyperuricemia when the drug was administered twice weekly; notably, the hyperuricemia was attributed to the concomitantly administered pyrazinamide. Other adverse reactions that occurred in 1-5% of patients included the following: hemoptysis, dizziness, hypertension, headache, gastrointestinal disorders, rash, cytopenias, hematuria, pyuria, and proteinuria (34).

Recently, Sanofi reported the presence of nitrosamine impurities in some manufacturing batches of rifapentine. These impurities have also been found in rifampicin, another tuberculosis medication. Low amounts of nitrosamines are common in water and foods, including cured and grilled meats, dairy products, and vegetables. Some types of nitrosamines have been shown to cause cancer in tested animals. Nitrosamine impurities may increase the risk of cancer if people are exposed to them above acceptable levels and for long periods. The nitrosamine compound found in rifapentine is 1-cyclopentyl-4-nitrosopiperazine (CPNP). Although there are no data available to directly assess the carcinogenic potential of CPNP, available information on closely related nitrosamine compounds has been used to calculate lifetime exposure limits for 1-cyclopentyl-4-nitrosopiperazine and CPNP. The FDA has established an exposure limit of 0.1 ppm for rifapentine; Daily exposures above this level for prolonged periods (decades) may potentially increase the risk of some types of cancer. Short-term use is not considered to pose an increased cancer risk.

Sanofi tested all batches of rifapentine and identified CPNP levels ranging from <1 ppm to up to 12 ppm. The company proposed a temporary limit of 20 ppm of CPNP in rifapentine. To mitigate or avoid shortages and help ensure patients have access to rifamycins, the FDA agreed that manufacturers may temporarily distribute rifapentine containing CPNP levels <20 ppm until they can reduce or eliminate the impurities. The World Health Organization and the STOP TB Partnership's Global Drug Facility endorsed this recommendation. No batches containing product >20 ppm will be used.

Risks associated with the use of isoniazid

The overall incidence of all adverse effects of isoniazid is approximately 5%, many of which do not require discontinuation of the medication. Peripheral neurotoxicity is dose-dependent and uncommon (<0.2%) at conventional doses. The risk of peripheral neuritis increases in malnourished individuals or those predisposed to neuritis due to other diseases. Concomitant administration of pyridoxine (vitamin B6) is recommended for these individuals (26).

Other nervous system reactions are rare at normal doses and include seizures, encephalopathy, optic neuritis, memory impairment, and psychosis. Gastrointestinal side effects include nausea, vomiting, and epigastric discomfort. Asymptomatic elevation of aminotransferases is common and occurs in 10-20% of people receiving isoniazid. However, severe idiosyncratic hepatic reactions are uncommon but are more likely in older people (up to 2.3% incidence of hepatitis in people over 50 years of age) and can be fatal. Daily alcohol consumption increases the risk of isoniazid-associated hepatotoxicity by approximately 4 times. To minimize this risk, we will measure liver enzymes before starting the intervention and, at the physician's discretion, these may be repeated at any time during the intervention.

Risks related to blood sample collection

Sometimes, having a blood test can cause some pain or discomfort. Occasionally, a small amount of bruising may occur on the skin where the blood was drawn. Trained members of the study team will collect the blood samples from the participants.

Risks associated with collecting samples using a swab.

Oral swab collection may cause discomfort, coughing, and changes in breathing rate. To minimize these risks, the collection will be performed by experienced nurses trained in this procedure.

Risks related to social stigma

The protocol can identify active cases of tuberculosis during recruitment. Although early diagnosis of tuberculosis provides individuals with the benefits of early diagnosis, the disease in Brazil, as in many countries, is potentially stigmatized. All TB cases must be reported through the Notifiable Diseases Information System (SINAN) and provided with free treatment. To minimize this risk, notifications will be forwarded to the competent authorities. in sealed envelopes.

Risks related to breaches of confidentiality regarding personal information.

The study team will obtain information on demographics, behaviors, and medical history during interviews; therefore, there is a potential risk of discomfort when answering questions regarding breaches of confidentiality related to personal information. Participant interviews will be conducted individually, in private rooms, with nurses experienced in research procedures and protocols, certified in good clinical practice, and trained to minimize discomfort associated with obtaining answers during interviews. We will maintain the confidentiality of personal information by implementing the data management procedures described in section 10 and will not share the data collected as part of the research protocol with health authorities, except in the situation of an active case of tuberculosis. Furthermore, access to individually identifiable information in the databases will be restricted to the PI and the data manager.

Risks related to the individualization of genetic data

We will sequence a coding region of the *NAT2* gene; this will generate individual genetic data. Because the sequence will be <1000 base pairs, it will not contain enough information to identify an individual. *NAT2* genotypes may have some association with bladder cancer risk, and knowing this may be distressing for participants. All laboratory samples will be coded and will not contain any identifying information for participants. *NAT2* genotyping data will be stored on encrypted, password-protected devices. We will make fully de-identified data available to NCBI in accordance with NIH Genomic Data Sharing requirements. We will inform participants of the results of the *NAT2* gene sequencing. *NAT2* genotypes have had some association with bladder cancer, and we will disclose this. Since slow acetylator phenotypes of *NAT2* are common, the effect size for cancer risk is small, and

there are no established means for screening or preventing bladder cancer, there is no indication for any specific intervention or diagnostic follow-up.

2.3.2 Known potential benefits

The proposed study will not provide direct benefits to research participants, as they would still be eligible for preventive therapy regardless of whether they participate in the study. They will receive the results of their *NAT2 tests*, but this has no proven benefits at this time. We believe that the benefits of the knowledge gained from this study for improving tuberculosis treatment outweigh the risks associated with the study protocol, which are minimal or can be effectively minimized by implementing the actions described in section 2.3.1.

2.3.3 Assessment of potential risks and benefits

In June 1998, the FDA approved rifapentine (RPT) for the treatment of tuberculosis, the first new drug approved for tuberculosis in over 25 years. Additionally, the 3HP regimen is registered with the US FDA and recommended for tuberculosis prevention by the CDC. The WHO recommends 3HP for low-burden, middle-income, and high-income countries in its integrated guidelines for the programmatic management of LTBI. Tolerance of the HP regimen was comparable to INH in the CPCRA/ACTG assay.

Isoniazid has been used in the treatment and prevention of tuberculosis for over 50 years, and its adverse event profile is well known. Skin rash, fever, jaundice, and peripheral neuritis are the most common adverse reactions related to INH. Simultaneous administration of pyridoxine (vitamin B6) prevents INH-related peripheral neuropathy, as well as almost all other nervous system disorders attributable to INH administration. In this study, if a participant develops symptoms of peripheral neuropathy, pyridoxine may be administered at a dose of 25 mg with each dose of INH. The pyridoxine dose may be increased to 50 mg with each dose of INH if symptoms of peripheral neuropathy persist.

RPT, like other rifamycins, causes an orange-red discoloration of body fluids. In studies where RPT was combined with INH and other tuberculosis medications, adverse reaction rates were similar between rifampicin and RPT, with increased hepatic aminotransferase activity in about 5% of patients (34).

In the TBTC 26 study, 3HP was also well tolerated among study participants compared to 9H. A summary of relevant adverse events and their frequencies is listed in Table 2.

Table 2 - Adverse drug-related events in RPT/INH trials for the treatment of LTBI.

Study	Population	Treatment completion		Adverse events		Comment
		3HP	93%	3HP	1%	
Schechter 2006(35)	Household contacts in Brazil	2RZ	94%	2RZ	10%	TDO, study terminated prematurely.
Martinson 2011(36)	HIV+ without treatment, South Africa	3HP	96%	3HP	8.7/100 PY	TDO, continuous INH is more toxic.
		6H	84%	6H	15.4/100 PY	

Sterling 2011(37)	Contacts, others at high risk, > 2 years, USA, Canada, Brazil, Spain	3HP	82.1%	3HP	8.2%	TDO, drug-related toxicity study
		9 AM	69.0%	9 AM	5.5%	
Belknap 2017(38)	USA, South Africa, Spain	3HP -TDO	87.2%	3HP - TDO	7.1%	AD+ text message
		3HP - AD	74.0%	3HP - AD	8.3%	
		3HP - AD +	76.4%	3HP - AD +	7.9%	
Observational studies						
Chan 2019(39)	Contacts >12 years Taiwan	3HP	83.9%	3HP	12.0%	TDO on 3HP
		9 AM	78.8%	9 AM	9.4%	
John 2019(40)	Health workers, South Korea	3HP	85.0%	3HP	15%	Directly observed therapy (DOT) used for 3HP (19% of cases with flu-like syndrome associated with 3HP)
		3HR	81.7%	3HR	10%	

Treatment completion rates and occurrence of adverse events in 3HP studies across various populations. Adverse events are reported differently based on published results. DOT = directly observed treatment; AD = self-administered therapy; number = number of months; H = INH; P = rifapentine; R = rifampicin; Z = pyrazinamide.

3. OBJECTIVES AND OUTCOMES OF THE STUDY

3.1 Objectives

3.1.1 Primary objectives

1. To evaluate the accuracy of a novel cartridge-based rapid molecular assay for *NAT2* *genotyping* using venous whole blood, digital capillary blood, and oral swabs.
2. To determine whether pharmacogenomics-guided weekly isoniazid dosing between slow and fast acetylators achieves equivalence to standardized dosing between intermediate acetylators.

3.1.2 Secondary objectives

1. To determine the diagnostic accuracy among slow acetylators.
2. To determine the diagnostic accuracy among rapid acetylators.
3. To evaluate the relationship between the variability of the *NAT2*, *AADAC*, *CYP2E1*, and *SLCO1B1* genes with gene expression and drug metabolism.
4. To evaluate gene expression signatures associated with the occurrence of systemic adverse drug reactions.
5. To evaluate the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification (25 mg/kg, maximum 1500 mg) among slow *NAT2* *acetylators* and compare it with the standard dosage (15 mg/kg, maximum 900 mg) among intermediate acetylators.

6. To evaluate the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification (5 mg/kg, maximum 300 mg) among slow *NAT2 acetylators* and compare it with the standard dose (15 mg/kg, maximum 900 mg) among intermediate acetylators.
7. To compare the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification with standard weekly dosing among *NAT2 rapid acetylators*.
8. To compare the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification with standard weekly dosing among slow *NAT2 acetylators*.
9. To compare the pharmacokinetics of standard weekly isoniazid dosing between rapid and intermediate *NAT2 acetylators*.
10. To compare the pharmacokinetics of standard weekly isoniazid dosing between slow and intermediate *NAT2 acetylators*.

3.1.3 Exploratory Objectives

- To evaluate the safety and tolerability of pharmacogenomics-guided isoniazid dose modification among *NAT2 rapid acetylators*.
- To evaluate the safety and tolerability of pharmacogenomics-guided isoniazid dose modification among slow *NAT2 acetylators*.

3.2 Outcomes

OBJECTIVE	OUTCOME AND MEASUREMENT
Primary	
1. To evaluate the accuracy of a novel cartridge-based rapid molecular assay for <i>NAT2 genotyping</i> using venous whole blood, digital capillary blood, and oral swabs.	<ol style="list-style-type: none"> 1. <i>NAT2</i> assay in detecting the correct polymorphism patterns compared to the sequencing reference standard. 2. Accuracy in predicting the acetylator genotype (slow, intermediate, fast) for each participant. <p>Measurements:</p> <ul style="list-style-type: none"> • Proportion of tests that provide a valid (not indeterminate) result. • <i>NAT2</i> sequencing reference standard.

<p>2. To determine whether pharmacogenomics-guided weekly isoniazid dosing between slow and fast acetylators achieves equivalence to standardized dosing between intermediate acetylators.</p>	<p>3. Equivalence of AUC₀₋₂₄, comparing the standard weekly dosage of INH (15 mg/kg) between intermediate acetylators with:</p> <p>A. increased dose (25mg/kg) among rapid acetylators; and</p> <p>B. Dose reduction (5mg/kg) among slow acetylators.</p> <p>Measurements:</p> <ul style="list-style-type: none"> • Wilcoxon rank sum tests
Secondary	
<ol style="list-style-type: none"> 1. To determine the diagnostic accuracy among slow acetylators. 2. To determine the diagnostic accuracy among rapid acetylators. 3. To evaluate the relationship between the variability of the NAT2, AADAC, CYP2E1, and SLCO1B1 genes with gene expression and drug metabolism. 4. To evaluate gene expression signatures associated with the occurrence of systemic adverse drug reactions. 5. To evaluate the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification (25 mg/kg, maximum 1500 mg) among slow <i>NAT2 acetylators</i> and compare it with the standard dosage (15 mg/kg, maximum 900 mg) among intermediate acetylators. 6. To evaluate the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification (5 mg/kg, maximum 300 mg) among slow <i>NAT2 acetylators</i> and compare it with the standard dose (15 mg/kg, maximum 900 mg) among intermediate acetylators. 7. To compare the pharmacokinetics of pharmacogenomics-guided isoniazid dose modification with standard weekly dosing among <i>NAT2 rapid acetylators</i>. 8. To compare the pharmacokinetics of pharmacogenomics-guided isoniazid 	<ol style="list-style-type: none"> 1. Maximum concentration of isoniazid (C_{max}) 2. Isoniazid concentration 24 hours after dosing (c_{24}) <p>Measurements:</p> <ul style="list-style-type: none"> • Wilcoxon rank sum tests • Wilcoxon sign classification test for paired data

<p>dose modification with standard weekly dosing among slow <i>NAT2 acetylators</i>.</p> <p>9. To compare the pharmacokinetics of standard weekly isoniazid dosing between rapid and intermediate <i>NAT2 acetylators</i>.</p> <p>10. To compare the pharmacokinetics of standard weekly isoniazid dosing between slow and intermediate <i>NAT2 acetylators</i>.</p>	
Exploratory	
<ul style="list-style-type: none"> To evaluate the safety and tolerability of pharmacogenomics-guided isoniazid dose modification among <i>NAT2 rapid acetylators</i>. To evaluate the safety and tolerability of pharmacogenomics-guided isoniazid dose modification among slow <i>NAT2 acetylators</i>. 	<p>1. Frequency of adverse events and serious adverse events by standardized grading criteria, following the Division of AIDS (DAIDS) Table for Classification of Adverse Events in Adults and Pediatrics, corrected version 2.1 (dated July 2017), available at: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf</p>

4. STUDY DESIGN

4.1 Overview

This is a multicenter, non-blinded, phase I/II randomized clinical trial (RCT) with three groups, encompassing individuals with an indication for treatment of LTBI. To evaluate the pharmacogenomics-adjusted pharmacokinetics of isoniazid, administered in combination with rifapentine, for tuberculosis chemoprophylaxis, nested within a diagnostic, observational, cross-sectional study to assess the accuracy of a rapid cartridge-based molecular assay for detecting single nucleotide polymorphisms in the *NAT2 gene*.

Participants will be randomized into three groups according to *NAT2* acetylation pattern and will receive the following treatment: **control group:** intermediate acetylators will receive a standard dose of 15 mg/kg (maximum 900 mg) of isoniazid and rifapentine orally weekly for 12 weeks; **group 1 rapid acetylators:** will receive a standard dose of isoniazid (15 mg/kg, maximum 900 mg) and, one week later, will receive an increased dose (25 mg/kg, maximum 1500 mg). Then, one week later, they will complete the remaining therapy with the standard dose; and **group 2 slow acetylators:** will receive a standard dose of isoniazid (15 mg/kg) and, one week later, will receive a lower dose (5 mg/kg, maximum 300 mg). Then, one week later, they will complete the remaining therapy with the standard dose.

For all participants, rifapentine will be administered in standard doses (15mg/kg up to 900mg). All participants will receive 50mg of vitamin B6, with a weekly dose. All medications will be administered orally.

There will be no randomization, since the groups will be formed according to the acetylation pattern of the *NAT2 enzyme*.

Follow-up of all participants will last 12 weeks. All participants will be examined by study physicians, undergo a complete blood count and liver function tests, and clinical evaluation before the start of the intervention. All participants will be assessed weekly in the first month and then monthly by study nurses and questioned about adverse events, new symptoms, or worsening of previous symptoms; any participant reporting the occurrence of the above events will be evaluated by the study physician. After the pharmacogenomics-guided dose (2nd dose of groups 1 and 2), participants will have blood samples collected for liver enzyme and INH pharmacokinetic evaluation. Participants are free to discontinue medication at any time, and physicians may decide to discontinue treatment based on risk assessment.

Unsolicited adverse events will be captured at any time the participant contacts the team. Suspected cases of hepatotoxicity may be monitored with the collection of biological material for laboratory analysis or the performance of diagnostic tests, as judged by the study physicians.

4.2 Justification of the dose

Rifapentine and INH dosage will be based on dosages given to individuals >50 kg in the BRIEF TB study and current CDC guidelines for both groups. Based on a meta-analysis of rifapentine pharmacokinetics in nine clinical trials of preventive therapy conducted by Hibma and colleagues (41), weight-range dosing will not be used.

Preventive therapy is associated with a risk of side effects, including fever, headache, dizziness, nausea, muscle/bone pain, and rash, with symptoms similar to those of the flu. There is a small risk of hepatotoxicity, hypersensitivity reactions, and neuropathy. According to CDC guidelines, approximately 4-5% of patients receiving 3HP experience these. These are risks that would be encountered during the normal course of preventive therapy as clinically indicated, except for rapid acetylators who will receive a single increased dose (25 mg/kg up to a maximum of 1500 mg, instead of 15 mg/kg up to a maximum of 900 mg).

We performed pharmacokinetic simulations at various doses, which supported this dose as possibly conservative, shown below. A daily dose of 20 mg/kg is currently being studied in an ACTG study, and daily doses of 16-18 mg/kg have been administered for months to MDR TB patients in randomized studies (42). The CDC-recommended weekly pediatric dose of INH is 20-40 mg/kg. It is important to note that C_{max} will not be elevated with the higher dose, but C₂₄ should be lower. INH C_{max} has not been shown to correlate with adverse events (43,44), while C₂₄ has shown a correlation.

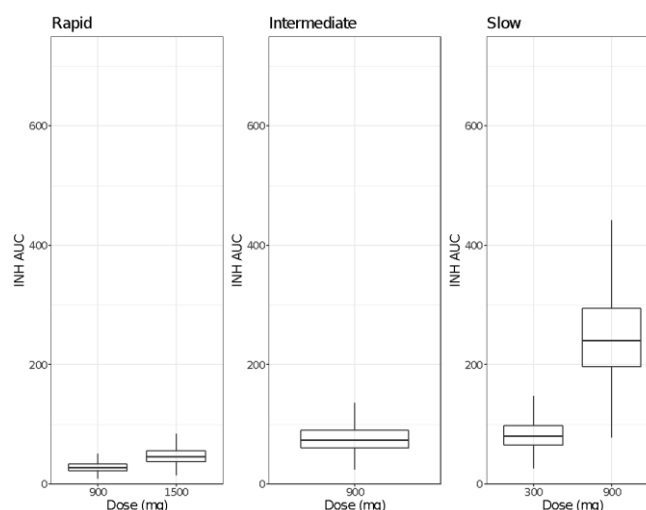


Figure 3 - INH AUC₀₋₂₄ by dose and acetylator profile, from 20,000 pharmacokinetic simulations.

4.3 Study population

Individuals eligible to receive TB chemoprophylaxis in Brazil, from five populations: A) HIV-infected individuals; B) healthcare professionals enrolled in a BCG revaccination trial (BRACE; CAAE 34101620.0000.0021); C) household contacts of confirmed cases of pulmonary tuberculosis, residing in the cities of Campo Grande/MS and Manaus/AM; D) Prison system workers and E) incarcerated population.

Two recruitment centers will be operating in Brazil: Campo Grande/MS, at the Faculty of Medicine of the Federal University of Mato Grosso do Sul (FAMED/UFMS), under the responsibility of Dr. Julio Croda, and Manaus/AM, at the Dr. Heitor Vieira Dourado Tropical Medicine Foundation (FMT/HVD), under the responsibility of Dr. Marcelo Cordeiro dos Santos.

4.4 Eligibility criteria

Participants will be assigned to one of the study groups based on their *NAT2* acetylation pattern only if they meet all inclusion criteria and none of the exclusion criteria.

4.4.1 Inclusion criteria

- Age 18 or older
- Eligible for preventive therapy for TB ¹according to the IGRA test result.

¹ Brazilian national guidelines recommend preventive therapy for individuals with IGRA or a positive tuberculin skin test who are TB contacts, people living with HIV, people with fibrotic radiographic abnormalities consistent with TB sequelae, those using steroids or TNF- α inhibitors, those with other immunosuppressive conditions, kidney disease, diabetes, low body weight, or calcified granulomas on chest X-ray. Tuberculin skin test size limits (e.g., ≥ 5 mm or 10 mm) depend on the risk factor. Additionally, individuals with PT/IGRA conversion are recommended to receive preventive therapy.

4.4.2 Exclusion criteria

- Evidence of active tuberculosis or currently being evaluated for possible active tuberculosis.
- Taking medications that interact with rifapentine (e.g., methadone, warfarin).
- Known intolerance or hypersensitivity to isoniazid or rifapentine.
- Prior treatment for active or latent tuberculosis for more than 14 days.
- Close contact with a case of INH- or RIF-resistant TB.
- Neutropenia (absolute neutrophil count <1000 cells/mm³).
- Clinical diagnosis of active liver disease or alcohol dependence.
- ALT or AST >3 times the upper limit of the test.

4.5 Lifestyle Considerations

Not applicable.

4.6 Screening failures

Screening failures are defined as participants who consent to participate in the study but who, during the screening procedures, are ineligible to continue in it. Therefore, they do not receive the intervention and are not randomized.

4.7 Recruitment and consent

designated team member who will provide them with detailed information about the research, outlining its objectives, risks, and benefits, and inviting them to participate. If they accept, consent will be obtained through the signing of an informed consent form.

Following the informed consent process, participants will answer a verbally administered questionnaire and undergo a chest X-ray performed by a radiology technician if they have not had an X-ray in the last three months. Those with TB symptoms or abnormal X-rays will undergo Xpert testing and sputum culture. Any participant with a positive Xpert test or sputum culture will be referred for TB treatment (provided free of charge and according to the guidelines of the National Tuberculosis Control Program) and reported to the Notifiable Diseases Information System (SINAN) of the Ministry of Health, and will not be allocated to the study. Any participant with an abnormal X-ray but a negative Xpert or sputum culture will be referred for evaluation by a study physician.

In this initial approach, a recruitment record will be used to capture the reason for non-inclusion, based on the eligibility criteria.

A peripheral venous blood sample (approximately 20 mL) will be collected for liver function tests (AST and ALT levels), complete blood count, and evaluation of polymorphisms in the NAT2, AADAC, CYP2E1, and SLC01B1 genes (through whole blood DNA extraction, PCR, and Min ION Nanopore sequencing). The data obtained will be used to assess the relationship between genetic variability, gene expression, and drug metabolism. We will also perform whole blood RNA extraction and transcriptome analysis to evaluate the expression of genes associated with adverse drug reactions. Oral swab samples and capillary blood samples will

also be obtained using lancets (stored in EDTA microtubes). and whole blood in a disc filter (*dried blood spot*) that will be stored in the Infectious Diseases Laboratory of UFMS (LAB DIP/UFMS) at -20 ° C, until NAT2 cartridge-based testing for the GeneXpert® platform. The results obtained through the GeneXpert platform will subsequently be compared with the results of NAT2 amplicon sequencing performed on the MinION Napore (at the time of allocation of intervention groups).

5 INTERVENTION

5.1 Groups

Control group - intermediate acetylators: will receive a standard dosage of 15mg/kg (maximum of 900mg) of isoniazid and rifapentine orally weekly for 12 weeks.

Group 1 - rapid acetylators: will receive a standard dose of isoniazid (15mg/kg, maximum 900mg) and, one week later, will receive an increased dose (25mg/kg, maximum 1,500mg). Then, one week after that, they will complete the remaining therapy with the standard dosage.

Group 2 - slow acetylators: will receive a standard dose of isoniazid (15mg/kg) and, one week later, will receive a lower dose (5mg/kg, maximum of 300mg). Then, one week after that, they will complete the remaining therapy with the standard dosage.

5.2 Intervention

5.2.1 Description of experimental products for research

Rifapentine	
	Rifapentine
Active substance and excipients	Excipients: calcium stearate, disodium EDTA, FD&C Blue No. 2 aluminum lake, hydroxypropylcellulose, hypromellose USP, microcrystalline cellulose, polyethylene glycol, pregelatinized starch, propylene glycol, sodium ascorbate, sodium lauryl sulfate, sodium starch glycolate, synthetic red iron oxide and titanium dioxide.
Trade or generic name	Prifitin®
Pharmaceutical form	Tablet
administrative route	Oral
Isoniazid	
	Isonicotinylhydrazine
Active substance and excipients	Excipients: colloidal silicon dioxide, lactose monohydrate, pregelatinized starch (corn), povidone and stearic acid.
Trade or generic name	Isoniazid

Pharmaceutical form	Tablet
administrative route	Oral

5.2.2 Dosage

Twelve doses will be administered to each participant, with a one-week interval. The standard dose for adults weighing more than 40 kg is 900 mg of rifapentine and 900 mg of isoniazid, orally. The second dose of isoniazid in groups 1 and 2 will be the experimental doses, adapted according to the *NAT2 acetylation pattern*, as described in section 4.1.

Additionally, to mitigate the potential occurrence of peripheral neuritis, all participants will receive 50mg of vitamin B6, along with the weekly dose of the regimen.

5.2.3 Dose modification

No dose modifications are permitted, other than those described in section 5.2.

5.2.4 Storage and distribution

Rifapentine

- Store at room temperature 25°C ±7°C
- Store in the original packaging to protect from light.
- Do not use the medicine after the expiry date printed on the packaging as “EXP”, which refers to the last day of the month listed.
- Any unused medication at the end of the study will be disposed of in accordance with local regulations.

Isoniazid

- Store at room temperature 25°C ±7°C
- Store in the original packaging to protect from light.
- Do not use the medicine after the expiry date printed on the packaging as “EXP”, which refers to the last day of the month listed.
- Any unused medication at the end of the study will be disposed of in accordance with local regulations.

5.2.5 Pharmacological considerations

Rifapentine

Rifapentine is a semi-synthetic derivative of rifamycin with a microbiological profile similar to that of rifampicin. Its structure differs from rifampicin by the presence of a cyclopentyl ring instead of a methyl group in the piperazinyl moiety. It has a longer half-life than rifampicin and, like rifampicin, rifapentine inhibits bacterial RNA synthesis by binding to the β subunit of DNA-dependent RNA polymerase.

Rifapentine is well absorbed from the gastrointestinal tract, with 70% bioavailability. When taken with food, its C_{max} and AUC increase by 40%–50% (34). It reaches peak serum

concentrations 5–6 hours after ingestion. Rifapentine and its 25-deacetyl metabolite are highly protein-bound, 97.7% and 93% respectively, primarily to albumin. Rifapentine is metabolized by an esterase enzyme found in the liver and blood to 25-deacetylrafapentine, a microbiologically active metabolite that contributes approximately 40% of the drug's overall activity. For *Mtb*, the MIC of 25-deacetylrafapentine is 0.25 µg /mL, while that of rifapentine is 0.05 µg /mL. The drug and the active metabolite have half-lives of 14-17 and 13 hours, respectively.

The drug is excreted in the bile and eliminated in the feces. Less than 10% of rifapentine is excreted in the urine in unchanged form. Rifapentine, like other rifamycins, induces CYP3A4, 2C8, and 2C9, which can lead to faster metabolism and elimination of many medications. Rifamycins are also known to induce the activity of phase II enzymes, such as glucuronosyltransferase and sulfotransferase, and may reduce the levels of drugs metabolized by these pathways.

Rifapentine (Priftin® · Sanofi) is available in 150mg tablets.

Isoniazid

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

Isoniazid is generally well absorbed; food and antacids decrease its absorption rate, but not its extent. Peak blood levels with isoniazid use of 3 to 5 µg /ml are obtained 30 minutes to 2 hours after ingestion of routine doses (45). It diffuses into all body fluids and cells and penetrates the caseous material of a tuberculoma or pulmonary cavity. In the liver, it is acetylated into 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 two metabolic phenotypes associated with the *NAT2 gene*, slow and fast acetylation, which are associated with race but not sex (46). The AUC of isoniazid among people with fast acetylation is 30% to 50% of that among people with slow acetylation.

Since isoniazid is well tolerated across a wide range of therapeutic doses, a single dose per body mass is recommended. Individuals with rapid acetylation achieve effective concentrations, while those with slow acetylation do not experience increased toxicity. The half-life ($t_{1/2}$) can vary from 1 hour in rapid acetylators ($t_{1/2} < 90$ min) to 3 hours in slow acetylators ($t_{1/2} > 90$ min). The normal dose of isoniazid for adults is 5 mg/kg once daily, up to a maximum of 300 mg once daily.

Isoniazid decreases the clearance of some drugs metabolized in the liver, particularly carbamazepine, phenytoin and diazepam (47). However, in the context of multidrug therapy including rifampicin, these potential drug interactions are of little importance because the effect of isoniazid is neutralized by the more potent opposing effect of rifampicin (48).

5.2.6 Administration of the experimental drug

Before starting treatment, each participant will receive counseling on treatment adherence, which will include information about the treatment, side effects, assistance with methods to remember to take the medication, and discussion of any anticipated problems while taking the medication.

Participants will receive weekly DOT (Directly Observed Therapy) intervention for 3 consecutive weeks. After taking the medication for the 3rd week, the team will give the remaining medication to the participant to complete the treatment, which they will then self-administer. Each week during the study, participants will be reminded to take all medications by phone call or WhatsApp. Exceptionally, participants belonging to the PPL (People with Disabilities) group will receive weekly visits for DOT intervention.

The study team will use measures to monitor treatment completion, including:

- Self-reported dosage at each monitoring visit;
- Tablet counting. Participants will bring any unused study medication to the research center in week 12. The study team will count the remaining tablets to estimate the number of scheduled doses taken.

Route/method of administration

- Oral administration, with direct dose therapy (DOT) for the first three weeks and self-administration until completion.

Overdosage/insufficient dosage or incorrect administration

Isoniazid

Signs and symptoms

Isoniazid overdose produces signs and symptoms 30 minutes to 3 hours after ingestion. Nausea, vomiting, dizziness, slurred speech, blurred vision, and visual hallucinations (including bright colors and strange patterns) are among the first manifestations. With severe overdose, respiratory problems and CNS depression are expected, rapidly progressing from stupor to deep coma, along with severe and intractable seizures. Severe metabolic acidosis, ketonuria, and hyperglycemia are typical laboratory findings.

Treatment

Untreated or inadequately treated cases of gross isoniazid overdose, 80 mg/kg to 150 mg/kg, can cause neurotoxicity and be fatal, but a good response has been reported in most patients who received appropriate treatment within the first few hours after ingestion of the drug.

For the asymptomatic patient

Drug absorption from the gastrointestinal tract may be decreased by administering activated charcoal. Gastric emptying should also be employed in asymptomatic patients. Protect the patient's airway when employing these procedures. Patients who acutely ingest more than 80 mg/kg should be treated with intravenous pyridoxine on a gram-for-gram basis

equal to the isoniazid dose. If an unknown quantity of isoniazid is ingested, consider an initial dose of 5 grams of pyridoxine administered over 30 to 60 minutes in adults.

For the symptomatic patient

Ensure adequate ventilation, support cardiac output, and protect the airway while treating seizures and attempting to limit absorption. If the isoniazid dose is known, the patient should initially be treated with a slow intravenous *bolus* of pyridoxine over 3 to 5 minutes, gram for gram, equal to the isoniazid dose. If the amount of isoniazid ingested is unknown, consider an initial intravenous *bolus* of 5 grams of pyridoxine in adults. If seizures continue, the pyridoxine dosage may be repeated. It would be rare for more than 10 grams of pyridoxine to be needed. The maximum safe dose of pyridoxine in isoniazid poisoning is unknown. If the patient does not respond to pyridoxine, diazepam may be administered. Phenytoin should be used with caution, as isoniazid interferes with phenytoin metabolism.

In general

Obtain blood samples for immediate determination of gases, electrolytes, BUN, glucose, etc.; blood typing and cross-comparison in preparation for possible hemodialysis.

Rapid control of metabolic acidosis

Patients with this degree of INH intoxication are likely to present with hypoventilation. Administration of sodium bicarbonate in these circumstances may exacerbate hypercarbia. Ventilation should be carefully monitored by measuring blood carbon dioxide levels and mechanically sustained if respiratory failure occurs.

Dialysis

Both peritoneal dialysis and hemodialysis have been used in the treatment of isoniazid overdose. These procedures are probably not necessary if seizure and acidosis control is achieved with pyridoxine, diazepam, and bicarbonate.

With measures based on initial and repeated determination of blood gases and other laboratory tests as needed, use meticulous respiratory care and other intensive care to protect against hypoxia, hypotension, aspiration, pneumonitis, etc.

Rifapentine

There is no experience with the treatment of acute rifapentine overdose at doses exceeding 1200 mg per dose.

In a pharmacokinetic study involving healthy volunteers (n = 9), single oral doses of up to 1200 mg were administered without serious adverse events. The only adverse events reported with the 1200 mg dose were heartburn (3/8), headache (2/8), and increased urinary frequency (1/8). In clinical trials, tuberculosis patients aged 20 to 74 years accidentally received continuous daily doses of 600 mg of rifapentine. Some patients received continuous daily dosing for up to 20 days without evidence of serious adverse effects. One patient experienced a transient elevation of ALT and glucose (the latter attributed to pre-existing diabetes); a second patient experienced mild pruritus. Although there is no experience with the treatment of acute rifapentine overdose, clinical experience with rifamycins suggests that gastric lavage to evacuate gastric contents (within a few hours of overdose), followed by

instillation of an activated charcoal paste into the stomach, may help to adsorb any remaining drug from the gastrointestinal tract.

Rifapentine and 25-desacetyl rifapentine bind to plasma proteins at rates of 97.7% and 93.2%, respectively. Rifapentine and related compounds excreted in the urine represent only 17% of the administered dose; therefore, hemodialysis or forced diuresis are not expected to increase the systemic elimination of unchanged rifapentine from the body of a patient with a Priftin overdose.

5.2.7 Product Liability

The study medication will be managed and controlled according to Standard Operating Procedures for distribution, storage, and responsibility. The study center will be responsible for maintaining records of all study products received, dispensed, returned, and destroyed (if unused or returned).

The rifapentine will be donated by the Ministry of Health. They will provide rifapentine (RPT, Priftin®) in boxes containing 3 blister packs of 8 tablets each (24 tablets of 150mg each).

Isoniazid will be obtained locally. Isoniazid is available in 100mg or 300mg tablets. The study will use tablets as they become available.

5.2.8 Concomitant medications and procedures

The principal investigator will review concomitant medications at screening, enrollment visits, and all clinical visits, tracking start and end dates and dose changes in the clinical assessment source document. Clinical research team members will track the list in a dedicated concomitant medication CRF.

Whenever a concomitant medication or study product is initiated, or the dose is changed, investigators should review the most recent package inserts for the concomitant medications and study products to verify the most current information on drug interactions, contraindications, and precautions.

All medications and procedures are permitted according to the inclusion and exclusion criteria.

A list of prohibited medications will be included; patients requiring prohibited medications will not be included. If the use of prohibited medications becomes necessary during treatment, the study medications will be discontinued, and the participant will be considered excluded from that point onward.

Rifapentine is a strong inducer of metabolizing enzymes, therefore there is a risk of drug interactions.

All concomitant medications, dosages, frequencies, and routes of administration will be recorded and entered into the data. Study researchers will consult the list of prohibited and precautionary medications (Appendix 1) before initiating any new medication to minimize the potential for drug interactions.

The first dose of rifapentine/isoniazid will be administered in the physician's office at the research site by a licensed healthcare professional. The office will be equipped with standard emergency resuscitation equipment, IV fluids, medications, and oxygen.

5.2.9 Discontinuation of the intervention

Participants who meet one or more of the following criteria will be discontinued from the study treatment:

- Any adverse clinical event, laboratory abnormality, intercurrent illness, or other medical condition or situation occurs such that continued administration of the study treatment is not in the participant's best interest; and
- Participant's request for premature discontinuation of study treatment.

Participants who discontinue treatment in the study will continue to receive follow-up visits as described in the assessment schedule.

Participants who withdraw their consent will not be included in the study.

The study investigator may discontinue a participant's treatment in the event of a serious or severe adverse event, or at any time if the investigator believes that discontinuation is in the participant's best interest.

For the purposes of the research, these participants should continue to be followed up in the study to determine the outcome according to the study's SOP, unless the participant withdraws their consent. If necessary, the participant should be referred to appropriate medical care facilities when the situation cannot reasonably be resolved by the study team.

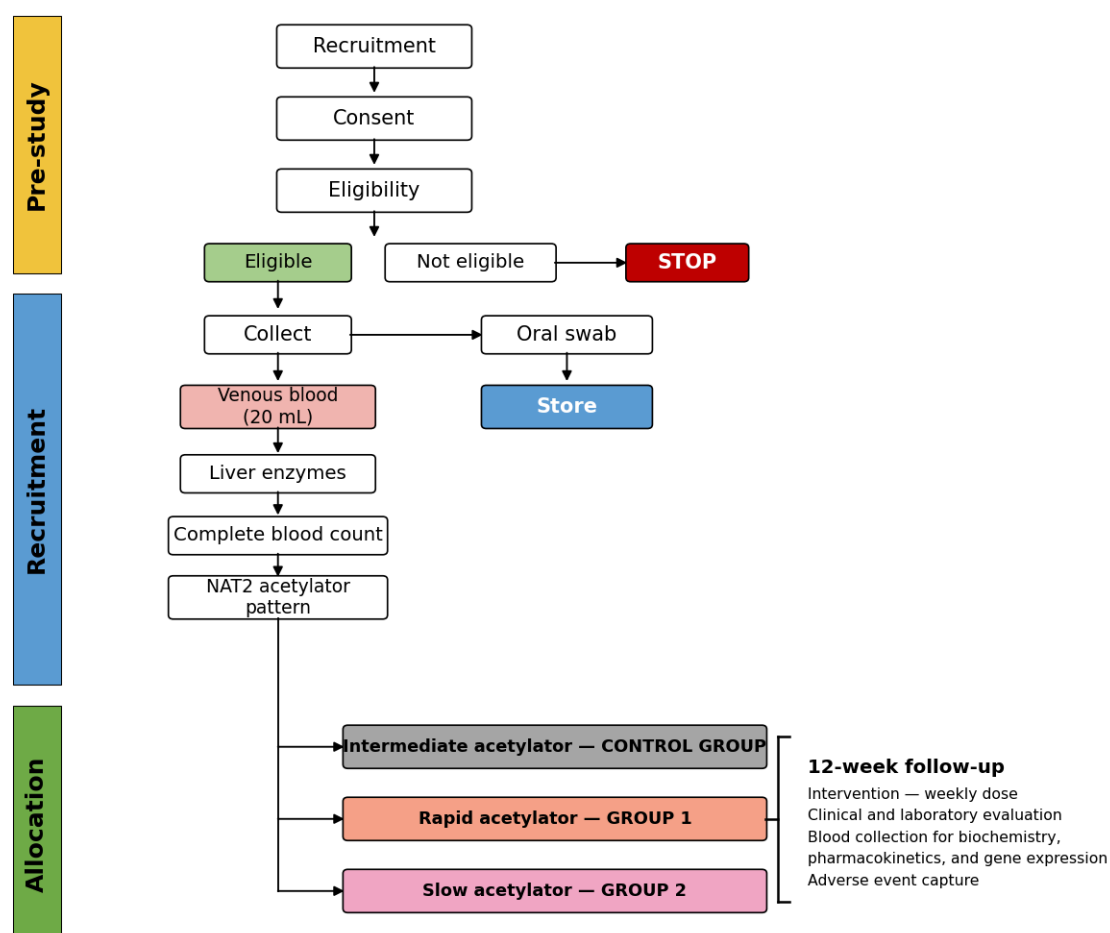
A participant may request premature discontinuation of study treatment. An early post-termination visit should be conducted in 14 days to assess any late-onset adverse events.

6. RANDOMIZATION AND BLINDING

No randomization or blinding procedures will be performed; only allocation to one of the groups will be used, according to the results of the *NAT2* acetylator pattern tests.

7. STUDY VISITS AND PROCEDURES

7.1 Flowchart



7.2 Participant activity schedule

	STUDY PERIOD													
	Pre-study	Inclusion and allocation	Follow-up											
	S ₋₁	S ₀	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	S ₁₁	S ₁₂
RECRUITMENT														
Consent														
Eligibility														
TB screening														
ALLOCATION														
INTERVENTIONS														
Rifapentine, B6														
Modified INH Dose for Slow/Fast Acetylators														

Standard dose of INH														
EVALUATIONS														
PAXgene collection														
Questionnaire														
Chest X-ray														
Blood collection (NAT2)														
Sputum collection														
Liver Function Tests*														
Complete blood count														
Pharmacokinetics														
Swab collection														
Capture of requested EA														
Clinical assessment														

Figure 4 - Study participant timeline.

*Liver function tests can be collected at any time, depending on the assessment of the study physician. The collection points shown on the schedule are the scheduled collections.

s = week (for example, s1 = first week).

INH = isoniazid

TB = tuberculosis

NAT2 = N-acetyltransferase 2

EA = adverse event

B6 = vitamin B6, pyridoxine

7.3 Description of procedures

The procedures related to recruitment, consent, screening, and allocation are described in sections 4 and 5 of this protocol.

Questionnaires

Pre-study

- Demographic data
- Health symptoms/complaints and adverse events at the time of the recruitment interview.
- Comorbidities: allergic diseases or allergic reactions, any systemic disease requiring continuous use of medication.
- Use of medications
- Risk factors: smoking, alcohol use, illicit drugs, hypersensitivity reaction.
- History of LTBI/TB: consistently positive tuberculin skin test, use of isoniazid or previous TB treatment.

Capture of solicited adverse events (weeks 1 to 4, 8 and 12)

- Questionnaire for collecting adverse events (expected and unexpected)

Blood samples

Prior to allocation (pre-study), 20 ml of venous whole blood will be collected for complete blood count, biochemistry (AST and ALT levels), and molecular analysis to assess the presence of polymorphisms and expression levels of the NAT2, AADAC, CYP2E1, and SLCO1B1 genes.

Pre-study blood samples will be analyzed before allocation. We will provide these results to participants in writing.

In weeks 2, Three and twelve (± 3 days) after allocation, peripheral venous blood samples (up to 45 mL) will be collected from participants for liver enzyme (AST and ALT) assays, pharmacokinetics, and gene expression analysis. Samples for pharmacokinetic testing will be collected at weeks 2 and 3 after the second and third doses. One EDTA or citrate tube will be collected at 1 hour, 2 hours, 8 hours, and 24 hours after dose administration. For gene expression analysis, one Paxgene tube will be collected at weeks 2 and 3 at the time of pharmacokinetic testing (8 hours).

After processing, the samples intended for pharmacokinetic testing will be stored at -80°C in the Infectious Diseases Laboratory of UFMS (LAB DIP/UFMS), including those from participants recruited at the Manaus/AM Center, until they are sent to Stanford University, where the tests will be performed. After the tests are completed, all samples will be destroyed.

Additional blood samples may be collected at the discretion of the study safety physician in the event of an adverse event, and other tests, in addition to those highlighted in this section, may also be performed at the physician's discretion.

Swab samples

Samples will be collected during the pre-study visit using Hydroflock® (Longhorn) swabs and stored in 500 μl of Primestore® MTM. Samples will be stored at -20°C in the LAB DIP/UFMS, including those from participants recruited at the Manaus/AM Center, until the NAT2 tests are performed using the new GeneXpert® platform cartridge.

Oral swab samples will be used to evaluate the accuracy of the cartridge-based assay and algorithm for identifying *NAT2 genotypes against a NAT2 amplicon sequencing reference standard*; therefore, these tests will not be performed in real time nor used to guide treatment. These results will be delivered to the participant only at the end of the study.

7.4 Notes on specific study visits

7.4.1 Unscheduled visit

If participants have any concerns related to adverse events, they can call the study team for advice and, if necessary, will be evaluated by a doctor.

7.5 Discontinuation procedures, participant withdrawal, and losses to follow-up

7.5.1 Discontinuation of treatment – the participant remains in the study for follow-up.

The experimental intervention is a twelve-dose therapy. If a participant changes their mind between doses, deciding they do not wish to receive further doses of the medication (but agrees to continue in the study during the follow-up period), they will be included in the analysis as intention-to-treat.

7.5.2 Withdrawal of consent – the participant withdraws from all participation in the study.

Participants may withdraw from the study at any time upon request. Withdrawal from the study will not affect access to standard treatment for LTBI.

For the safety of all participants completing the study, reasonable efforts should be made to perform protocol-specified safety assessments to capture new safety events and to evaluate existing unresolved safety events after withdrawal.

A Case Report Form (CRF) will be used to capture the date the participant withdraws their consent.

7.5.3 Loss to follow-up

Those who, for any other reason, leave the study before completing the 12 weeks of follow-up will be considered lost to study.

7.5.4 Replacements

Participants who have been allocated cannot be replaced.

7.5.5 Conclusion of the study

A participant is considered to have completed the study if they have completed all phases, including the last visit or the last scheduled procedure shown on the activity schedule.

The end of the study is defined as the completion of the last visit or procedure shown in the study activity schedule. At this stage, the Principal Investigator will ensure that the Ethics Committee, as well as all regulatory and funding agencies, have been notified.

This study may be temporarily suspended or terminated prematurely if there is sufficient reasonable cause. If the study is terminated or suspended prematurely, the Principal Investigator will immediately inform the study participants, the Ethics Committee, the funding agency (where applicable), and the regulatory agencies, providing the reason(s) for the termination or suspension.

Circumstances that may justify termination or suspension include, but are not limited to:

- Determining an unexpected, significant, or unacceptable risk for participants.
- Insufficient compliance with protocol requirements.
- Data that is not sufficiently complete and/or assessable.

- Demonstration of effectiveness that would justify the interruption.
- Determination that the primary outcome has been met.
- Determination of futility

In case of concerns regarding security, protocol compliance, or data quality, the study may be resumed as soon as the issues have been addressed satisfactorily by the sponsor, the Ethics Committee, the funding agency, and/or regulatory agencies.

7.5.6 Continuation of therapy

Since the treatment consists of 12 doses, no additional therapy is planned.

8. SECURITY MONITORING AND REPORTING

8.1 Definitions

Adverse Event (AE)

An adverse event (AE) is any undesirable medical occurrence in a participant who received an investigational product and does not necessarily have a causal relationship with the study treatment. For this study, only certain adverse events are recorded, specifically serious adverse events, as defined below, and non-serious adverse events of interest specified in section 8.2.

Adverse reaction (AR)

Any unintended adverse response to an investigational drug related to any dose administered.

Serious Adverse Event (SAE) / Serious Adverse Reaction (SAR)

An EAG is any medical occurrence that:

- results in death; or
- presents a risk to life; or
- Requires hospitalization or extension of existing hospitalization;
 - Hospitalization will only be considered an EAG (Emergency Adverse Event) if it requires an overnight stay. Outpatient care will not be considered an EAG.
- Results in persistent or significant disability/incapacity; or
- It is a congenital anomaly / birth defect.

Note: Life-threatening risk refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that, hypothetically, could have caused death if it had been more severe.

Medical and scientific judgment must be exercised when deciding whether an adverse event should be classified as serious in other situations. Significant medical events that are not immediately fatal or that do not result in death or hospitalization, but that may compromise the participant or require intervention to prevent one of the other outcomes listed in this definition from also being considered serious.

For this study, all AEGs will be collected from allocation until one month after discontinuation of study medications.

Unexpected Adverse Event

An event not described as an adverse reaction in the investigational medicinal product leaflet or in the package insert.

8.2 Gathering and obtaining information about adverse events

8.2.1 EAG Capture

Adverse events will be collected throughout the duration of the study. Participants will have weekly access to a member of the research team to report any suspected adverse event and will be followed up until resolution or stabilization.

In the questionnaires for weeks 1 to 4, 8, and 12, we will ask participants if they have any complaints about the use of the medication. They will also be asked if they have been hospitalized, suffered any accidents, or used any new medication. In addition, adverse events (AEs) will be documented based on physical examination findings, clinically significant laboratory results, or other documents relevant to the participant's safety.

For this study, all EAGs will be collected from allocation up to 12 weeks post-allocation.

8.2.2 Non-serious EA capture

Non-serious AE of interest is captured:

- The day of administration or delivery of the investigational product, or clinical evaluation, as recorded by the study team.
- Through weekly contacts
- Through spontaneous contact between the participant and the team.

8.3 EA Documentation

For the purposes of this study, the investigator or delegate is responsible for recording all adverse events, regardless of their relationship to the study medications, with the following exceptions:

- Conditions that are pre-existing at the time of recruitment and do not deteriorate will not be considered adverse events.
- Abnormal laboratory values will not be considered adverse events unless they are deemed clinically significant by the investigator and documented as such.
Each AE's documentation at the CRF will include:
 - Date of occurrence of the adverse event.
 - Participant number or code.
 - Event number or code (EAG).
 - Classification of the adverse event (index or subsequent).
 - Discrimination of the occurrence (examples: febrile neutropenia, pneumonia, among others).

- Type of AEAG ("death", "life-threatening", "need for hospitalization", "prolonged hospitalization", "significant damage", "permanent damage", "congenital anomaly", "at the researcher's discretion", "other").
- Causality with the product under investigation ("not related", "possibly related", "probably related", "definitely related").
- Detailed description of the assistance provided to the participant.
- Date of last update.
- Participant status at the date of the last update ("in progress", "recovered without damage", "recovered with damage", and "deceased").
- Description of the discontinuation of research participants.

Changes in the severity of an adverse event (AE) will be reported. AEs characterized as intermittent will be documented for each episode. All AEs will be followed up until adequate resolution, whenever possible.

8.4 Assessing the causal relationship of a participant's AE

All non-serious adverse events (AEs) of interest and generalized adverse events (GAEs) must have their relationship to the study intervention analyzed by the study physician (or a delegate) who will assess the AE based on the temporal relationship and their clinical judgment. The degree of certainty about causality will be classified using the categories below.

The relationship between the event and the study intervention will be evaluated as follows:

Code	Causal relationship	Description
1	Unrelated	EA is clearly not related to the intervention.
2	Unlikely	EA is dubiously related to the intervention.
3	Possible	EA may be related to the intervention.
4	Likely	The EA is probably related to the intervention.
5	Definitive	EA is clearly related to intervention.

Figure 5 - Causal relationship between adverse event and investigational product.

8.5 Assessing the severity of a participant's adverse event

The study physician (or a delegate) will be responsible for assessing the severity of an adverse event (AE). The determination of the severity of all AEs must be made by the researcher based on medical judgment and severity categories from grade 1 to 5, as defined below, with the following exception:

- Elective surgery planned at the time of recruitment.
- Hospitalization due to disease progression will not be considered an adverse event for the purposes of this study.

Degree	Gravity	Description
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Grade 1	Light	Asymptomatic or with mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate	Moderate; minimal, local or non-invasive intervention indicated; limitation of age-appropriate activities related to daily living.
Grade 3	Serious	Serious or clinically significant, but not immediately life-threatening; hospitalization or prolonged hospitalization is indicated; disabling; limitation of self-care in daily activities.
Grade 4	Threat to life	Life-threatening consequences; urgent intervention indicated.
Grade 5	Fatal	Death related to AE

Figure 6 - Severity of adverse events.

8.6 Security Event Report

The principal investigator must comply with the applicable regulatory requirements regarding AEG notification, as defined by ANVISA in RDC No. 9, of February 20, 2015, and by the CEP/CONEP system, as defined in Circular Letter No. 13/2020.

8.6.1 Report to ANVISA

The principal investigator is responsible for reporting any unexpected adverse events (AEOs) to ANVISA within 48 hours, provided that their causality is possible, probable, or related to the product under investigation.

The report must be submitted digitally via: http://formsus.datasus.gov.br/site/formulario.php?id_aplicacao=3961. Additional information regarding the monitoring of the aforementioned adverse events must be included in the form within 8 calendar days from the reporting date.

8.6.2 Report to the Ethics Committee/CONEP

The CEP (Brazilian Postal Code) must be notified of all AEs (expected or not) through the Plataforma Brasil (Notification) after the event has ended or as soon as possible in case of death. The legislation does not establish a deadline. For this protocol, we will use:

- A. 10 days in case of death, 7 days in case of a causal link with the product under investigation.
- B. 6 months in other cases.

The report should contain:

1. Date of occurrence of the adverse event.
2. Participant number or code.
3. Event number or code (EAG).
4. Classification of the adverse event (index or subsequent).
5. Discrimination of the occurrence (examples: febrile neutropenia, pneumonia, among others).
6. Type of SAE ("death", "life-threatening", "need for hospitalization", "prolonged hospitalization", "significant damage", "permanent damage", "congenital anomaly", "at the researcher's discretion", "other");

7. Causality with the product being investigated or research procedure ("unrelated", "possibly related", "probably related", "definitely related").
8. Detailed description of the assistance provided to the participant.
9. Date of last update.
10. Participant status at the date of the last update ("in progress", "recovered without damage", "recovered with damage", and "deceased").
11. Description of the discontinuation of research participants.

If the report refers to more than one event or participant, it should be written in chronological order. As other adverse events are optional reports, we will not report them, but we will keep a complete record of them at the research center.

9. CLINICAL MANAGEMENT

9.1 Clinical management of adverse events

This study will classify toxicity and adverse events according to the Division of AIDS (DAIDS) Table for Classification of Adverse Events in Adults and Pediatrics, corrected version 2.1 (dated July 2017), available at: <https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf>

9.2 Toxicity Management

9.2.1 Gastrointestinal

For nausea/vomiting and/ or diarrhea \geq grade 3, or for grade 2 toxicity if symptoms were not present at the previous consultation, all study medications should be discontinued until symptoms have disappeared. Reintroduce study medications cautiously. Antiemetic and antidiarrheal medications may be used at the investigator 's discretion. Laboratory tests should be ordered as clinically indicated.

Every effort should be made to reintroduce the study medications after symptoms improve or remit.

9.2.2 Cutaneous

For grade 2 or 3 cutaneous events occurring after the first dose of HP, all study medications should be discontinued until the toxicity resolves. Study medications should be reintroduced with caution.

A grade 4 skin or mucocutaneous rash is a serious toxicity and all study medications should be permanently discontinued.

The study medications may be discontinued at the researcher 's discretion . Similarly, temporarily interrupted study medications may be reintroduced at the center's researcher 's discretion .

9.2.3 Rifampicin hypersensitivity syndrome (RHS)

The signs and symptoms of RHS may include hypotension, urticaria, angioedema, acute bronchospasm, conjunctivitis, thrombocytopenia, neutropenia, or flu-like syndrome (weakness, fatigue, muscle pain, nausea, vomiting, headache, fever, chills, aches, rash, itching, sweating, dizziness, shortness of breath, chest pain, cough, syncope, palpitations). Anaphylaxis has been reported.

This syndrome has been associated with intermittent administration of rifampicin and was reported in a rifapentine and isoniazid regimen study (49). In the study, 26 flu-like and systemic drug reactions were reported by 3.5% of 3HP recipients compared to 0.4% of 9h recipients. More recently, among healthy volunteers who received HP and dolutegravir, 2 of 4 volunteers experienced rifamycin hypersensitivity syndrome (fever, hypotension, elevated transaminases), interrupting the study (50). No hypersensitivity reactions were found with daily HP in the BRIEF TB study. We will carefully monitor participants for signs and symptoms of hypersensitivity reactions.

For participants who develop signs or symptoms of RHS:

- Continue the study medication regimen.
- Assess RHS through clinical evaluation and laboratory tests, including a comprehensive metabolic panel, complete blood count with differential, and other tests necessary to rule out likely alternative diagnoses. (For example, if symptoms suggest influenza, a nasopharyngeal aspirate for viral testing may be sent).
- If a Grade 3 or higher adverse event meets the RHS definition (and the symptoms are not clearly attributable to an alternative diagnosis), permanently discontinue the study drug regimen and notify ANVISA.

9.2.4 Drug-associated fever

If \geq grade 3, all study medications should be discontinued until the participant is afebrile. Study medications should be reintroduced cautiously. If fever recurs upon reintroduction, study medications should be permanently discontinued.

9.2.5. Liver toxicity

All medications in the study will be permanently discontinued if any of the following liver chemistry criteria are met:

- ALT $\geq 3 \times$ ULN and bilirubin $\geq 2 \times$ ULN ($>35\%$ direct bilirubin; bilirubin fractionation required)

NOTE: Serum bilirubin fractionation should be performed if the test is available. If the test is not available, record the presence of detectable urinary bilirubin on the dipstick, indicating direct bilirubin elevations and suggesting liver injury. If the test is not available and an individual meets the criterion of total bilirubin $\geq 2 \times$ ULN, the event will meet the criteria for hepatic arrest;

- ALT $\geq 3 \times$ ULN with symptoms or worsening of acute hepatitis or hypersensitivity, such as fatigue, nausea, vomiting, pain or tenderness in the right upper quadrant, fever, rash, or eosinophilia, or
- ALT $\geq 5 \times$ ULN; regardless of symptoms

In the case of liver toxicity, appropriate assessments for alternative causes may be carried out (viral hepatitis, alcohol exposure, etc.).

9.2.6 Peripheral Neuropathy

Participants with baseline peripheral neuropathy <grade 2 as defined by DAIDS classification table: Patients with AIDS may be included in the study but should be carefully monitored for neuropathy progression.

For grade 1 or 2, continue the study medications and monitor the participant more frequently for progression of peripheral neuropathy. Consider initiating or increasing the dose of vitamin B6.

For grade 3 or 4, discontinue all study medication until toxicity resolves to grade <2. If peripheral neuropathy does not remit despite discontinuation of study medications, study medications may be reintroduced at the discretion of the center investigator.

9.2.7 Other toxicities

For toxicities that do not fall into one of the scenarios above, the management will be as follows:

Levels 1 and 2: participants may continue HP at the researcher 's discretion with careful monitoring.

Grades 3 and 4: study medications should be discontinued until symptoms resolve (or until ≤grade 2, or within normal limits). Study medications may be permanently discontinued at the investigator 's discretion.

If the study medications are discontinued for any reason, participants will have 16 weeks from enrollment to complete at least 11 doses of HP. If the treatment interruption lasts more than 4 weeks, the participant will need to be re-examined for TB before restarting HP.

9.3 Criteria for discontinuing study medications

9.3.1 Criteria for permanent discontinuation

- Discontinuation for an individual participant
- Drug-related toxicity requiring discontinuation of treatment.
- Diagnosed with active tuberculosis
- Conclusion of the study events
- Participant's request to terminate treatment.
- Clinical reasons deemed fatal by the physician, even if not addressed in the toxicity section of the protocol.
- Acquires HIV infection during the study.

9.3.2 Premature discontinuation of the study

- Participant's request to withdraw
- Request from the primary care provider if he/she feels the study is no longer of interest to the participant.
- A participant deemed by the researcher to be at significant risk of failing to comply with the protocol guidelines, causing harm to themselves or seriously compromising the validity of the study results.

All participants who discontinue therapy will be followed up according to their assigned schedule until the end of the study.

10. DATA AND INFORMATION MANAGEMENT

10.1 Overview

The principal investigator is responsible for storing essential documents for data management and maintaining a specific record of the Essential Documents related to data management.

The principal investigator is responsible for maintaining adequate and accurate files of any relevant source documents, including observations or other data related to participants. Source documents must be attributable, legible (including any changes or corrections), contemporaneous, original, accurate, complete, consistent, durable, and available. Changes to source documents (printed and electronic) must be traceable, must not obscure the original entry, and must be explained when necessary. The local principal investigator will also maintain accurate case report forms (CRFs) (i.e., data collection forms), when applicable, and will be responsible for ensuring that data collected and reported are accurate, legible, complete, timely entered, and durable. To maintain data integrity, any changes to the data (printed and electronic copy) must be traceable, must not obscure the original entry, and must be explained when necessary.

Anyone delegated to collect data, perform data entry, or sign off on data integrity will be logged in the delegation log and trained to perform these tasks and functions related to the study.

10.2 Data Management

10.2.1 Data generation (source documents)

In this study, the following types of data will be collected:

- Personal identification information (names, dates of birth, contact details)
- Confidential information, including health data (medical history, participant eligibility, adverse reactions, and other notes, as appropriate).
- unidentified data from laboratory tests

10.2.2 Source Document Plan

The source documents for this study will include questionnaires completed by the participant or researcher; data recorded from automated instruments, laboratory reports, and signed information and consent forms.

10.2.3 Data collection, use, storage and access methods

Data collection methods

The data for this study will be collected and entered using the REDCap electronic database. REDCap is a secure web-based application for building and managing online surveys and databases. REDCap can be accessed at <https://redcap.matogrossodosul.fiocruz.br>

Use of data

The data will be used for the analyses specified in the protocol and the Statistical Analysis Plan.

After the study is completed and analyzed, the data will be retained for 15 years, the mandatory period for archiving clinical research data in Brazil.

Storage and access

The printed data will be stored at UFMS (Federal University of Mato Grosso do Sul) when originating from participants recruited in Campo Grande/MS, and at FMT/HVD (Faculty of Medicine/Hospital Heitor Vieira Dourado) when originating from participants recruited in Manaus/AM, in locked rooms in a secure location accessible only to the research team.

Electronic data will be securely stored in the FIOCRUZ-MS REDCap database system and in files stored on FIOCRUZ network file servers, with nightly backups. Files containing private or confidential data will be stored only in locations accessible only to appropriate designated members of the research team.

REDCap is hosted on the FIOCRUZ infrastructure and is subject to the same security and backup regime as other systems (e.g., network file servers). Data backup is performed nightly on a local backup server, with a monthly tape backup and external storage. REDCap maintains an audit trail of data creation/update/deletion events that are accessible to project users with permission to view them. Access to REDCap will be provided by a FIOCRUZ user account or (for external collaborators) by a REDCap user account created by the FIOCRUZ system administrator. The permissions granted to each user within each REDCap project will be controlled and will be the responsibility of the trial team, with this task delegated by the Principal Investigator. REDCap has a feature that makes adding and removing users and managing user permissions simple. All data transmissions between users and the REDCap server are encrypted. The instructions for data entry in REDCap must be read and the training record signed before personnel begin data entry in REDCap.

Authorized representatives from the sponsoring institution, as well as representatives from the Ethics Committee and regulatory agencies, may inspect all documents and records that must be maintained by the researcher for the participants in this study. The research center will allow access to such records.

10.2.4 Data Confidentiality

The confidentiality of participants is strictly maintained in trust by the principal investigator, the participating researchers, the research team, and UFMS. This confidentiality extends to cover tests on biological samples, in addition to clinical information related to the participants.

To preserve confidentiality and reduce the risk of identification during the collection, analysis, and storage of data and information, the following will be done:

- (1) The number of private/confidential variables collected for each individual was minimized. The data collected will be limited to what is necessary to meet the primary and secondary objectives.

- (2) The participant's data and samples will be identified through the use of a unique number assigned to the study participant ("re-identifiable").

The principal investigator is responsible for storing a master file of identifiable data with the participant ID in REDCap; access is managed by restricting user permissions to research team members and authorized individuals.

- (3) Separation of functions responsible for managing identifiers and those responsible for content analysis. The data will be analyzed by members of the research team, who will receive anonymized data identified only by the participant's ID in the study.

10.2.5 Quality assurance

A data dictionary for REDCap will be used to minimize data entry errors. Data quality control checks (e.g., checking for invalid characters, invalid dates, data that is not consistent with data in other data fields) and data cleaning will be performed regularly by trained members of the research team. Any discrepancies will be reported to the principal investigator and addressed in a timely manner.

Quality control checks will be carried out regularly by the data management team, who will highlight all questions to the principal investigators and physicians of the study.

10.2.6 Archiving - data and document retention

The data will be securely stored on the FIOCRUZ server (restricted access) and/or locked in a secure room at UFMS (for physical data) for 5 years after the study is completed, in accordance with CONEP requirements.

Julio Croda will be the custodian during the archiving period, and members of the research team will have access to the stored data. The records must not be destroyed without the written consent of the principal investigator.

10.2.7 Data Sharing

The research resources generated by this funding will be distributed free of charge, as available, to qualified academic researchers for non-commercial research. The data generated from this project will be disseminated through presentations at scientific meetings and publications. All participating researchers and their associated institutions have committed to publishing, without a defined time limit, all relevant scientific information they obtain during this project. The principal investigators and the data manager will coordinate the sharing of information between the collaborators of this protocol and the Health Departments of Campo Grande/MS and Manaus/AM, since tuberculosis is a notifiable disease in Brazil.

Unpublished information will be made available to interested parties upon written request or by email to the principal investigators. Sharing unpublished information may be subject to confidentiality issues related to collaboration with scientists at academic and other public sector institutions in the U.S. and Brazil, and would need to be discussed with the appropriate institutions before any request is granted.

All institutions and researchers will adhere to the NIH Grant Policy on Sharing Unique Research Resources, including the "Sharing Biomedical Research Resources: Principles and Guidelines for NIH Grant and Contract Recipients," issued in December 1999 [https://grants.nih.gov/grants/intell-property_64FR72090.pdf]. Specifically, material transfers would be made without more restrictive terms than in the Simple Charter or MTA and without scope for requirements. Should any intellectual property arise that requires a patent, we guarantee that the technology will remain widely available to the research community as per the NIH Principles and Guidelines document.

Anonymous data generated from *NAT2 sequencing* will be deposited into GenBank within six months of completion of final sequencing data or acceptance of first publication, whichever comes first, without restrictions on publication or other dissemination. In addition, INH PK data will be submitted. We anticipate that sequencing will be completed during year 2 of the proposed research.

10.2.8 Long-term custody (after the end of the archiving period)

Julio Croda will be the long-term custodian of the archive. If the principal investigator becomes unable to perform this task, all custodian responsibilities will fall to the sponsor.

10.2.9 Data retrieval and linking

This study aims to obtain some research data from existing service data sources (SINAN, BRACE study). In some cases, participant consent may allow datasets to be retrieved without the need for connection keys, such as in medical record consultations in cases of care provided at the hospital unit.

10.2.10 Sample management: additional considerations on data management

Data and information for biological samples will be managed as described above, with the following additional considerations.

Samples will be processed, stored, and data recorded at LAB DIP/UFMS, including samples from participants recruited in Manaus/AM. Samples will be identified by means of barcoded tubes or the participant's unique identification in the study. No identifying information will be stored on sample labels. Samples will be securely stored in temperature-controlled freezers and liquid nitrogen tanks, as appropriate for the sample type. Access to samples will be restricted to the study team. Samples will be used for the analyses specified in the protocol. For tests requiring equipment or technical expertise not available in Campo Grande/MS, selected samples may be sent to collaborating laboratories outside Campo Grande (interstate and/or international) for additional testing. Sample shipment for testing will be carried out by a team accredited by the International Air Transport Association (IATA) with temperature control (e.g., ice pack, dry ice) as appropriate for the sample type.

The samples will be retained for the duration of the study period, and Julio Croda will be the custodian of the biological samples. All samples not used immediately for the laboratory evaluations described in the previous sections will be cryopreserved as described above.

11. STUDY SUPERVISION

The principal investigator is responsible for supervising any individual or party to whom they have delegated study tasks. They must provide ongoing supervision and documentation of their supervision. To meet this Good Clinical Practice requirement, a group will be responsible for the day-to-day management of the study and will include, at a minimum, the PI, study coordinator, and research nurse. The group will review all aspects of the study's conduct and progress, ensuring there is a forum to identify and address issues.

11.1 Independent Data and Safety Monitoring Committee (DSMB)

As this is a Phase I pharmacokinetic study involving fewer than 100 participants and without blinding, we will not convene an independent data and safety monitoring board. We are delivering a high dose of isoniazid to a group of participants (rapid acetylators; targeted enrollment = 22) administered as a single dose. We will follow up with the participant 24 hours after dose administration, check liver function tests and contact them by phone 72 hours later, and see them again after 7 days to repeat liver function tests. We will monitor adverse events continuously, including conducting a formal review of all adverse events after the first 5, 10, and 15 rapid acetylators have received this dose to assess any safety signals. We will report all adverse events at these intervals to the IRB and ask for their recommendation on whether to continue. Furthermore, if any serious adverse event occurs at any time, we will stop recruitment and consult the Ethics Committee to determine whether we should proceed with the study.

11.2 Quality control and assurance

Both the Sponsor and the researchers principals have responsibilities regarding quality management.

Jason Andrews, Julio Croda and Marcelo dos Santos will be jointly responsible for overseeing all aspects of the study, including the protection and security of all study data. Dr. Croda and Dr. Marcelo will supervise. The study team (nurses, laboratory technicians, and radiology technicians) will be at their respective centers. Results from approved tests (QuantiFERON, X-ray, sputum Xpert and culture, when performed, and clinical evaluation) will be reported immediately. Any screened or enrolled individuals with active TB will be referred for free treatment.

In the event of a non-conformity that significantly affects the protection of the human participant or the reliability of the results, the principal investigator will conduct a root cause analysis and develop a corrective and preventive action plan.

12. STATISTICAL METHODS

***NAT2* SNP molecular rapid assay**

The primary outcome for the validation assay is the accuracy of the cartridge-based *NAT2* assay in detecting the correct polymorphism patterns compared to the sequencing reference standard. The *NAT2* assay provides a read indicating whether 0, 1, or 2 SNPs are present at each of the 5 positions in the *NAT2* gene. These polymorphisms are 191G>A, 282C>T, 341T>C, 590G>A, and 857G>A. The primary metric will be the accuracy in correctly identifying the number of SNPs present at all positions for each individual. For example, if an

individual is alternating homozygous at 282, heterozygous at 341, and wild-type (no polymorphisms) for 191, 590, and 857, the accurate read for that individual for polymorphisms at the 5 positions would be 0, 2, 1, 0, 0. Reporting any other pattern, even if correct in 4 out of 5 positions, would be considered inaccurate. As a secondary metric, we will characterize the accuracy in the SNP report by position for each of the 5 positions.

A key secondary outcome will be the accuracy in predicting the acetylator genotype (slow, intermediate, fast) for each individual. This step will involve evaluating the SNP pattern, using the algorithm to predict the acetylator type of non-phased SNPs and comparing the prediction to the sequencing reference pattern. This differs from the primary outcome in two ways.

First, accurate SNP detection does not guarantee accurate haplotype resolution; for example, an individual with two SNPs on one chromosome and one SNP on each of the two chromosomes will appear identical to non-phased data, the former being an intermediate acetylator while the latter would be a slow acetylator. The haplotype prediction algorithm uses globally representative data to predict phased haplotypes from non-phased data.

Secondly, inaccuracies in SNP detection can still result in the same acetylator prediction. For example, if an individual has 4 SNPs and only 3 are detected, they may still be correctly classified as a slow acetylator.

For each of these results, we will perform cartridge-based assay analyses using whole blood, digital capillary blood, dried blood smears, and oral swabs, reporting: 1) the proportion of assays that provide a valid (non-indeterminate) result; and 2) the accuracy of each sample type compared to the *NAT2 sequencing reference standard*.

12.1.1 Analytical approach

Precision in describing the polymorphism pattern, SNP detection by site, and acetylator type are descriptive statistics and will be reported as proportions. We will report exact 95% confidence intervals around each estimate. We will test the hypothesis that precision exceeds 95% using a one-sample exact binomial test. All analyses will be performed using R (R Foundation for Statistical Computing).

12.1.2 Subgroup analyses

We will perform subgroup analyses in which we report: 1) diagnostic accuracy among slow acetylators; and 2) diagnostic accuracy among fast acetylators.

12.1.3 Sample size

Based on our preliminary data using whole blood (100% accuracy; n=23), we expect the accuracy in correctly identifying polymorphism patterns and predicting the acetylator genotype from whole blood to be >95%. To test this hypothesis with 90% power, assuming a true accuracy of 99%, 300 samples will be needed. **participants** (80% power would require 99 participants) (see Figure 4). We predict comparable accuracy (~99% and >95%) for predicting the acetylator genotype and accuracy at each SNP position, therefore the power for each of these hypothesis tests will be the same.

The proportion of samples that give a valid and accurate result from other sample types (finger prick capillary blood, oral swabs) may be lower due to varying concentrations

and DNA. We plan to test 60 samples of each type. Assuming that 5% of the tests provide an invalid (or inaccurate) result, the accuracy around this estimate would be $\pm 5.5\%$.

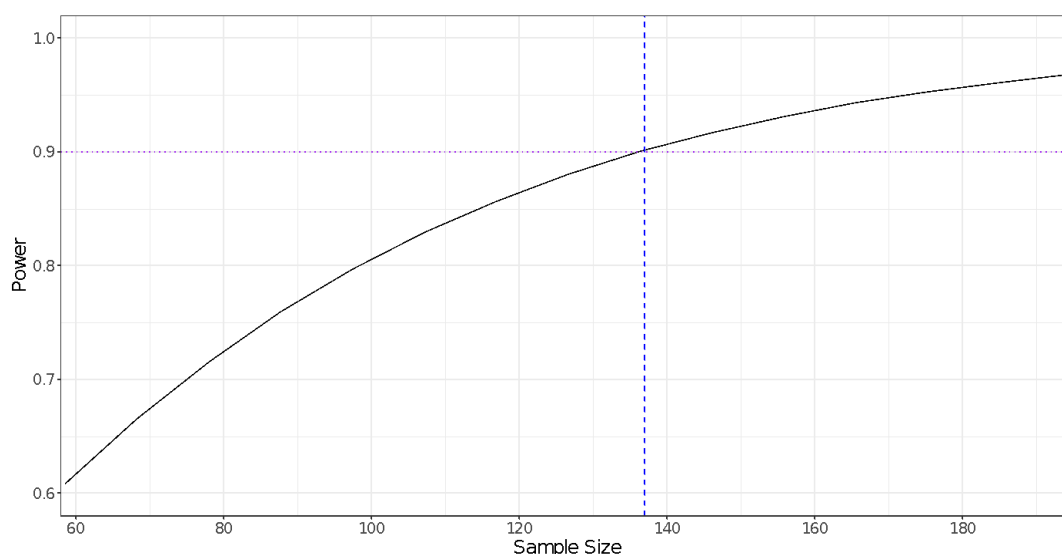


Figure 7 - Power for a one-tailed test with precision > 95% assuming a true precision of 99%, by sample size.

12.2 Pharmacokinetics of isoniazid guided by pharmacogenomics

12.2.1 Hypotheses

Primary

1. Isoniazid at 25 mg/kg among the fast *NAT2 acetylators* achieves an AUC_{0-24} equivalent compared to isoniazid at 15 mg/kg among the intermediate acetylators.
2. Isoniazid at 5 mg/kg among the slow *NAT2 acetylators* achieves AUC_{0-24} equivalence compared to isoniazid at 15 mg/kg among the intermediate acetylators.

Secondary

1. Isoniazid at 15 mg/kg will result in lower AUC_{0-24} in rapid acetylators compared to standard acetylators.
2. Isoniazid at 15 mg/kg will result in a higher AUC_{0-24} in slow acetylators compared to standard acetylators.
3. Isoniazid at 5 mg/kg among slow *NAT2 acetylators* will reduce the proportion of individuals with $C_{24} > 0.15 \mu\text{g/ml}$ compared to isoniazid at 15 mg/kg among the same individuals.

Exploratory

1. Isoniazid at 25 mg/kg among rapid *NAT2 acetylators* will not increase the frequency of adverse events compared to isoniazid at 15 mg/kg among intermediate acetylators.

12.2.2 Analytical Approach

For the primary hypothesis test comparing AUC_{0-24} between participants receiving pharmacogenomics-guided isoniazid dosing between fast or slow acetylators with intermediate acetylators at the standard dosage, we will perform Wilcoxon rank-sum tests.

The null hypothesis in strict terms is that the probability of a randomly drawn AUC₀₋₂₄ measurement from one group being greater than a measurement from the other is equal to 0.5. Our study hypothesis here is equal to the null hypothesis, so larger p-values provide more support for our hypothesis that the modified dosing achieves equivalence between the two groups. As described below, we will power the study such that if large differences in AUC₀₋₂₄ were present, we would be able to detect them and reject the null hypothesis with this test.

For the secondary hypotheses that assess whether standard dosing between fast and slow acetylators results in differences in AUC₀₋₂₄ compared to intermediate acetylators, we will similarly compare these distributions using Wilcoxon rank-sum tests. Since we do not expect AUC₀₋₂₄ to be higher among fast acetylators (or lower among slow acetylators) under standard dosing, we will perform one-sided tests. For fast acetylators, we will test the null hypothesis that AUC₀₋₂₄ is not lower compared to intermediate acetylators, and for slow acetylators, we will test the null hypothesis that AUC₀₋₂₄ is not higher compared to intermediate acetylators.

For secondary hypotheses assessing whether AUC₀₋₂₄ differs in modified doses compared to the standard dose in the same patient between fast and slow acetylators, we will compare the distributions using a Wilcoxon signed-rank test for paired data, with the null hypothesis of no difference in AUC₀₋₂₄.

For the secondary hypotheses that assess whether the proportion of individuals with C₂₄ > 0.15 µg/ml is lower among slow acetylators after their isoniazid dose reduction compared to their standard dose, since these are paired observations with the same individual, we tested the null hypothesis that the proportion is not lower with dose reduction using an exact one-tailed McNemar test.

We will report descriptive statistics of PK parameters (AUC₀₋₂₄, C_{max}, C₂₄) by median and interquartile range, and adverse events by proportion of individuals (with exact binomial confidence intervals) experiencing any adverse events and specific adverse events. We will compare adverse events between individuals who received dose adjustment with intermediate acetylators using Fisher's exact test, but we do not expect to have sufficient power to detect differences in adverse events for this pharmacokinetic study. We will stratify all analyses by HIV status and sex to test their impact as a biological variable.

12.2.3 Sample size

For the primary hypothesis test of the equivalence of modified doses between fast and slow acetylators compared to intermediate acetylators at the standard dose, to achieve 90% power to detect a difference in the two distributions of 1 standard deviation, 20 participants would be needed in group 1, 20 participants in group 2, and 26 participants in the control group (Table 3).

Table 3 - Sample size according to the equivalence margin and statistical power for the intervention arm (reference arm size in parentheses).

Equivalence margin	Power		
	70%	80%	90%
0.75 SD	23 (30)	27 (36)	34 (45)
1.0 SD	13 (17)	15 (20)	20 (26)

1.25 SD	9 (11)	10 (13)	13 (17)
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This power calculation is supported by a parametric equivalence estimation procedure and a non-parametric equivalence estimation procedure, based on the Wilcoxon test, both reporting 90% power for this sample size and equivalence margin. We specify a larger sample size in the intermediate acetylators arm because they will be included in two comparisons (with slow acetylators and fast acetylators), and therefore greater inclusion of this arm will increase statistical efficiency. We propose allocating 10 Additional participants were included in each group to account for any attrition or loss of specimens (control group 36; group 1, 32; and group 2, 32).

For the secondary hypotheses that assess whether standard dosing between fast and slow acetylators results in differences in AUC₀₋₂₄ compared to intermediate acetylators, we will have greater power to detect minor effects, as we will perform the one-tailed Wilcoxon test. The power will be 94% with the sample size above for each of these comparisons.

For the secondary outcome comparing the proportion of slow acetylators with C24 > 0.15 µg/ml, which is lower among slow acetylators with reduced doses compared to the standard dose, assuming that 60% of slow acetylators have C24 > 0.15 µg/ml and that this proportion reduces to 25% (equivalent to the population distribution), we will have 56% power to detect this reduction. The study will not be expanded to include this parameter.

13. ETHICS AND DISCLOSURE

13.1 Research ethics approval and authorization from local agencies

This protocol and the informed consent form, and any subsequent amendments, will be reviewed and approved by the research ethics committees involving human subjects, both in Brazil (CEP/CONEP) and in the United States (IRB Stanford University), before the start of the research. The study procedures will not begin before ethical approval.

13.2 Changes to the protocol

This study will be conducted according to the current version of the protocol. Any change to the protocol or the Informed Consent Form that affects the scientific intent, the study design, participant safety, or may affect participants' willingness to continue participating in the study is considered an amendment and, therefore, will be drafted and filed as an amendment to this protocol and/or ICF. All such changes will be submitted to the Ethics Committee for approval before being implemented.

13.3 Deviations and violations of protocol

All deviations from the protocol will be recorded in the CRF and must be reported to the principal investigator or delegate, who will assess their seriousness.

Deviations deemed to significantly affect the rights of a study participant, or the reliability and robustness of the data generated in the clinical study will be reported as violations. Reports will be made in a timely manner (the principal investigator must review and submit it to the Ethics Committee along with the semi-annual report).

In cases where non-compliance significantly affects participant protection or the reliability of results, a root cause analysis will be conducted, and a corrective and preventive action plan will be developed. Where serious deviations or violations of the protocol identify protocol-related problems, the protocol will be reviewed and, where indicated, amended.

14. CONFIDENTIALITY

The confidentiality of the participant is strictly maintained by the researcher, research team, and the sponsoring institution and its agents. This confidentiality extends to cover testing on biological samples, in addition to clinical information related to the participants.

The study data and all other information generated will be kept strictly confidential. No information about the study or the data will be disclosed to unauthorized third parties without the prior written approval of the sponsoring institution. Authorized representatives of the sponsoring institution may inspect all documents and records that must be maintained by the researcher. Participating clinical trial centers will allow access to these records.

All laboratory samples, assessment forms, reports, and other records leaving the site will be identified only by the participant's identification number (ID) to maintain participant confidentiality.

Clinical information will not be disclosed without the participant's written permission, except as necessary for monitoring by the Ethics Committee or regulatory agencies.

15. REFUND OF PARTICIPANTS

Expenses incurred in participating in the study, such as food or transportation, will be reimbursed. The amount will not be considered substantial, and the reimbursement system will be designed to reduce the risk of the reimbursement being considered compensation or an incentive to the study participant.

16. FINANCIAL DISCLOSURE AND CONFLICTS OF INTEREST

This is a research study funded by development funds, without the participation of industries with commercial interests, and the funders will have no role in the study design, data collection and analysis, publication decisions, or manuscript preparation. UFMS has no commercial interest in the manufacture and marketing of any of the drugs under study.

17. DISSEMINATION AND TRANSLATION PLAN

The study results will be reported to participants after the analysis is complete.

The results of this study will be submitted to peer-reviewed journals, presented at conferences, and may form part of academic theses.

The principal investigator is primarily responsible for publishing the study results.

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APPENDIX 1

Medications that interact with isoniazid

- Carbamazepine
- Chlorzoxazone
- Disulfiram
- Ketoconazole
- Phenytoin
- Warfarin
- Theophylline
- Selective serotonin reuptake inhibitor antidepressants (e.g., citalopram, fluoxetine, paroxetine, sertraline)

Medications that interact with rifapentine

1. Antiarrhythmics
 - a. Disopyramide
 - b. Mexiletine
 - c. Quinidine
 - d. Tocainide
2. Antibiotics
 - a. Chloramphenicol
 - b. Clarithromycin
 - c. Dapsone
 - d. Doxycycline
 - e. Fluoroquinolones
3. Anticoagulants
 - a. Warfarin
4. Anticonvulsants
 - a. Phenytoin
5. Antimalarials
 - a. Quinine
6. Antipsychotics
 - a. Haloperidol
7. Azole antifungals
 - a. Fluconazole
 - b. Itraconazole
 - c. Ketoconazole
8. Barbiturates
 - a. Phenobarbital
9. Benzodiazepines
 - a. Diazepam
10. Propranolol beta-blockers

- a. Diltiazem
- 11. Calcium channel blockers
 - a. Nifedipine
 - b. Verapamil
- 12. Cardiac glycoside preparations
 - a. Digoxin
- 13. Corticosteroids
 - a. Prednisone
- 14. Fibrates
 - a. Clofibrate
- 15. Oral hypoglycemic agents
 - a. Sulfonylureas
- 16. Hormonal Contraceptives / Progestins
 - a. Ethinylestradiol
 - b. Levonorgestrel
- 17. Immunosuppressants
 - a. Cyclosporine
 - b. Tacrolimus
- 18. Methylxanthines
 - a. Theophylline
- 19. Narcotic analgesics
 - a. Methadone
- 20. Phosphodiesterase-5 (PDE-5) inhibitors
 - a. Sildenafil
- 21. Thyroid preparations
 - a. Levothyroxine
- 22. Tricyclic antidepressants
 - a. Amitriptyline
 - b. Nortriptyline