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Early View

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# Optimizing pyrazinamide for the treatment of tuberculosis

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**Summary (256-character, including spaces)**

The activity of pyrazinamide, a critical drug for tuberculosis treatment, increases as drug concentrations go up, but optimizing this drug alone is unlikely to result in treatment shortening. Rather, rifampicin dosing must go up in parallel.

**Target journal: European Respiratory Journal**

**Key Words** : Pyrazinamide, pharmacokinetics, pharmacodynamics, toxicity

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**ABSTRACT** (239 words, limit 244, structured)

Pyrazinamide is a potent sterilizing agent that shortens the treatment duration needed to cure tuberculosis. It is synergistic with novel and existing drugs for tuberculosis. The dose of pyrazinamide that optimizes efficacy while remaining safe is uncertain, as is its potential role in shortening treatment duration further.

Pharmacokinetic data, sputum culture, and safety laboratory results were compiled from TBTC Studies 27 and 28 and PanACEA MAMS-TB, multi-center Phase 2 trials in which participants received rifampicin (range 10-35 mg/kg), pyrazinamide (range 20-30 mg/kg), plus two companion drugs. Pyrazinamide pharmacokinetic-pharmacodynamic (PK/PD) and PK-toxicity analyses were performed.

In TBTC studies (n=77), higher pyrazinamide maximum concentration (Cmax) was associated with shorter time to culture conversion (TTCC) and higher probability of two-month culture conversion (p-value < 0.001). Parametric survival analyses showed that relationships varied geographically, with steeper PK-PD relationships seen among non-African than African participants. In PanACEA MAMS-TB (n=363), TTCC decreased as pyrazinamide Cmax increased and varied by rifampicin AUC (p-value < 0.01). Modeling and simulation suggested that very high doses of pyrazinamide (>4500 mg) or increasing both pyrazinamide and rifampicin would be required to reach targets associated with treatment shortening. Combining all trials, liver toxicity was rare (3.9% with Grade 3 or higher liver function tests, LFT), and no relationship was seen between pyrazinamide Cmax and LFT levels.

Pyrazinamide's microbiologic efficacy increases with increasing drug concentrations. Optimizing pyrazinamide alone, though, is unlikely to be sufficient to allow tuberculosis treatment shortening; rather, rifampicin dose would need to be increased in parallel.

## INTRODUCTION

Pyrazinamide is a potent sterilizing agent against *Mycobacterium tuberculosis*. It is unique in its activity against semi-dormant bacilli in acidic environments and against bacilli that remain viable despite unfavorable local conditions and antibiotic pressure, so-called "persisters" that must be eliminated to cure tuberculosis disease (1).

Currently-recommended "short-course" treatment for drug-sensitive tuberculosis remains lengthy. Isoniazid, rifampicin, pyrazinamide, and ethambutol are given for two months (intensive phase), followed by isoniazid and rifampicin for four months (continuation phase). The addition of pyrazinamide to rifampicin and isoniazid during the intensive phase of therapy allows for treatment shortening from 9 to 6 months (2, 3). Whether or not optimization of pyrazinamide—giving it for longer, increasing the dose, or pairing it with synergistic drugs-- can contribute to a regimen that cures tuberculosis more quickly is unknown (4).

In the World Health Organization (WHO) 1984 treatment guidelines, the recommended daily dose of pyrazinamide was 35 mg/kg (in keeping with studies that showed its treatment shortening benefit). In 2003, WHO reduced the recommended daily dose to 25 mg/kg; the rationale was left unstated (5). In some studies, low pyrazinamide exposures have been associated with worse outcomes (6, 7). However, pyrazinamide can cause liver injury at high doses given for prolonged periods (8, 9). The relationship between pyrazinamide exposures and either efficacy or, on the flip side, hepatotoxicity is not firmly established (10).

The Tuberculosis Trials Consortium (TBTC) is a multinational trials network funded by the US Centers for Disease Control and Prevention (CDC). TBTC conducted two clinical trials assessing the substitution of moxifloxacin for a first-line agent-- Study 27 (S27, moxifloxacin substituted for ethambutol) and Study 28 (S28, moxifloxacin substituted for isoniazid) (11, 12). The Pan African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA), funded by the European & Developing Countries Clinical Trial Partnership (EDCTP), conducted a multi-arm multi-stage (MAMS-TB) trial assessing combinations that included higher-dose rifampicin, moxifloxacin, and an investigational drug, SQ-109 (13). We used PK-PD modeling to assess the relationships between pyrazinamide exposures and efficacy or hepatotoxicity using data from these trials.

## MATERIAL and METHODS

### Study Design

We included PK, safety, and efficacy data from participants in S27, S28, and MAMS-TB (11-13)-- all randomized Phase 2 clinical trials involving adults with sputum smear-positive, drug-susceptible, pulmonary TB-- to establish the relationship between exposure and efficacy as well as exposure and toxicity.

In S27 (NCT00140309), during the intensive phase of treatment, all participants received isoniazid (H), rifampicin (R) and pyrazinamide (Z), and they were randomized to receive either moxifloxacin (M) (HRZM) or ethambutol (E) (HRZE) (11). Dosing was daily for the first two weeks, followed by three- or five-times per week. PK sampling was conducted during initial daily dosing. In S28 (NCT00144417), the arms were HRZE vs MRZE (12), and dosing was five

times per week. In both trials, pyrazinamide was 20-25 mg/kg (1000-2000mg), and rifampicin was at standard dose, 10 mg/kg, for 24 weeks (**Supplemental Table S1**). In S27 and S28, after the intensive phase, participants transitioned to standard continuation phase treatment with rifampicin and isoniazid. Sputum cultures and safety testing, including liver function tests (LFT) [aspartate aminotransferase (AST) and total bilirubin] were collected at weeks 12, 16, and 24. Over the studies' duration, 850 sputum samples were inoculated on liquid media (2 participants in S27, all participants in S28) or on both solid and liquid media (7 participants in S27).

In MAMS-TB (NCT01785186), participants were randomized to receive regimens containing standard- or higher-dose rifampicin, SQ109 (Q), or moxifloxacin, plus other first-line drugs (13). Regimens were $HR_{10}ZQ$, $HR_{20}ZQ$, $HR_{20}ZM$, $HR_{35}ZE$, and $HR_{10}ZE$ (subscript indicates mg/kg dose); doses were given 7 days/week. pyrazinamide was given at 25-30 mg/kg (800-2000mg) (**Supplemental Table S2**). Study treatment (including pyrazinamide) was given for 12 weeks, then patients were transitioned to rifampicin and isoniazid to complete 26 weeks of treatment. Sputa for culture were collected weekly up to week 12, then at weeks 14, 17, 22, and 26. Liquid culture data were used in our analyses. Safety assessments, including AST, alanine aminotransferase (ALT), and total bilirubin, were performed at weeks 1, 2, 4, 6, 9, 12, and 14.

Trials were conducted according to Good Clinical Practice. Written, informed consent was obtained from participants, and ethical and regulatory approvals were obtained at local and national levels.

**Pyrazinamide PK and Minimal Inhibitory Concentration (MIC) Assessments**

Intensive PK sampling was performed in a subset of participants in S27 and S28 in Uganda, South Africa, or the United States (n=72). PK sampling was performed pre-dose, and 1, 2, 6, 12, and 24 hours post-dose, and so that PK values would reflect steady state measures, these were collected at least 10 days after beginning treatment. Pyrazinamide PK analysis was performed using a validated gas chromatography assay with mass selective detection (14). For MIC determinations, isolates were stored at baseline. The pyrazinamide MIC of participants' isolates were determined using the BD BACTEC MGIT 320 system. Pyrazinamide MIC testing was performed using the standard method described in the package insert, except that in addition to the standard test concentration of 100 mcg/mL, three additional concentrations were tested (25, 50, and 75 mcg/mL) (15).

In MAMS-TB, PK sampling was performed in a subset of participants (20/arm) four weeks after commencing therapy. Samples were collected pre-dose and 1, 2, 3, 4, 6, 8, 12, and 24h post-dose. pyrazinamide bioanalysis was performed using high performance liquid chromatography (13). MICs were not measured.

**Population PK/PD Modeling**

*Pyrazinamide with standard-dose rifampicin*

Using PK data from S27 and S28, a nonlinear mixed effects (NLME) model was previously developed to characterize pyrazinamide population PK ( Supplemental Table 3) (14). The existing model was used to generate post-hoc Bayesian estimates of secondary PK parameter

values ($AUC_{0\text{-}24hr;}$ and maximum concentration ($C_{max}$)) in NONMEM (version 7.4.3, ICON, Gaithersburg, MD) for each participant, taking into account an individual's PK data and characteristics (e.g. dose, weight, sex, age). Pharmacodynamic indices (PD indices) were calculated using MIC data (e.g. $AUC_{0\text{-}24hr}$/ MIC or Cmax/MIC). Cox proportional hazards regression analysis in R program (version: 3.6.1; package: survival 2.44) was performed to assess the relationship between pyrazinamide PK and PD indices vs. outcomes (time to sputum culture conversion, probability of culture conversion by eight weeks of treatment). The PK parameter with the best fit was included in final models. Variables with p-values less than $< 0.1$ in univariate models or factors known to be associated with culture conversion were tested in multivariate models (e.g. sex, ethnicity, cavity status, regimen). After exploring relationships with Cox modeling, we then proceeded to parametric survival analysis, a more sophisticated modeling technique that allows for evaluation of predictors' influence on both the shape and scale of the survival curve (16) (Supplemental materials, Section A). Hazard function was defined by scale and shape parameters; covariates were tested on scale and shape parameters in analyses.

*Pyrazinamide with standard vs. higher-dose rifampicin*

A population PK model for pyrazinamide was developed based on PK data from MAMS-TB using NONMEM software to generate primary PK parameters (Supplemental materials, Section A, Supplemental Table 4, Supplemental Figure 1). The relationship between pyrazinamide Cmax or $AUC_{0\text{-}24hr}$ and treatment outcomes was assessed in similar fashion to S27 and S28 in R program (version). A number of covariates were evaluated for inclusion in multivariate models (baseline mycobacterial load, weight, HIV status, age, sex, radiographic findings, rifampicin

PK). Data were analyzed using parametric survival analyses (Supplemental materials, Section A). PK-PD assessments were restricted to twelve weeks.

*Dosing and efficacy simulations*

Final survival models with covariates for S27 and S28 and, separately, for MAMS-TB, were used to simulate scenarios to investigate the probability of 8- or 12-week culture conversion reaching certain targets (e.g. 90% and 95%) for different dosing strategies, assuming that high rates of early culture conversion are a prerequisite for a regimen that will effectively shorten treatment (Supplemental materials, Section A). Simulations of 500 trials were conducted for each scenario.

**PK-toxicity Modeling**

We evaluated the relationship between pyrazinamide PK parameters (e.g. Cmax or AUC) and change in LFTs from baseline on the basis of their relevance. Linear regression was conducted to measure the association between pyrazinamide Cmax and individual maximal LFT values in R program (version version: 3.6.1). Multiple R-squared and p-value were calculated individually for ALT, AST and total bilirubin.

**RESULTS**

**Study Population**

**Table 1** shows demographic and dose information for the 72 participants in the PK substudies of S27 and S28. In MAMS-TB, data for 363 participants were available and used in safety assessments. 96 individuals participated in the PK substudy and had concentration data sufficient

to produce PK estimates (characteristics in Table 2); data for 86 subjects had dose and time of dose recorded adequately for population PK analysis.

**Table 1.** Demographic, treatment, and clinical characteristics among participants enrolled in the pharmacokinetic sub-study of Tuberculosis Trials Consortium Studies 27 and 28

| | **Study 27 participants (n=9)** | **Study 28 participants (n=63)** | **All study participants (n=72)** |
|---|---|---|---|
| **Demographic Factors** | | | |
| Age, years | 50 (37-55) | 33(26-38) | 33 (27-42) |
| Female sex | 1 (11%) | 12 (19%) | 13 (18%) |
| Enrollment from Africa* | 0 (0%) | 37 (59%) | 37 (51%) |
| Race | | | |
| Black | 2 (22%) | 40 (63%) | 42 (58%) |
| White | 7 (78%) | 22 (35%) | 29 (40%) |
| Asian | 0 (0%) | 1 (1.6%) | 1 (1.4%) |
| Hispanic Ethnicity | 7 (78%) | 18 (29%) | 25 (35%) |
| **Intensive Phase Treatment** | | | |
| HRZE | 7 (78%) | 15 (24%) | 22 (31%) |
| HRZM[a] | 2 (22%) | --- | 2 (3%) |
| MRZE[b] | --- | 48 (76%) | 48 (67%) |
| Thrice weekly therapy[c] | 4 (44%) | --- | 4 (6%) |
| Pyrazinamide dose (mg) | 1000 (1000-1500) | 1500 (1000-1500) | 1500 (1000-1500) |
| Pyrazinamide dose (mg/kg) | 19.7 (18.6-23.9) | 22.9 (20.2-25.4) | 22.9 (19.9-25.3) |
| **Clinical Factors** | | | |
| Cavity on baseline chest X-ray | 5 (56%) | 50 (79%) | 55 (76%) |
| HIV positive | 1 (11%) | 2 (3%) | 3 (4%) |
| Weight (kg) | 54.1 (53.3-74.0) | 57.0 (51.1-63.0) | 56.7 (51.3-63.3) |

*Uganda or South Africa

Data presented are median (interquartile range) for age, weight, and dose and n (%) for all other factors.

[a] HRZM not used in Study 28

[b] MRZE not used in Study 27

[c] In Study 27, PK sampling was performed during the first two weeks of therapy, when dosing was daily; after that, some patients received thrice-weekly dosing

Abbreviations: HRZE: isoniazid-rifampicin-pyrazinamide-ethambutol intensive phase regimen; HRZM: isoniazid-rifampicin-pyrazinamide-moxifloxacin intensive phase regimen; MRZE: moxifloxacin-rifampicin-pyrazinamide-ethambutol intensive phase regimen; TB: tuberculosis; BMI: body mass index; HIV: human immunodeficiency virus

**Table 2.** Demographic, treatment, and clinical characteristics among participants enrolled in the pharmacokinetic sub-study of PanACEA MAMS-TB

| | **All study participants (n=96)** |
|---|---|
| **Demographic Factors** | |
| Age (years), median (IQR) | 34.5 (28.9-39.2) |
| Male sex | 67 (70 %) |
| **Race** | |
| Black | 83 (86%) |
| Mixed | 13 (14%) |
| **Intensive Phase Treatment** | |
| HRZE | 19 |
| HR35ZE | 20 |
| HRZQ | 19 |
| HR20ZQ | 19 |
| HR20ZM | 19 |
| Pyrazinamide dose (mg), median (IQR) | 1200 (1200, 1600) |
| Pyrazinamide dose (mg/kg), median (IQR) | 25.7 (24.0, 28.3) |
| **Clinical Factors** | |
| Weight (kg), median (IQR) | 54.0, (48.9, 56.5) |
| BMI, median (IQR) | 19.2, (17.6, 20.5)* |
| BMI<18.0 kg/$m^2$ | 25 (26%)* |
| HIV positive | 2 (2%) |
| Cavity on baseline chest X-ray | 66 (68.8%) |

*1 patient had height missing

**PK and MIC Results**

444 plasma samples were used in PK analyses from S27 and S28. Post-hoc Bayesian estimates of pyrazinamide PK parameters and PD indices are in Table 3. Predicted PZA Cmax ranged from 15 to 55 ug/mL; only 18 (25%) of participants had $C_{max}$ above 35 ug/mL. Pyrazinamide MICs were 25, 50 and 75 µg/ml (27, 29 and 4 participants, respectively). 846 plasma PK samples from MAMS-TB were used in PK assessments (Supplemental Figure 1). Predicted pyrazinamide Cmax ranged from 30 to 51 ug/mL; 55 (64.0%) of participants had $C_{max}$ above 35 ug/mL (Table 3). Time to maximum concentration (Tmax) median was 4h (range 3-6h). Observed rifampin Cmax varied depending on the dose level. Parameter estimates for the final population PK models are in Supplemental Tables S3 and S4 (14).

**Table 3.** Post hoc Bayesian estimates of pyrazinamide PK parameters from TBTC Studies 27 and 28 and PanACEA MAMS-TB.

| Parameter | Median | IQR |
|---|---|---|
| **TBTC Trials (68 participants with MIC data)** | | |
| **Pyrazinamide Pharmacokinetic Parameters** | | |
| Predicted $C_{MAX}$ (µg/ml) | 29.2 | (25.6, 35.0) |
| Predicted $AUC_{0\text{-}24hr}$ (µg*hr/ml) | 306 | (261, 357) |
| **Pharmacodynamic Parameters** | | |
| Predicted $AUC_{0\text{-}24hr}$/MIC | 8.35 | (5.36, 12.7) |
| Predicted $C_{MAX}$/MIC | 0.775 | (0.549, 1.18) |

| | | | |
|---|---|---|---|
| **PanACEA MAMS Trial (86 participants)** | | | |
| **Pyrazinamide Pharmacokinetic Parameters** | | | |
| Predicted $C_{MAX}$ (μg/ml) | | 37.2 | (33.4, 40.9) |
| Predicted $AUC_{0\text{-}24hr}$ (μg*hr/ml) | | 331 | (278, 398) |
| **Rifampicin Pharmacokinetic Parameters** | | | |
| Observed $C_{MAX}$ (μg/ml) | Control: HR10ZE | 5.56 | (5.08, 7.26) |
| | Arm 1: HR35ZE | 26.7 | (23.6, 32.1) |
| | Arm 2: HR10ZQ | 3.65 | (2.49, 5.04) |
| | Arm 3: HR20ZQ | 12.1 | (9.81, 13.2) |
| | Arm 4: HR20ZM | 12.1 | (9.83, 14.4) |
| Observed $AUC_{0\text{-}24hr}$ (μg*hr/ml) | Control: HR10ZE | 23.4 | (17.4, 29.3) |
| | Arm 1: HR35ZE | 164 | (131, 199) |
| | Arm 2: HR10ZQ | 18.3 | (10.8, 23.5) |
| | Arm 3: HR20ZQ | 66.3 | (56.7, 82.9) |
| | Arm 4: HR20ZM | 61.7 | (50.5, 78.7) |

**PK-PD of pyrazinamide, with Standard-dose rifampicin (TBTC Trials)**

*Time to culture conversion*. In multivariate Cox regression analyses, the only significant predictors of time to culture conversion were pyrazinamide PK parameters (Cmax p=0.046 or AUC p=0.015). In the more complex parametric survival analyses, pyrazinamide Cmax and geographic site were the only covariates that improved the fit of the Weibull time-to-culture-conversion model significantly (pyrazinamide Cmax influenced the shape parameter, and geographic site influenced both scale and shape parameters) (Figure 1, Supplemental Figure 2a,

Supplemental Table 5). Efficacy improved over the full range of clinically-observed values of pyrazinamide Cmax, without plateau (Figure 2, top).

*Probability of culture conversion*. There was a positive relationship between pyrazinamide Cmax and two-month culture conversion in non-African but not African participants; however, there was a positive relationship between Cmax and probability of culture conversion by 3 months across groups (Supplemental Table 6). Simulations show that to achieve culture conversion by 2 months in 90% of participants, pyrazinamide Cmax of 43 and 93 ug/mL would be needed for non-African and African participants, respectively. Average daily doses of 1800mg and 4600mg are required to achieve these targets (Table 4A).

**Table 4**. (A) Clinically-observed maximal concentration (Cmax) and associated drug doses (90%CI) that would be required to achieve 90 and 95% culture conversion on solid media by 2 months of treatment in TBTC trials and (B) Doses (and clinically-observed Cmax) of pyrazinamide and rifampicin that would be needed to achieve 90% or 95% culture conversion on liquid media by (i) two months or (ii) three months of treatment, using PanACEA MAMS-TB data.

**A)**

| **Enrollment Site** | **90% Culture Conversion** | **95% Culture Conversion** | |
|---|---|---|---|
| **Non-Africa** | 43 μg/mL | 54 μg/mL | **Observed Cmax** |
| **Africa** | 93 μg/mL | 120 μg/mL | |
| **Non-Africa** | 1800 mg (2800, 6000) | 2200 (1400, 4800) | **Expected Dose Level** |
| **Africa** | 4600 (3000, 8400) | 5800 (3800, 8800) | |

**B)**

| Pyrazinamide Dose (mg)* | Pyrazinamide Cmax (μg/mL) | i. Rifampicin AUC (for conversion by 2 months) (μg*hr/mL) | ii. Rifampicin IF AUC (for conversion by 3 months) (μg*hr/mL) | %Culture Conversion |
|---|---|---|---|---|
| 1500 | 42 | >688 | >450 | 95% |
| 3000 | 83 | >352 | >=113 | 95% |
| 1500 | 42 | >588 | >349 | 90% |
| 3000 | 83 | >251 | >=13 | 90% |

*for reference, median weight in MAMS-TB was 54 kg

**PK-PD of pyrazinamide, with Higher-dose rifampicin (PanACEA MAMS-TB trial)**

In Cox regression models, pyrazinamide Cmax or $AUC_{0\text{-}24hr}$ were associated positively with time to culture conversion (p=0.0067 and 0.73, respectively). In parametric survival analysis, several factors were correlated with the scale parameter in the Weibull model (age, ethnicity, weight, baseline mycobacterial load, HIV status, pyrazinamide Cmax, rifampicin $AUC_{0\text{-}24hr}$), and several factors correlated with the shape parameter (age, ethnicity, baseline mycobacterial load, pyrazinamide Cmax). The final model, which included rifampicin $AUC_{0\text{-}24hr}$ and pyrazinamide Cmax on scale and pyrazinamide Cmax on shape, demonstrated a significant exposure-response relationship for pyrazinamide that depended on rifampicin exposures (Supplemental Tables 7 and 8, Figure 3, Supplemental Figure 2b). Table 4B shows the doses of pyrazinamide and exposures of rifampicin needed to achieve 2-month or 3-month culture conversion proportions of 90, or 95% on liquid media. For context, in MAMS-TB, doses of 10, 20, and 35 mg/kg of rifampicin achieved median $AUC_{0\text{-}24hr}$ values of 20.6, 61.7, and 164.2 ug*hr/mL (13).

**PK-toxicity Analysis**

One of 72 participants in the TBTC trials and 12 of 363 participants in MAMS-TB had LFT values greater than 3 times the upper limit of normal during TB treatment. In TBTC trials, no association could be shown between pyrazinamide Cmax and AST or total bilirubin (Multiple R-squared = 0.023 and 0.00070, p-value = 0.19 and 0.82, respectively) (Figure 4A); Median Cmax in those who had LFT>3x normal was 28.9 ug/mL versus 29.7 ug/mL in those who did not. Similarly, there was not an association between pyrazinamide Cmax and ALT, or AST, in MAMS-TB (Multiple R-squared = 0.00063, 0.00026 and 0.019, p-value = 0.64, 0.76 and 0.16, respectively) (Figure 4B). Median Cmax was 35.3 μg/mL in those who had LFT>3x normal and 37.2 μg/mL in those who did not.

**DISCUSSION**

Pyrazinamide is a standard component of first-line TB treatment, yet the 'right dose' is not established. In this study using data from three international Phase 2 clinical trials, higher concentrations of pyrazinamide were associated with higher culture conversion rates at 2 and 3 months of treatment. These analyses suggest that current dosing may be insufficient to maximize efficacy (17). In the trials that enrolled from geographically-diverse settings, parametric survival modeling revealed that PK-PD relationships differed for participants from African vs. non-African sites. To achieve targets associated with treatment-shortening, drug doses that are beyond the range of tolerability would likely be needed in African patients in the absence of other new drugs. Modeling and simulation showed that increasing doses of both rifampicin and pyrazinamide appears to be a more promising strategy. The range of pyrazinamide

concentrations was broad, yet elevations in liver enzymes were rare, and there was not an association between pyrazinamide levels and hepatotoxicity.

Pyrazinamide is an important sterilizing agent. Early on, it was highly effective in two-drug combinations with isoniazid, provided it was given at a high enough dose and for sufficient duration (8, 18-22). Following the discovery of rifampicin, adding pyrazinamide to rifampicin-containing regimens reduced relapses (2, 23-25), and pyrazinamide became an essential part of current "short-course" treatment. Giving it during the first eight weeks allows for the shortening of treatment from nine to six months. In the trials demonstrating its treatment shortening activity, though, the doses given were 30-40 mg/kg daily, not the currently-recommended 20-25 mg/kg for adults (26-28).

In our study, culture conversion rates increased with increasing pyrazinamide exposure both when pyrazinamide was combined with standard-dose rifampicin or higher-dose rifampicin, and the best activity was seen when exposures of both drugs were high, demonstrating that there was an observable exposure-response relationship even when the companion drug was a potent sterilizing agent given at a high dose. Interestingly, in the TBTC studies, PK-PD relationships were different for non-African and African participants. African participants tended to have higher baseline extent of disease (higher likelihood of 3+ sputum smear grade or large lung cavities) than non-Africans, but these factors were not significant in our multivariate models, and there are likely other unobserved factors contributing to lower treatment response. This same phenomenon of lower treatment response, even after adjusting for known risk factors, was seen in the larger S28 study population and in TBTC Studies 29 and 29X and remains unexplained

(29, 30). While optimizing pyrazinamide in the context of first-line therapy is important, pyrazinamide also has a role in multidrug-resistant TB treatment; treatment outcomes are significantly worse if the MDR-TB strain is pyrazinamide-resistant (28). Pyrazinamide also enhances the activity of new and investigational drugs, namely bedaquiline, delamanid, and pretomanid, so optimization of pyrazinamide may be valuable in multiple contexts (31-33).

Our study was not the first modern study to find that pyrazinamide PK influenced treatment outcomes. In our study, Cmax was the covariate identified in the final PK-PD model as the most informative. However, we note that Cmax and AUC are highly correlated and each has a strong association with outcomes; depending on the sampling strategy of a given study, which influences how well that parameter is estimated, one might have a modestly stronger correlation or better precision. For example, single samples can sometimes fail to capture $C_{max}$ well. In the TBTC Studies, CV% for Cmax and AUC were similar at 23.1% and 26.1%, respectively, so variability in these estimates was similar. In a study in Botswana, after adjusting for HIV infection and CD4 cell count, patients with pyrazinamide Cmax less than 35 ug/mL (a putative pyrazinamide PK target) had a 3.4-fold higher risk of poor treatment outcome (6). In South Africa, pyrazinamide AUC was a top predictor of poor long-term outcomes (7). In children with and without HIV in India, low pyrazinamide and rifampicin Cmax were associated with unfavourable outcomes (34). In a recent meta-analysis, low pyrazinamide concentrations were shown to increase the risk of poor outcomes with relative risk of 1.73 (35). At currently-recommended doses, a high proportion of patients do not have drug concentrations that reach 35 ug/mL, let alone a proposed alternative target of 58 ug/mL (10), and the evidence base for selection of a 20-25 mg/kg dose is limited. Likely the optimal dose for this drug lies somewhere

between 35 and 45 mg/kg. Higher doses would produce exposures exceeding 5000 ug*h/mL (see below) in some patients (29, 36). At the current dose, we are undertreating many.

Pyrazinamide commonly causes arthralgias, but its most dreaded toxicity is liver injury. In early studies, doses of at least 40-50 mg/kg given for 24 weeks or longer caused unacceptable rates of liver toxicity (5-10%), while rates were substantially lower (2-5%) if the duration or dose was reduced (37). Currently, doses of 30-40 mg/kg daily are well-tolerated as part of MDR-TB treatment (38). In a meta-analysis involving 4490 individuals, risk of liver toxicity did not appear to increase as a function of drug exposure until exposures were quite high (weekly AUC of > 5000 ug*h/mL) (39). Pyrazinamide toxicity appears to be idiosyncratic up until a point, after which dose-related increases in liver toxicity are seen (10). The mechanism for and contributing factors to pyrazinamide-associated liver injury are not clearly understood and may differ for different companion drugs (40). Consistent with previous reports, in our study, median weekly AUC were 2100 (TBTC) and 2310 (PanACEA MAMS-TB) ug*h/mL, and liver injury was rare and not related to exposure in the ranges seen.

Our study has limitations. Because of differences in culture methodology and covariates collected between TBTC and PanACEA trials, we could not combine PK-efficacy data into a single model. Model-predicted doses assumed proportional dose effects at doses higher than those observed, which may not be the case. In our parametric survival analyses, we did not consider interval censoring. The concentration that increases risk of liver toxicity could not be determined, as liver toxicity was rare and the dose range limited. Adjusting pyrazinamide PK parameters for isolates' MIC values did not improve model fit; likely this is because the MIC

range was narrow, MICs were not measured precisely, and many participants did not have MIC data. In one TBTC trial, some patients had intermittent dosing- sensitivity analyses suggested removing those patients did not change model parameters or fit. Lastly, predicting an effective treatment-shortening regimen using microbiologic data from Phase 2 trials is an uncertain science; while 90-95% culture conversion on solid media by 8 weeks of treatment has been proposed, there are no well-validated prediction models using liquid culture (41).

It is important that the dose of each drug in a TB treatment regimen be optimized. Higher-dose rifampicin and pyrazinamide have the potential to shorten TB treatment. Simply prolonging the duration over which pyrazinamide is given was not sufficient to reduce treatment duration from 6 to 4 months in historical trials (4). Using parametric survival modeling and trial simulations, we discovered that increasing just the pyrazinamide dose does not seem as though it will improve outcomes in the hardest-to-treat patients (29). Indeed, the predicted doses of pyrazinamide that would be required as part of the standard regimen to produce rapid and sustained culture conversion (90% conversion by two months, for example) for all patients were high and would not be safe. Whether or not an enhanced multidrug regimen containing high-dose rifampicin (e.g. $\geq$35 mg/kg) and higher-dose pyrazinamide (e.g. 30-40 mg/kg) will be sufficient to meaningfully reduce TB treatment duration must be explored prospectively, with attention to safety and tolerability.

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## FIGURE LEGENDS

**Figure 1.** Visual predictive checks of the PK/outcome Weibull survival models for TBTC S27/28 (A) and PanANCEA MAMS trials stratified by covariates identified in the survival model (B).

**Figure 2.** Among participants taking combination treatment including pyrazinamide and standard-dose rifampicin in TBTC Studies 27 and 28, the relationship between maximum drug concentration (mcg/mL) and proportion with culture conversion to negative by 2 months of treatment. The median Cmax with drug doses of 1000mg, 1500mg, and 2000mg are shown in the vertical dash lines, and the observed range of Cmax values is contained within the vertical grey lines. In Panel a, the grey ribbon shows the 90% confidence interval of the proportion with culture conversion to negative with the black line as the median. In Panel b, the relationship between Cmax and two-month culture conversion is shown for African vs. non-African participants.

**Figure 3.** Simulated relationship between culture conversion on liquid medium by 2 months of treatment with maximum concentrations (Cmax) of pyrazinamide and area under the curve ($AUC_{0\text{-}24hr}$) of rifampicin from PanACEA MAMS trial. Black dots are Cmax values of pyrazinamide and rifampicin obtained by population PK models. Notes: for patients whose PK concentrations were not measured, pyrazinamide Cmax values were imputed using the population PK model and rifampicin AUC values were imputed using geometric mean values of the regimen taken.

**Figure 4.** (A) Distribution and regression of individual maximal AST (left) and maximal total bilirubin (right) versus observed Cmax of pyrazinamide in TBTC 27 and 28 trials. (B) Distribution and regression of individual maximal ALT (left), individual maximal AST (middle), and individual total bilirubin (right) versus of pyrazinamide Cmax in PanANCEA MAMS trial.

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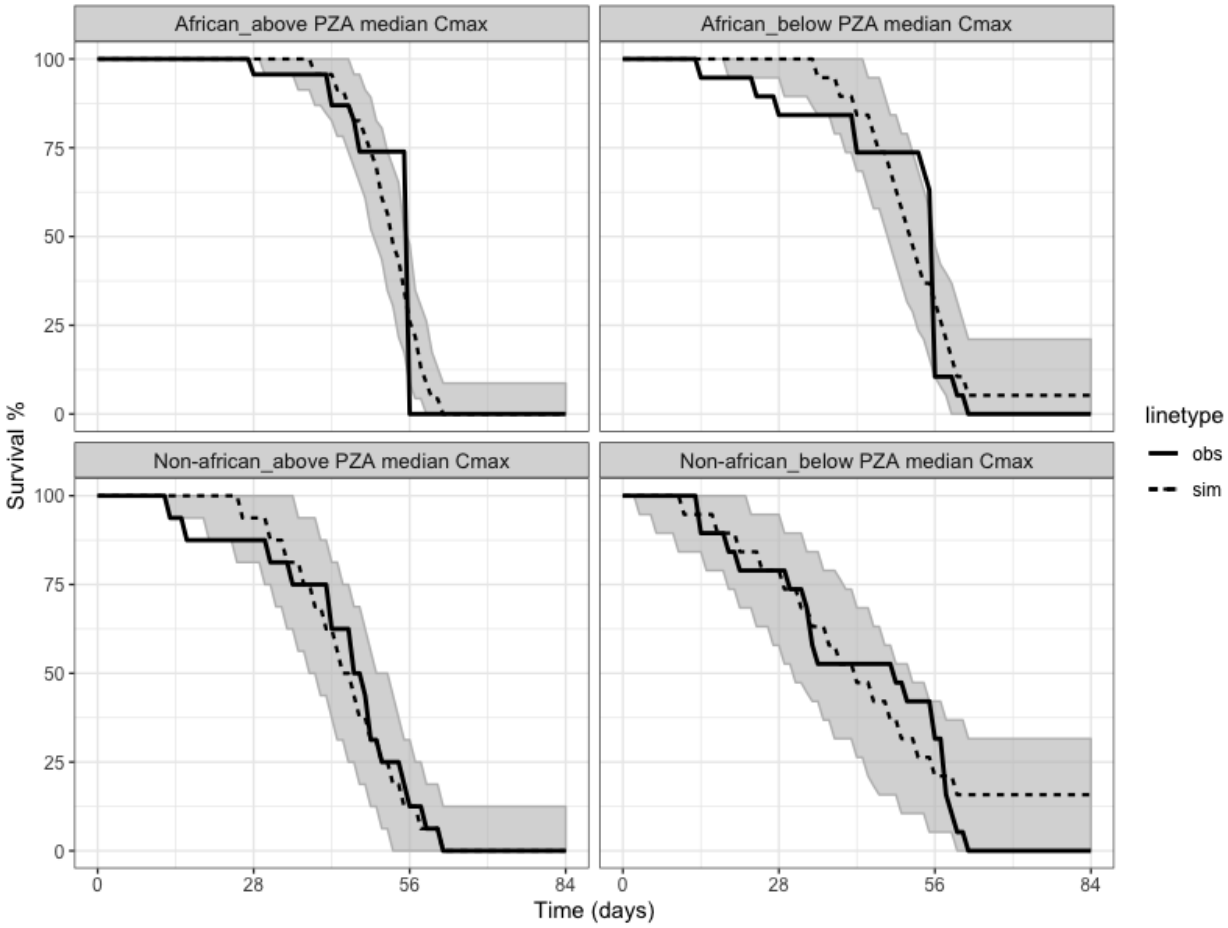
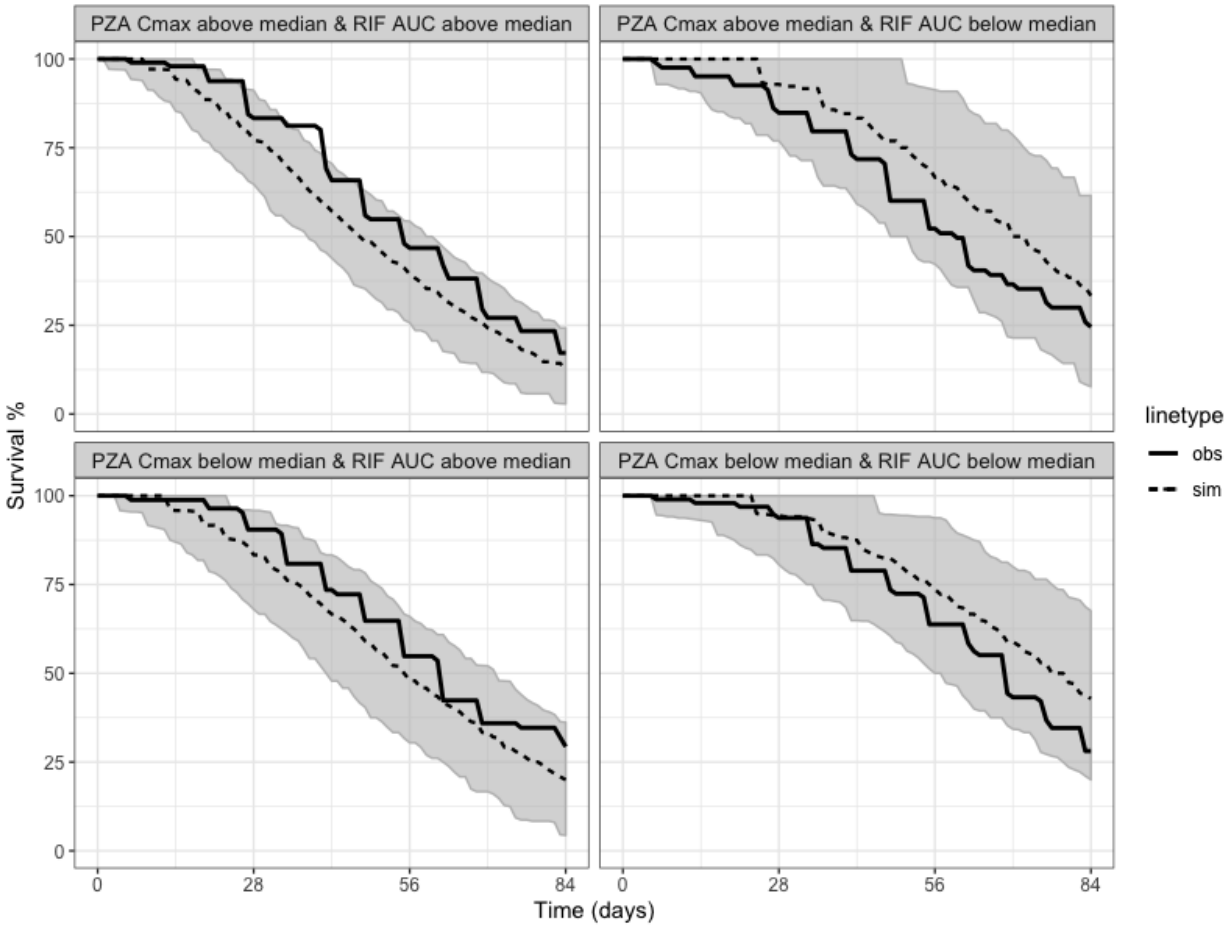
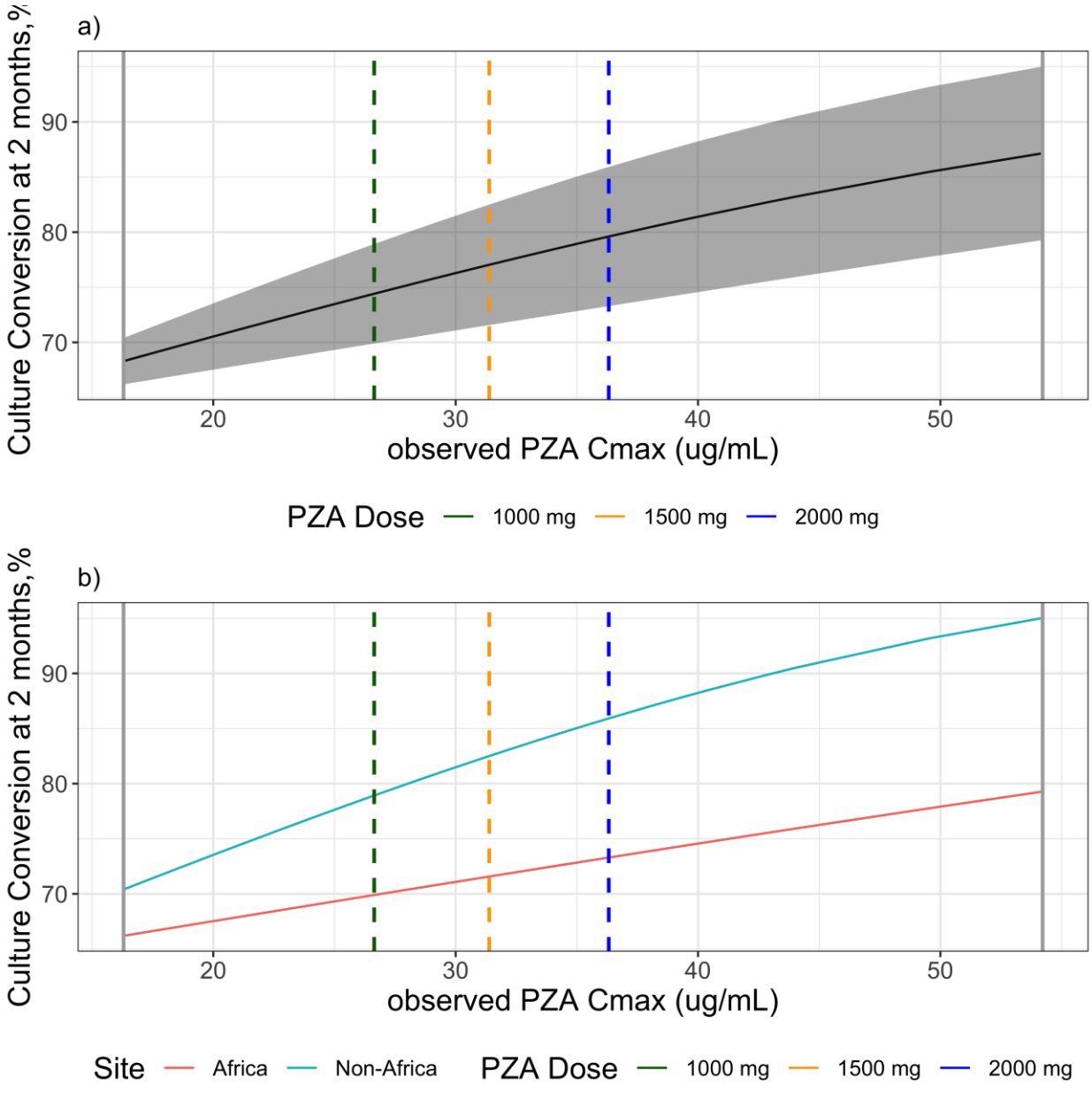
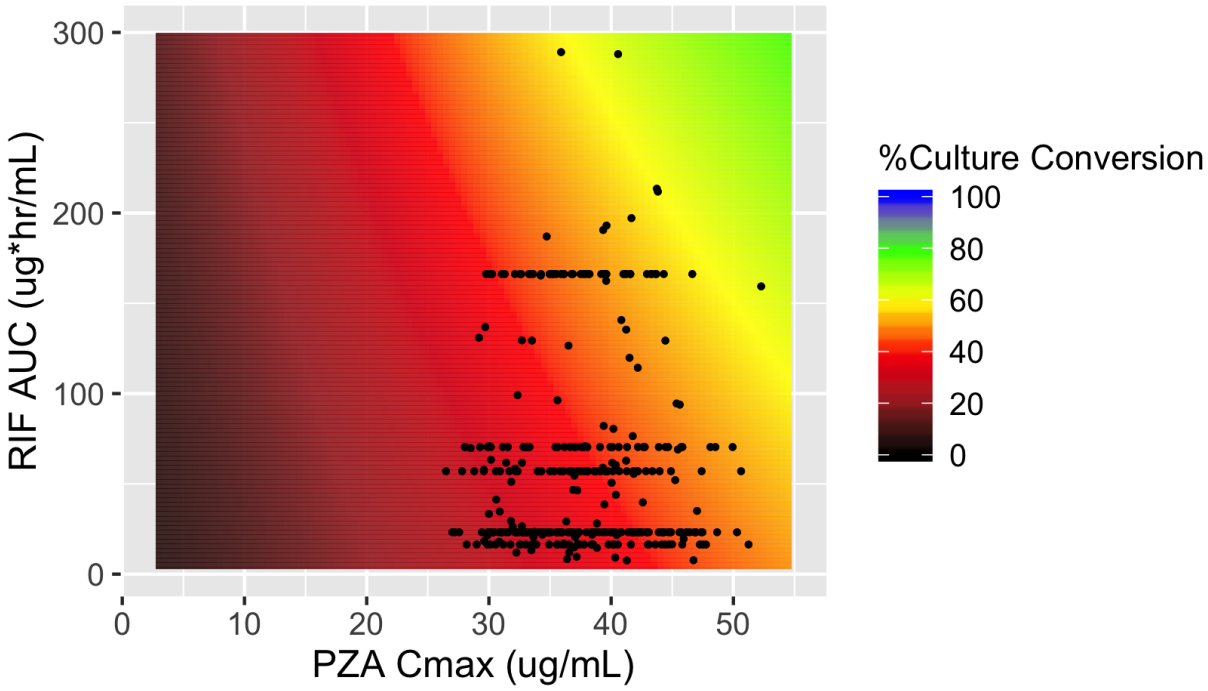
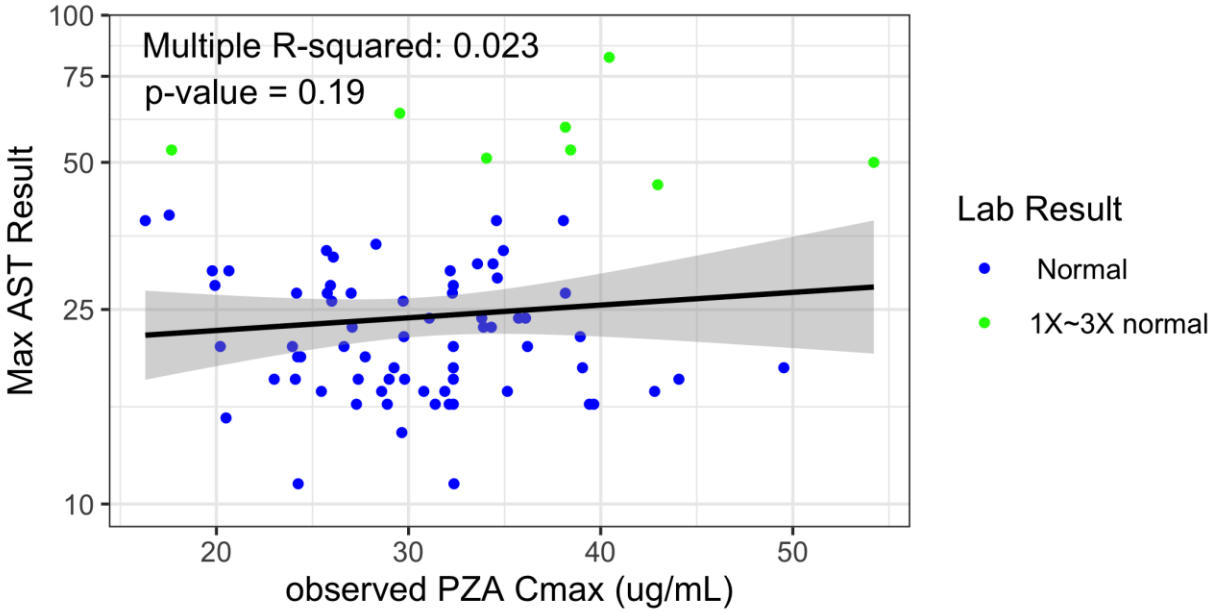
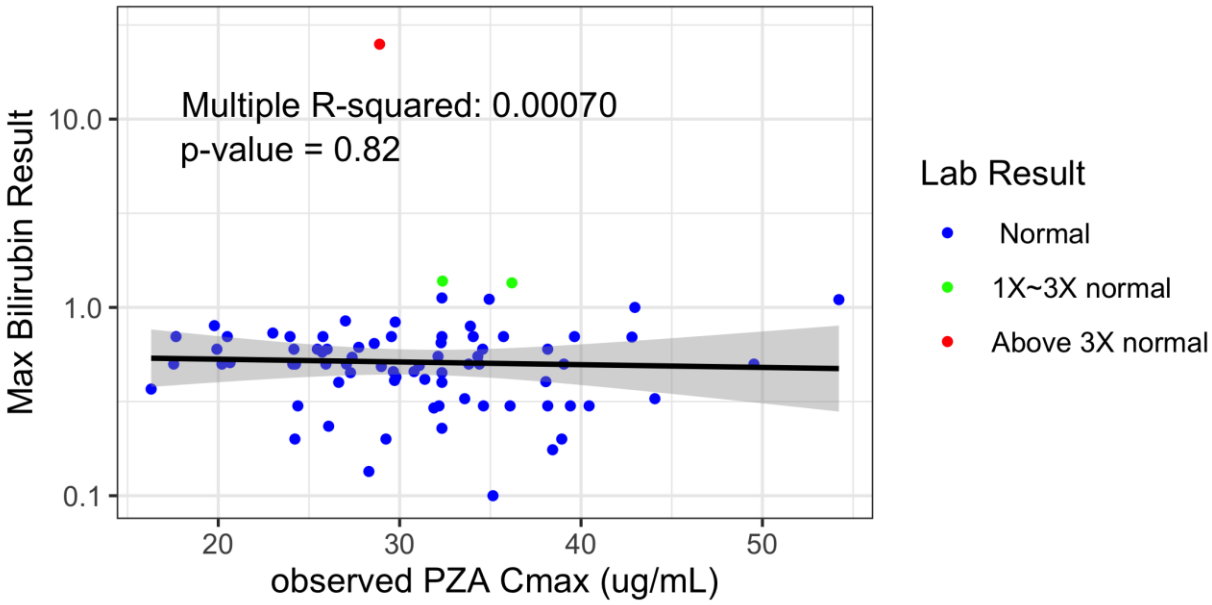

African_above PZA median Cmax
African_below PZA median Cmax
Non-african_above PZA median Cmax
Non-african_below PZA median Cmax
Survival %
100
75
50
25
0
0
28
56
84
Time (days)
linetype
obs
sim

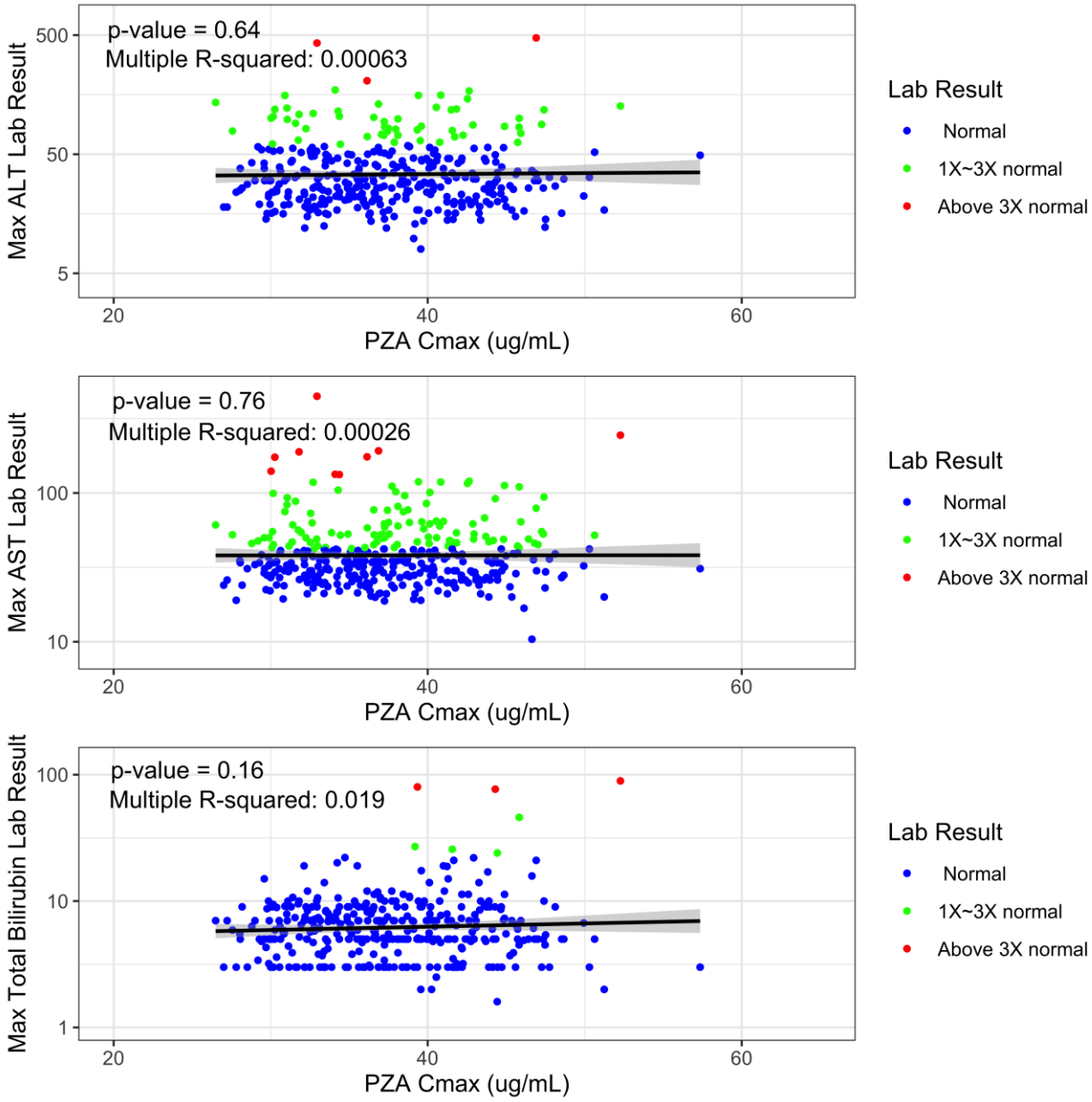

PZA Cmax above median & RIF AUC above median
PZA Cmax above median & RIF AUC below median
PZA Cmax below median & RIF AUC above median
PZA Cmax below median & RIF AUC below median
Survival %
100
75
50
25
0
0
28
56
84
Time (days)
linetype
obs
sim


a)
Culture Conversion at 2 months,%
90
80
70
20
30
40
50
observed PZA Cmax (ug/mL)
PZA Dose
1000 mg
1500 mg
2000 mg
b)
Culture Conversion at 2 months,%
90
80
70
20
30
40
50
observed PZA Cmax (ug/mL)
Site
Africa
Non-Africa
PZA Dose
1000 mg
1500 mg
2000 mg


300
200
100
0
RIF AUC (ug*hr/mL)
0
10
20
30
40
50
PZA Cmax (ug/mL)
%Culture Conversion
100
80
60
40
20
0


Multiple R-squared: 0.023
p-value = 0.19
Max AST Result
100
75
50
25
10
20
30
40
50
observed PZA Cmax (ug/mL)
Lab Result
Normal
1X~3X normal


Multiple R-squared: 0.00070
p-value = 0.82
Max Bilirubin Result
10.0
1.0
0.1
20
30
40
50
observed PZA Cmax (ug/mL)
Lab Result
Normal
1X~3X normal
Above 3X normal


p-value = 0.64
Multiple R-squared: 0.00063
Max ALT Lab Result
500
50
5
20
40
60
PZA Cmax (ug/mL)
Lab Result
Normal
1X~3X normal
Above 3X normal
p-value = 0.76
Multiple R-squared: 0.00026
Max AST Lab Result
100
10
20
40
60
PZA Cmax (ug/mL)
Lab Result
Normal
1X~3X normal
Above 3X normal
p-value = 0.16
Multiple R-squared: 0.019
Max Total Bilirubin Lab Result
100
10
1
20
40
60
PZA Cmax (ug/mL)
Lab Result
Normal
1X~3X normal
Above 3X normal

## SUPPLEMENTAL MATERIALS

### A. Development of the PK/PD models for the analyses of TBTC and PanACEA MAMS trials

The population PK analysis was performed using the non-linear mixed effects modeling approach using NONMEM (version 7.4.3; ICON plc, Gaithersburg, MD, USA) [1]. The R-based version of Xpose (version 4.7 and higher) was used to produce standard goodness-of-fit plots. Perl (version 5.18.2; http://www.perl.org) and PsN were used for model evaluation and automatic covariate model-building [2].

One- and two- compartment models with a first-order absorption with or without time delay, or transit compartment absorption and linear or nonlinear elimination rate constant were tested using PsN and NONMEM. A lognormal distribution for inter-individual variability (IIV) was included and additive and/or proportional models for the residual error were evaluated. The first-order conditional estimation with interaction method (FOCEI) was applied and the model-building procedure and model selection was based on the comparison of full versus reduced models using the log-likelihood criterion (the difference in the minimum OFV between hierarchical models was assumed to be Chi-square distributed with degrees of freedom equal to the difference in the number of parameters between models), goodness-of-fit plots (e.g. relevant residuals against time randomly distributed around zero), and scientific plausibility of the model. The stability of NONMEM models was assessed on the basis of acceptable basic goodness-of-fit plots, number of significant digits $\geqslant 3$ for all estimated parameters, successful covariance step, estimates of typical patient parameters ($\Theta$'s) not close to a boundary, and stability check

performed for a selected basic model (the model finds the global minimum when the initial values are altered in each direction [i.e. each parameter, one at a time] by a large factor [10 in this analysis]).

The identification of covariates was undertaken using 'Stepwise Covariate Model-Building' (SCM) using PsN and NONMEM. This method involved stepwise testing of linear and dichotomous relationships on categorical covariates, and linear, hockey-stick, and exponential relationships on continuous covariates in a forwards inclusion (change in objective function value [ΔOFV] of 3.84; $p < 0.05$ for 1 degree of freedom [DF]) and backwards exclusion (ΔOFV of 6.63; $p < 0.01$ for 1 DF) procedure. The detected covariate effects were included in the final model if the relationship is qualitatively meaningful and clinically significant with a cutoff of 20%.

Visual predictive check was conducted to evaluate whether the final model with estimated fixed-effect parameters and covariates adequately describe data. In general, 500 Monte Carlo simulation replicates of the original dataset were generated using the final model. The data were plotted versus time along with the summary statistics computed from the simulated data with 5th, 50th, and 95th percentiles including uncertainty. The coincidence between the original data and simulated data demonstrated the predictive ability of fixed effects parameters in the final model.

To evaluate the relationship between drug treatment and time to stable culture conversion, uni- and multivariate cox regression using R program (version: 3.6.1; package: survival 2.44) and

parametric survival analyses using PsN and NONMEM were conducted with time to culture positive as endpoint to assess the relationship between the outcome and covariates. The NONMEM estimation methods for parametric survival analysis used were first-order (FO). Model building and selection process was similar as described above.

Uni-and multivariate cox regression and parametric survival analyses were conducted for TBTC trials data to assess the relationship between the outcome and multiple variables, including sex, ethnicity, cavity status, regimen, dose and MIC of PZA, as well as predicted and observed Cmax and $AUC_{0\text{-}24hr}$ of PZA. Three models were tested in the parametric survival analysis, including exponential, Weibull and Gompertz distributions. The final optimal model was selected based on the statistical significance (p-value < 0.05). For TBTC S27/28 trials, compared to exponential and Weibull distributions, Gompertz distribution was the best fit to describe the treatment outcome data ($\Delta$OFV = -136.024 with $\Delta$df = 1 and $\Delta$OFV = -24.668 with $\Delta$df = 0, respectively). However, even though Gompertz distribution was better in terms of OFV, there was no improvement in goodness of fit. As such, Weibull distribution was selected as a more parsimonious model to describe the data.

Similar, uni-and multivariate cox regression and parametric survival analyses were conducted with time to culture positive as endpoint for PanACEA MAMS trials data to assess the relationship between the outcome and covariates using R program (version: 3.6.1; package: survival 2.44). The covariates in this analysis included PK secondary parameters of PZA and RIF, including Cmax and $AUC_{0\text{-}24hr}$ for both PZA and RIF, as well as multiple covariates, such

as sex, weight, HIV status, cough, baseline mycobacterial load, age, ethnicity, percentage of lung involved in chest x-ray, cavitary disease status, and adherence. For PanANCEA MAMS trial, compared to exponential and Gompertz distributions, Weibull distribution was the best fit to describe the treatment outcome data ($\Delta$OFV = -70.56 with $\Delta$df = 1 and $\Delta$OFV = -57.47 with $\Delta$df = 0, respectively).

In the parametric survival analysis, these covariates listed above were tested on scale ($\lambda$) and shape ($\beta$) parameters of Weibull distribution model for all three trials to characterize the hazard rate (hz) as indicated by the equation below.

$$hz = \lambda \times \beta \times (\lambda \times t)^{\beta-1}$$

The link between between hz and overall survival is established through the cumulative hazard (HZ) as indicated by the following expression.

$$OS = e^{-HZ}$$

For TBTC trials, sex, ethnicity, cavity status, regimen and MIC of PZA were evaluated as categorical covariates, and predicted and observed Cmax and $AUC_{0\text{-}24hr}$ of PZA were tested as continual covariates. In PanACEA trial, all four covariates were tested as the continuous one. The resultant final model contained covariates that met the predefined statistical criteria. In addition, covariates would only be retained on the basis of their relevance, in view of the purpose of the model.

In SCM analysis, when the covariate Africa is added to SHAPE in a linear relationship, the OFV decreased by 15.743 (Δdf of 1, p-value < 0.01). Then on top of the relationship of Africa added on SHAPE, the covariate Cmax_obs was found to be in a linear relationship with SHAPE and decrease the OFV by 10.910 (Δdf of 1, p-value < 0.01). When Africa was added to BASE in a linear relationship on top of the first two covariates, the OFV was decreased by 6.268 (Δdf of 1, p-value < 0.05).

In SCM analysis, when the covariate PZA Cmax is added to BASE in a linear relationship, the OFV decreased by 8.366 (Δdf of 1, p-value < 0.01); when the covariate RIF $AUC_{0\text{-}24hr}$ is added to BASE in an exponential relationship, the OFV decreased by 5.246 (Δdf of 1, p-value < 0.05).

For TBTC trials, the VPC was stratified on covariates identified in the modeling process, including site and observed PZA Cmax, whereas Cmax of PZA and $AUC_{0\text{-}24hr}$ of RIF as the identified covariate was the stratification factor for PanACEA MAMS trial.

**Supplemental Table 1**. Doses of tuberculosis drugs given in Tuberculosis Trials Consortium studies 27 and 28

| | **Study 27** | | **Study 28** |
|---|---|---|---|
| **Drug** | **Dose for daily therapy** | **Dose for thrice-weekly therapy** | |
| Moxifloxacin | 400 mg | 400 mg | 400 mg |
| Rifampin | | | |
| ≤ 45 kg | 450 mg | 450 mg | 450 mg |
| > 45 kg | 600 mg | 600 mg | 600 mg |
| Isoniazid | 300 mg | 15 mg/kg, max. dose - 900 mg | 300 mg |
| Pyrazinamide | | | |
| < 40 kg | --- | --- | 25 mg/kg rounded to nearest 500 mg[+] |
| 40-55 kg | 1000 mg | 1500 mg * | 1000 mg |
| 56-75 kg | 1500 mg | 2500 mg * | 1500 mg |
| 76 – 90 kg | 2000 mg | 3000 mg * | 2000 mg |

| | | | |
|---|---|---|---|
| > 90 kg | --- | --- | 2000 mg |
| Ethambutol | | | |
| < 40 kg | --- | --- | 15 mg/kg rounded to nearest 100 mg |
| 40-55 kg | 800 mg | 1200 mg * | 800 mg |
| 56-75 kg | 1200 mg | 2000 mg * | 1200 mg |
| 76 – 90 kg | 1600 mg | 2400 mg * | 1600 mg |
| > 90 kg | --- | --- | 1600 mg |

* maximum dose, regardless of weight

[+] for pyrazinamide dosing in patients < 40 kg, 1000 mg typically used instead of 500 mg

**Supplemental Table 2**. Doses of tuberculosis drugs given in PanACEA MAMS

| | **Weight Band 1: 30-37 kgs** | **Weight Band 2: 38-54 kg** | **Weight Band 3: 55-70kg** | **Weight Band 4: >70kg** |
|---|---|---|---|---|
| Control<br>HRZE | RHZE: 2 tablets<br>Vit B6: 1 tablet | RHZE: 3 tablets<br>Vit B6: 1 tablet | RHZE: 4 tablets<br>Vit B6: 1 tablet | RHZE: 5 tablets<br>Vit B6: 1 tablet |
| Arm 1 ($R_{35}$):<br>$HR_{35}ZE$ | RHZE: 2 tablets<br>R300: 3 tablets<br>Vit B6: 1 tablet | RHZE: 3 tablets<br>R150: 1 tablet<br>R300: 3 tablets<br>Vit B6: 1 tablet | RHZE: 4 tablets<br>R300: 5 tablets<br>Vit B6: 1 tablet | RHZE: 5 tablets<br>R300: 7 tablets<br>Vit B6: 1 tablet |
| Arm 2 (Q):<br>HRZQ | RHZ: 2 tablets<br>Q: 2 tablets<br>Vit B6: 1 tablet | RHZ: 3 tablets<br>Q: 2 tablets<br>Vit B6: 1 tablet | RHZ: 4 tablets<br>Q: 2 tablets<br>Vit B6: 1 tablet | RHZ: 5 tablets<br>Q: 2 tablets<br>Vit B6: 1 tablet |
| Arm 3 ($R_{20}Q$):<br>$HR_{20}ZQ$ | RHZ: 2 tablets<br>R150: 2 tablets<br>Q: 2 tablets<br>Vit B6: 1 tablet | RHZ: 3 tablets<br>R150: 3 tablets<br>Q: 2 tablets<br>Vit B6: 1 tablet | RHZ: 4 tablets<br>R150: 4 tablets<br>Q: 2 tablets<br>Vit B6: 1 tablet | RHZ: 5 tablets<br>R150: 5 tablets<br>Q: 2 tablets<br>Vit B6: 1 tablet |
| Arm 4 ($R_{20}M$)<br>$HR_{20}ZM$ | RHZ: 2 tablets<br>R150: 2 tablets<br>M: 1 tabl<br>Vit B6: 1 tablet | RHZ: 3 tablets<br>R150: 3 tablets<br>M: 1 tabl<br>Vit B6: 1 tablet | RHZ: 4 tablets<br>R150: 4 tablets<br>M: 1 tabl<br>Vit B6: 1 tablet | RHZ: 5 tablets<br>R150: 5 tablets<br>M: 1 tabl<br>Vit B6: 1 tablet |

| | | | | |
|---|---|---|---|---|
| | | | | |
| All treatment arms: continuation phase | RH: 2 tablets<br>Vit B6: 1 tablet | RH: 3 tablets<br>Vit B6: 1 tablet | RH: 4 tablets<br>Vit B6: 1 tablet | RH: 5 tablets<br>Vit B6: 1 tablet |

RHZ=: 150 mg rifampicin, 75 mg isoniazid and 400 mg pyrazinamide;

RHZE = 150 mg rifampicin, 75 mg isoniazid, 400 mg pyrazinamide and 275 mg ethambutol

R150 = 150 mg rifampicin

R300 = 300 mg rifampicin

M = 400 mg Moxifloxacin

Q = 300 mg SQ109

Vit B6 = Pyridoxine 25mg

RH: 150 mg rifampicin, 75 mg isoniazid

**Supplemental Table 3**. **Pyrazinamide population PK model using TBTC data**

| PK Parameter | Definition | Model Estimates | |
|---|---|---|---|
| | | **Population Estimates (RSE%)** | **Inter-individual Variability, CV% (RSE%)** |
| $k_a$ ($hr^{-1}$) | Linear absorption rate | 3.63 (12) | 220 (22) |
| CL/F at 70 kg (L/hr) * | Clearance from the central compartment | 5.06 (3) | 23 (9) |
| V/F at 70 kg (L) * | Volume of distribution of the central compartment | | |
| Females | Female Volume of distribution | 46.5 (4) | 10.9 (15) |
| Males | Male Volume of distribution | 54.2 (2) | |
| Proportional error (%) | Proportional percentage of the residual error | 10 (7) | -- |
| Additive error ($mg/L^{-1}$) | Additive error of the residual error | 0.94 (8) | -- |

* Allometric scaling was used to describe the effect of body weight on both V/F and CL/F, using fixed exponents of 1 and 0.75, respectively: $CL/F = TVCL/F * (WT/70)^{0.75} * \exp(\eta_2)$; $V/F = TVV/F * (WT/70)^{1} * \exp(\eta_3)$.

**Supplemental Table 4.** **Pyrazinamide population PK model using PanACEA MAMS data**

| PK Parameter | Definition | Model Estimates | |
|---|---|---|---|
| | | **Population Estimates (RSE%)** | **Inter-individual Variability, CV% (RSE%) [shrinkage]** |
| MTT (hr) | Mean time from the first transit compartment to the absorption compartment | 1.23 (7) | 58.2 (8) [3%] |
| CL/F (L/hr) | Clearance from central compartment | 4.03 (3) | 24.8 (8) [2%] |
| V/F (L) [a] | Volume of distribution of central compartment | 44.2 (2) | 7.1 (39%) [53%] |
| VCWT (1/kg) | Weight effect coefficient on volume of distribution | 0.016 (13) | -- |
| VCSEX | Sex effect coefficient on volume of distribution | -0.164 (19) | -- |
| Proportional error (%) | Proportional percentage of the residual error | 20.4 (6) | -- |
| Additive error ($mg/L^{-1}$) | Additive error of the residual error | 0.01 FIX | -- |

[a] Typical value for $V_c$ = 44.2 * (1 + 0.016 * (body weight - 54)) * (1 - 0.164 * gender) x exp (η2); for gender, female = 0, and male = 1

**Supplemental Table 5. Survival Analysis with Covariates using TBTC data**

| Parameters | Definition | Estimates (RSE%) |
|---|---|---|
| $\lambda$ ($day^{-1}$) | Typical value for Weibull scale parameter | 0.0183 (1%) |
| $\beta$ | Typical value for Weibull shape parameter | 9.24 (28%) |
| $\lambda$_africa | Effect of non-Africa vs Africa on Weibull scale ($\lambda$) | 0.134 (43%) |
| $\beta$_Cmax (mL/ug) | Slope of linear relationship between PZA Cmax and Weibull shape ($\beta$) | 0.0434 (25%) |
| $\beta$_africa | Effect of non-Africa vs Africa on Weibull shape ($\beta$) | -0.605 (21%) |

shape = $\beta$ * (1+ 0.0434*(Cmax – 31.08)) * (1 - 0.605 * site): for site, Africa = 0 and Non-Africa = 1;

scale= $\lambda$ * (1 + 0.134 * site): for site, Africa = 0 and Non-Africa = 1

**Supplemental Table 6. Culture Conversions at 8 and 12 weeks in TBTC trials, by geographic area and drug exposure**

| | **Culture Conversion at 2 months ($5^{th}$ and $95^{th}$ percentile), %** | **Culture Conversion at 3 months ($5^{th}$ and $95^{th}$ percentile), %** |
|---|---|---|
| **Covariates: Africa and observed Cmax** | | |
| Non-africa, high Cmax (n=16) | 87.5 (68.8, 100) | 100 (81.3, 100) |
| Non-africa, low Cmax (n=19) | 78.9 (57.9, 94.7) | 84.2 (65.7, 100) |
| Africa, high Cmax (n=23) | 73.9 (52.2, 89.2) | 100 (91.3, 100) |
| Africa, low Cmax (n=19) | 68.4 (47.4, 89.5) | 94.7 (78.9, 100) |
| **Covariate: Observed Cmax only** | | |
| high Cmax (n=39) | 79.5 (66.7, 89.7) | 94.9 (87.2, 100) |
| low Cmax (n=38) | 73.7 (57.9, 86.8) | 86.8 (73.7, 94.7) |

(**Note**: high or low Cmax is defined as above or below median value of Cmax, respectively.)

**Supplemental Table 7**. **Survival Analysis with Covariates using PanACEA MAMS data**

| **Parameters** | **Definition** | **Estimates (RSE%)** |
|---|---|---|
| $\lambda$ ($day^{-1}$) | Typical value for Weibull scale | 0.011 (5%) |
| $\beta$ | Typical value for Weibull shape | 1.54 (4%) |
| $\lambda$_PZA_ $C_{max}$ (mL/ug) | Slope of linear relationship between PZA $C_{max}$ and Weibull scale ($\lambda$) | 0.0207 (42%) |
| $\lambda$_RIF_$AUC_{0\text{-}24hr}$ (mL/(ug*hr)) | Exponent of exponential relationship between RIF $AUC_{0\text{-}24hr}$ and Weibull scale ($\lambda$) | 0.0017 (56%) |

scale= $\lambda$ * (1+ 0.0207 * (PZACmax – 37.17)) * ($exp^{0.0017*(RIFAUC0\text{-}24hr - 39.72)}$)

**Supplemental Table 8. Culture Conversions at 8 and 12 weeks in PanACEA MAMS, by pyrazinamide and rifampicin drug exposure.**

| **PanACEA trials** | **Culture Conversion at 2 months ($5^{th}$ and $95^{th}$ percentile), %** | **Culture Conversion at 3 months ($5^{th}$ and $95^{th}$ percentile), %** |
|---|---|---|
| **Covariates: PZA Cmax and RIF $AUC_{0\text{-}24hr}$** | | |
| highPZA Cmax_highRIF **$AUC_{0\text{-}24hr}$** (n=99) | 60.6 (45.7, 74.3) | 87.5 (75.8, 97.1) |
| highPZA Cmax_lowRIF **$AUC_{0\text{-}24hr}$** (n=83) | 33.3 (8.69, 57.8) | 66.7 (38.5, 92.3) |
| lowPZA Cmax highRIF **$AUC_{0\text{-}24hr}$** (n=84) | 52.0 (34.0, 59.6) | 80.0 (63.8, 95.6) |
| lowPZA Cmax_lowRIF **$AUC_{0\text{-}24hr}$** (n=97) | 26.3 (6.25, 50.0) | 51.7 (32.4, 80.0) |

(**Note**: high or low Cmax/$AUC_{0\text{-}24hr}$ is defined as above or below median value of Cmax/$AUC_{0\text{-}24hr}$, respectively.)

**Supplemental Figure 1. Visual Predictive Checks of the popPK model for PZA for TBTC S27/28 trials.**

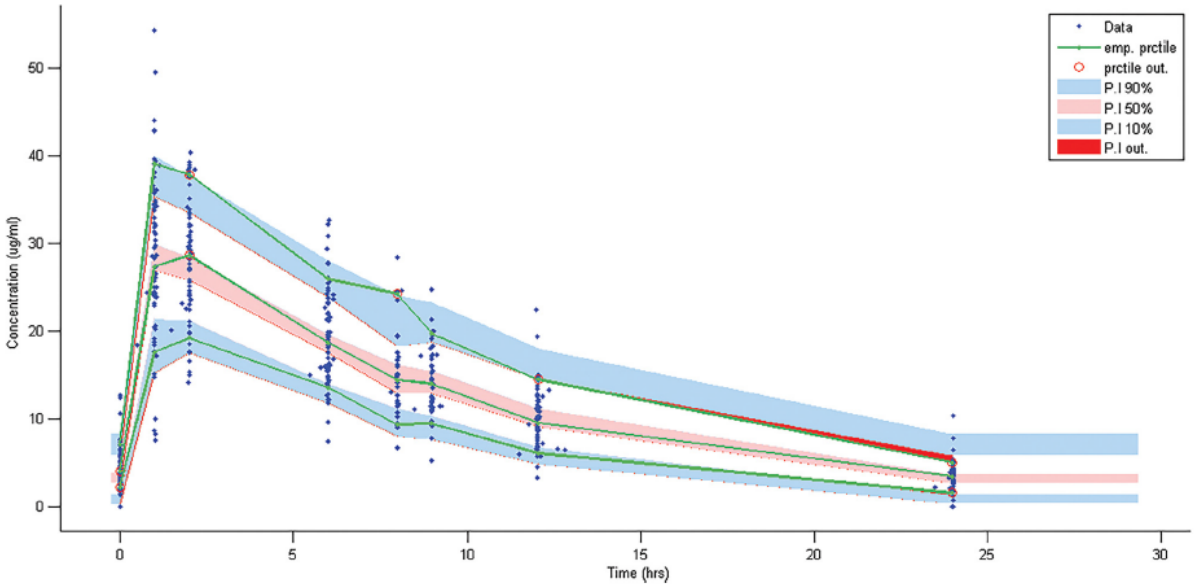
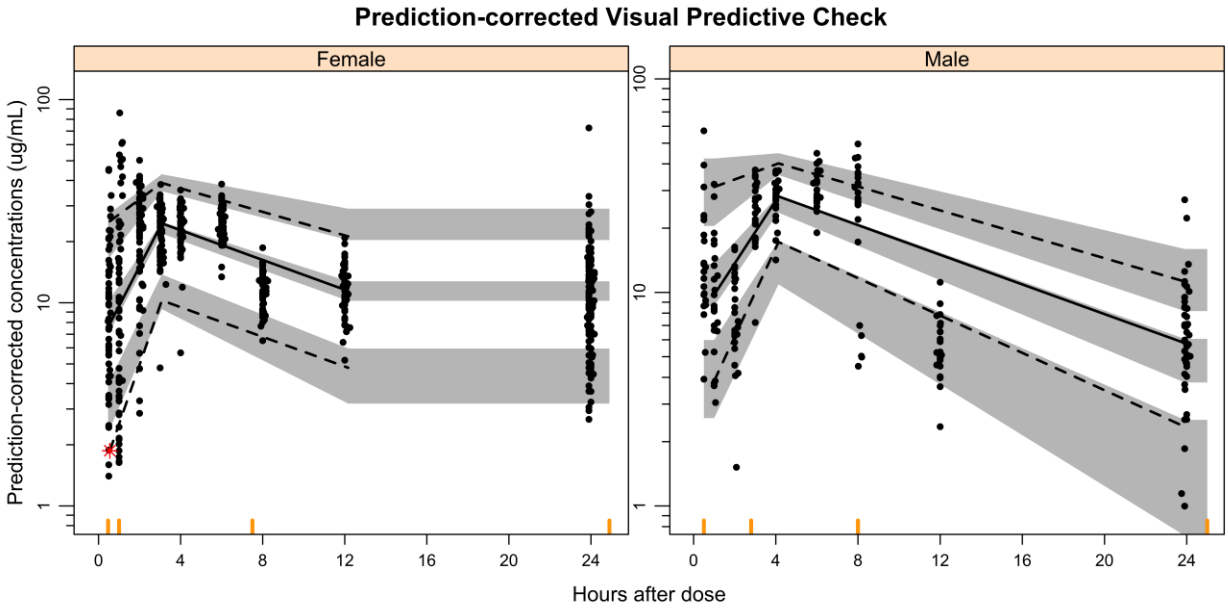


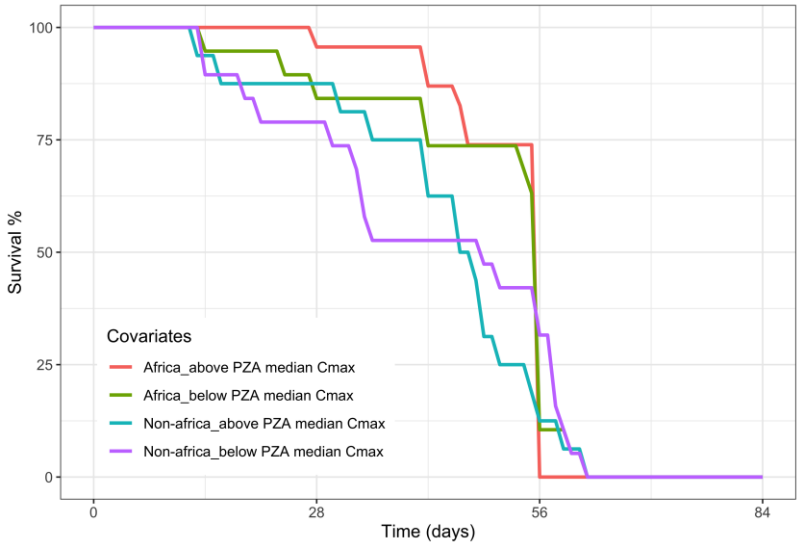

**FIG 3** Visual predictive check (VPC) for PZA concentration versus time on the basis of 1,000 Monte Carlo simulations. Solid green line, the 10th, 50th, and 90th percentiles of the observed data; shaded regions, 90% confidence interval around the 10th, 50th, and 90th percentiles of simulated data; blue diamonds, observed concentrations. emp. prctile, empiric percentile; prctile out., percentile outside of the bounds; and P.I, prediction interval.

**Supplemental Figure 2. Visual Predictive Checks of the popPK model for PZA for PanACEA MAMS-TB trial.**

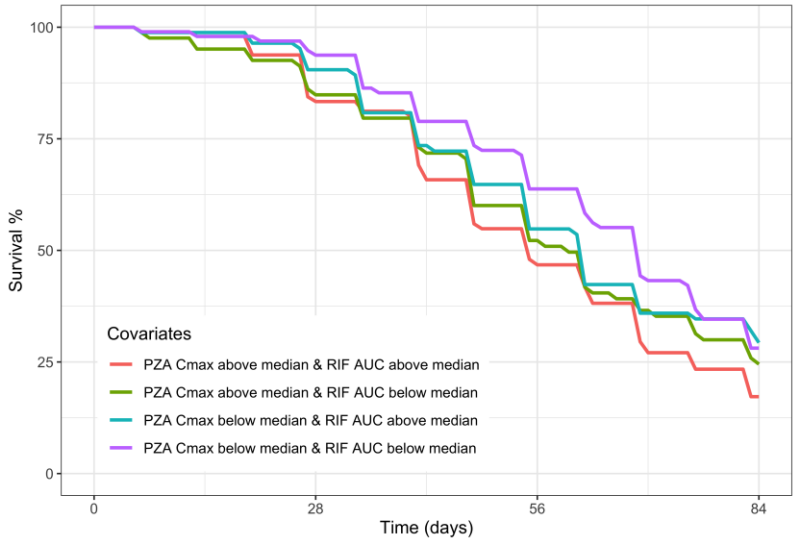


**Supplemental Figure 3. Kaplan Meier Plot of Outcome Data Stratified by Covariates for TBTC S27/28 (a) and PanACEA Trials (b).**

**a) TBTC S27/28 trials**




**b) PanACEA MAMS-TB trial**



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