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 (Θ'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 (ΔOFV = -136.024 with Δdf = 1 and ΔOFV = -24.668 with Δdf = 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 (ΔOFV = -70.56 with Δdf = 1 and ΔOFV = -57.47 with Δdf = 0, respectively).

In the parametric survival analysis, these covariates listed above were tested on scale (λ) and shape (β) 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 HRZE	RHZE: 2 tablets Vit B6: 1 tablet	RHZE: 3 tablets Vit B6: 1 tablet	RHZE: 4 tablets Vit B6: 1 tablet	RHZE: 5 tablets Vit B6: 1 tablet
Arm 1 (R_{35}): $HR_{35}ZE$	RHZE: 2 tablets R300: 3 tablets Vit B6: 1 tablet	RHZE: 3 tablets R150: 1 tablet R300: 3 tablets Vit B6: 1 tablet	RHZE: 4 tablets R300: 5 tablets Vit B6: 1 tablet	RHZE: 5 tablets R300: 7 tablets Vit B6: 1 tablet
Arm 2 (Q): HRZQ	RHZ: 2 tablets Q: 2 tablets Vit B6: 1 tablet	RHZ: 3 tablets Q: 2 tablets Vit B6: 1 tablet	RHZ: 4 tablets Q: 2 tablets Vit B6: 1 tablet	RHZ: 5 tablets Q: 2 tablets Vit B6: 1 tablet
Arm 3 ($R_{20}Q$): $HR_{20}ZQ$	RHZ: 2 tablets R150: 2 tablets Q: 2 tablets Vit B6: 1 tablet	RHZ: 3 tablets R150: 3 tablets Q: 2 tablets Vit B6: 1 tablet	RHZ: 4 tablets R150: 4 tablets Q: 2 tablets Vit B6: 1 tablet	RHZ: 5 tablets R150: 5 tablets Q: 2 tablets Vit B6: 1 tablet
Arm 4 ($R_{20}M$) $HR_{20}ZM$	RHZ: 2 tablets R150: 2 tablets M: 1 tabl Vit B6: 1 tablet	RHZ: 3 tablets R150: 3 tablets M: 1 tabl Vit B6: 1 tablet	RHZ: 4 tablets R150: 4 tablets M: 1 tabl Vit B6: 1 tablet	RHZ: 5 tablets R150: 5 tablets M: 1 tabl Vit B6: 1 tablet

All treatment arms: continuation phase	RH: 2 tablets Vit B6: 1 tablet	RH: 3 tablets Vit B6: 1 tablet	RH: 4 tablets Vit B6: 1 tablet	RH: 5 tablets 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%)
λ (day^{-1})	Typical value for Weibull scale parameter	0.0183 (1%)
β	Typical value for Weibull shape parameter	9.24 (28%)
λ_africa	Effect of non-Africa vs Africa on Weibull scale (λ)	0.134 (43%)
β_Cmax (mL/ug)	Slope of linear relationship between PZA Cmax and Weibull shape (β)	0.0434 (25%)
β_africa	Effect of non-Africa vs Africa on Weibull shape (β)	-0.605 (21%)

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

scale= λ * (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%)**
λ (day^{-1})	Typical value for Weibull scale	0.011 (5%)
β	Typical value for Weibull shape	1.54 (4%)
λ_PZA_ C_{max} (mL/ug)	Slope of linear relationship between PZA C_{max} and Weibull scale (λ)	0.0207 (42%)
λ_RIF_$AUC_{0\text{-}24hr}$ (mL/(ug*hr))	Exponent of exponential relationship between RIF $AUC_{0\text{-}24hr}$ and Weibull scale (λ)	0.0017 (56%)

scale= λ * (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.

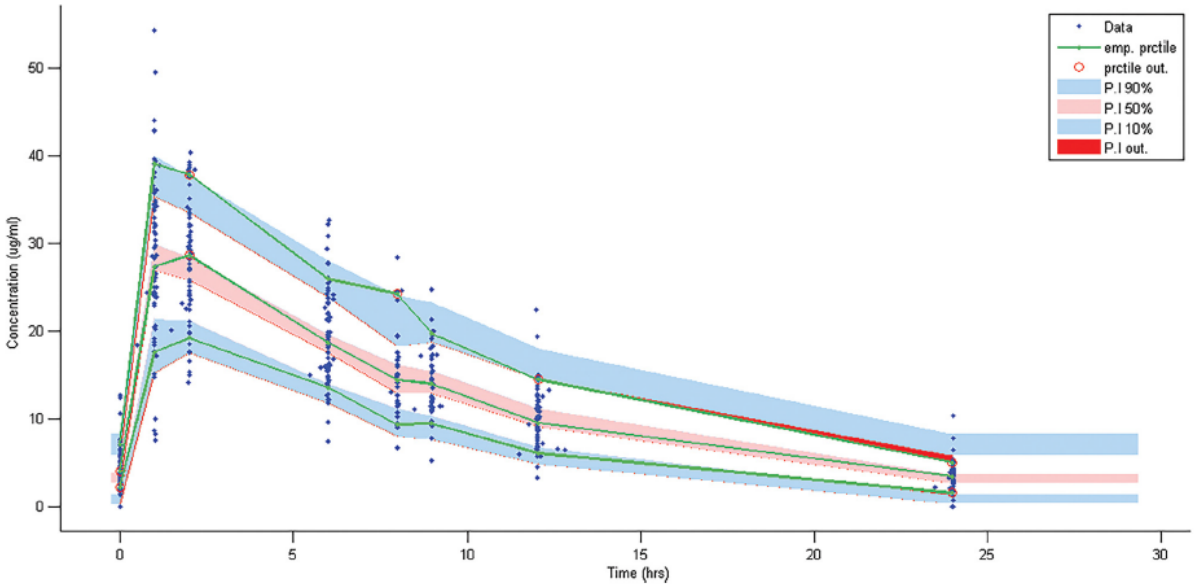
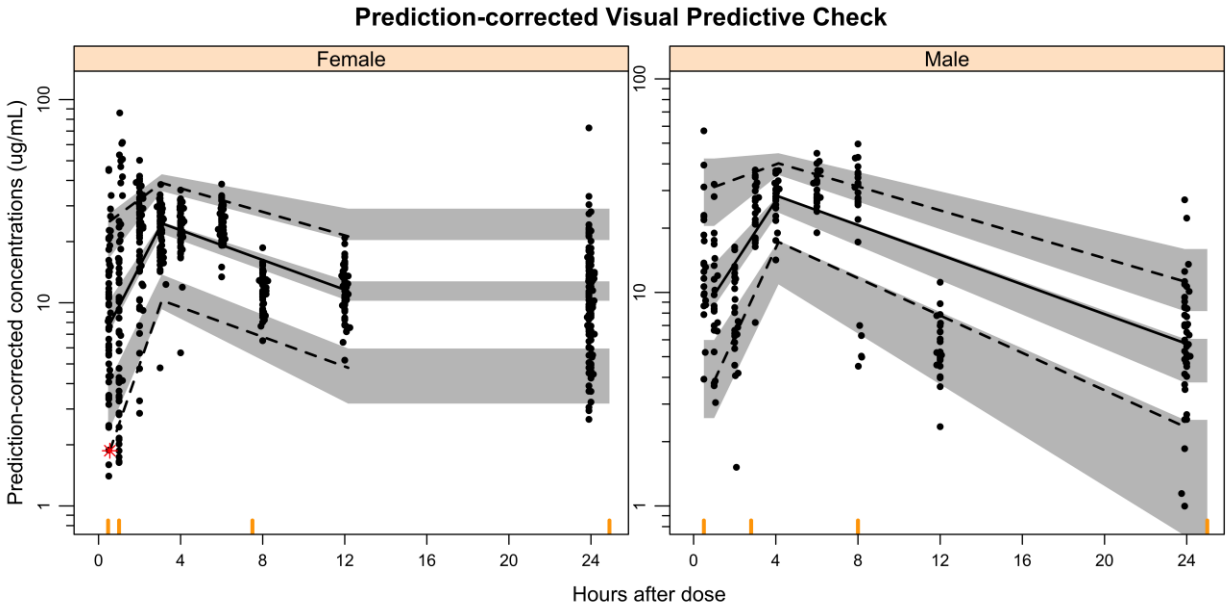


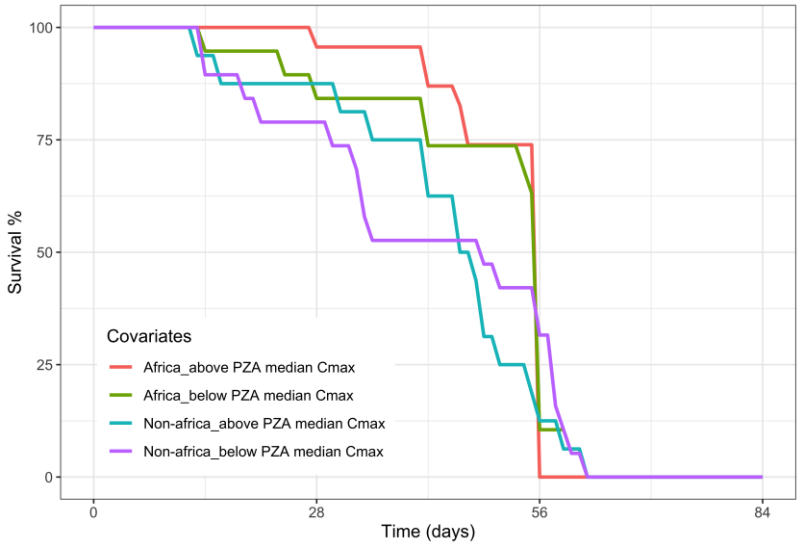
FIG 3 Visual predictive check (VPC) for PZA concentration versus time on the basis of 1,000 Monte Carlo simulations. Solid green line, the 10th, 50th, and 90th percentiles of the observed data; shaded regions, 90% confidence interval around the 10th, 50th, and 90th percentiles of simulated data; blue 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.

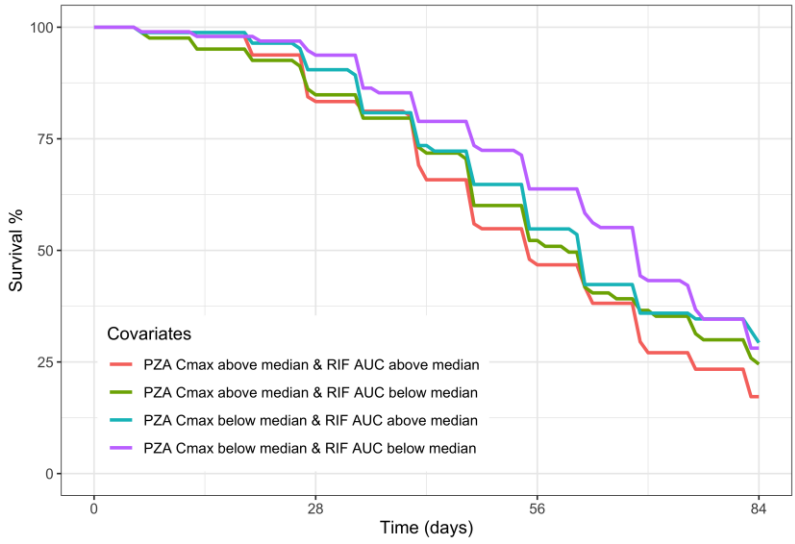


Supplemental Figure 3. Kaplan Meier Plot of Outcome Data Stratified by Covariates for TBTC S27/28 (a) and PanACEA Trials (b).

a) TBTC S27/28 trials



b) PanACEA MAMS-TB trial



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