Longitudinal PKPD biomarkers correlate with treatment outcome in drug sensitive pulmonary tuberculosis; a population pharmacokinetic-pharmacodynamic analysis

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Summary: Systemic exposure to isoniazid and rifampicin correlates with bacillary clearance from sputum and bacillary clearance from sputum correlates with tuberculosis treatment outcome. These findings show the potential of longitudinal PKPD biomarkers for trial design and clinical decision making.

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Abstract

Background: This study aims to explore relationships between baseline demographic covariates, plasma antibiotic exposure, sputum bacillary load and clinical outcome data to help improve future TB treatment response predictions.

Methods: Data were available from a longitudinal cohort study in Malawian drug sensitive TB patients on standard therapy, including steady-state plasma antibiotic exposure (154 patients), sputum bacillary load (102 patients), final outcome (87 patients) and clinical details. Population pharmacokinetic and pharmacokinetic-pharmacodynamic models were developed in the software package $NONMEM$. Outcome data were analysed using univariate logistic regression and Cox proportional hazard models in R, a free software for statistical computing.

Results: Higher isoniazid exposure correlated with increased bacillary killing in sputum (*p<0.01*). Bacillary killing in sputum remained fast, with later progression to biphasic decline, in patients with higher rifampicin $AUC_{0\text{-}24}$ (*p<0.01*). Serial Sputum Colony Counting negativity at month 2 (*P<0.05*), isoniazid C_{MAX} (*p<0.05*), isoniazid C_{MAX}/MIC (*p<0.01*) and isoniazid $AUC_{0\text{-}24}$/MIC (*p<0.01*) correlated with treatment success but not with remaining free of TB. Slower bacillary killing (*P<0.05*) and earlier progression to biphasic bacillary decline (*p<0.01*) both correlate with treatment failure. Post-treatment recurrence only correlated with slower bacillary killing (*P<0.05*).

Conclusions: Patterns of early bacillary clearance matter. Static measurements such as month 2 sputum conversion and pharmacokinetic parameters such as C_{MAX}/MIC and $AUC_{0\text{-}24}$/MIC were predictive of treatment failure, but modelling of quantitative longitudinal data was required to assess the risk of recurrence. Pooled individual patient data analyses from larger datasets are needed to confirm these findings.

Introduction

Tuberculosis (TB) caused an estimated 1.2 million deaths worldwide from 10 million clinical cases in 2018.[1] There is an urgent need for short, effective treatments. The current standard treatment of drug-sensitive TB remains long at six months and efforts to shorten standard TB treatment have been unsuccessful.[2–4] Potent early anti-bacterial activity from short-course moxifloxacin-containing regimens did not translate into non-inferior treatment success rates compared to standard of care regimens in Phase III clinical trials.[2] Ongoing efforts to tackle the problem include new trials of novel drug combinations (e.g. SimpliciTB, ClinicalTrials.gov Identifier: NCT03338621) and consideration of treatment individualisation or stratification, targeting shorter treatment to the patients who are most likely to be cured (e.g. TRUNCATE-TB, ClinicalTrials.gov Identifier: NCT03474198). Both approaches would benefit from a biomarker that predicts final treatment outcome during the early weeks of treatment. This currently does not exist and treatment stratification is currently focused on baseline measurements. Progress would be accelerated by improved understanding of relationships between antibiotic exposure for individual antimicrobial drugs, serial quantitative bacterial load measurements and clinical outcomes. Statistical and mathematical modelling has already enhanced our understanding of the aforementioned relationships.[5–7] Several pharmacodynamic (PD) and pharmacokinetic-pharmacodynamic (PKPD) models have been developed to study the correlation between drug concentrations and the bactericidal effect of treatment.[6–11] These models enable prediction of early drug effect, but analyses become difficult when bacterial loads fall below the limit of quantification. Month two and month three culture sputum culture conversion have been proposed as moderately good predictors of long-term treatment failure or relapse risk between treatment arms of early phase clinical trials.[5] However, the predictive value of culture conversion is weak for individual patient care,[12] hampering robust translation to drug development and clinical programmes.

Our overarching hypothesis is that integrating models describing correlations between individual antibiotic exposure and early anti-bacterial effect with models relating early anti-bacterial effect to

treatment outcome will improve our understanding of treatment response and contribute to improved outcome predictions. The aim of this study was to explore predictors for treatment outcome using baseline demographic, steady-state plasma antibiotic exposure and serial sputum bacillary load data from drug sensitive TB patients. Specific goals were to 1.) fit pharmacokinetic (PK) models to concentration-time data for each of the first-line anti-tuberculosis drugs, 2.) fit a population PKPD model to sputum bacterial load-time data, and 3.) explore associations between early bacillary clearance from sputum and treatment success and recurrence of TB.

Materials and methods

Study population and study design

Data from patients recruited to a longitudinal cohort study of clinical, pharmacological, and bacteriological responses to TB therapy conducted in Queen Elizabeth Central Hospital in Blantyre, Malawi were analysed. The Liverpool School of Tropical Medicine and the College of Medicine Research Ethics Committee, University of Malawi gave ethical approval for this study. Longitudinal PD and outcome data from this patient cohort has been published.[13] The current study extends that work with PKPD analyses.

Consenting adults aged 16-65 years with sputum smear-positive pulmonary TB were eligible. Exclusion criteria included haemoglobin<6g/dL, creatinine>177 μmol/l, total bilirubin>51 μmol/l, alanine transaminase >200 IU/l, clinical status suggestive of imminent mortality, pregnancy, prior TB treatment within five years, concurrent corticosteroid therapy or baseline resistance to rifampicin and isoniazid using the Genotype MTBDRplus 2.0 line probe assay (LPA, Hain Life Sciences). Patient characteristics have been reported previously.[13] Alcohol consumption (any beer or spirits) and smoking were reported as binary covariates based on practice at the time of study recruitment. Percentage of abnormal lung-field observed on baseline chest radiographs (CXR) was assessed by consensus of two independent readers. On binary variables (e.g. presence / absence of cavities): the reviewers met to reach consensus on any discrepancies. On continuous variables

(e.g. % lung-field affected), an average of the two scores was taken. Participants received daily Fixed Dose Combination (FDC) tablets containing a standard first-line WHO-approved regimen according to the Malawi Ministry of Health National TB Control Programme Manual 6th Edition 2007. The detail is as follows: For the Intensive Phase of therapy fixed dose RZHE tablets (rifampicin 150mg, isoniazid 75mg, pyrazinamide 400mg, ethambutol 275mg) were used. For the Continuation Phase, RH tablets (rifampicin 150mg, isoniazid 75mg) were used. Weight bands for both phases were: 30-37kg - 2 tablets, 37-54kg - 3 tablets, 54-74kg - 4 tablets, $$74kg - 5 tablets. Adherence was monitored by direct questioning and pill counts. All patients had point of care HIV serology. Anti-retroviral therapy (ART) was provided per national protocols.

Clinical endpoint definitions

An endpoint of stable cure was reported for patients who were clinically well with two consecutive negative sputum cultures by the End of Treatment (EOT) and no TB recurrence in the subsequent 12 months. Treatment failure was reported for patients with positive sputum culture at EOT. TB recurrence was reported for patients who appeared to be cured at EOT but re-presented with TB disease, i.e. reinfection or relapse, in the next 12 months. The LPA was repeated on positive EOT or post-treatment *Mycobacterium tuberculosis* (*Mtb*) isolates from treatment failures or TB recurrences respectively.

Bacterial load measurement

Patients had overnight sputum samples collected on day 0, 2, 4, 7, 14, 28, 49, and 56 of treatment. Auramine-phenol microscopy was done on direct and concentrated sputum smears at all time points. Two 1ml aliquots were used for sputum bacillary load measurement on solid (Serial Sputum Colony Counting [SSCC]) and liquid (Mycobacterial Growth Indicator Tube [MGIT]) culture. All patients submitted spot sputum samples after 5 months of therapy (EOT samples) to assess bacteriological cure. Those with ongoing or recurrent symptoms submitted post treatment samples to test for relapse. Standard bacteriological methods for SSCC (results reported as $\log_{10}$ colony

forming units (CFU)/ml) and MGIT (results reported as days-to-positivity but analysed in this paper as a binary positive/negative result at month 1 and 2) were as previously described.[13] Only patients who contributed at least two bacterial load measurements could be included in PKPD models, as a bacterial clearance rate cannot be calculated from a single measurement.

Minimum Inhibitory Concentration (MIC) assays

Mtb isolates from baseline sputum cultures of all patients were stored at -70^{o}C for bioanalysis on UKMYC sensititre plates (Thermofisher). Plates for this project were custom-configured by the manufacturer; the wells contained dry microdilutions of lyophilised antibiotics across concentrations which allowed careful MIC titration within the drug-susceptible range (two antibiotic-free control wells; doubling dilutions from 16-0.015 mg/L for rifampicin and isoniazid; doubling dilutions from 8-0.25mg/L for ethambutol).

Mtb Isolates were revived in Middlebrook 7H9 broth, then sub-cultured onto Middlebrook 7H11 agar. Colonies were emulsified in 0.2% saline-tween with glass beads, and vortexed for 30 seconds. Turbidity was adjusted to 0.5 McFarland Standard. 100 ul from each suspension were transferred into 11ml Middlebrook 7H9 broth, supplemented with oleic acid-albumin-dextrose-catalase, to give an inoculum of 10^5 cfu/ml. 100 ul of this material from each isolate were inoculated, in duplicate, into plate wells covering all concentrations of all drugs. Plates were covered with permanent plastic seals and incubated at 37°C in 5% CO_2. Plates were checked for contamination at 24 and 48 hours, then monitored at 10, 14, and 21 days. Results were read when growth was clearly visible in the antibiotic-free control wells. For each antibiotic, the lowest concentration with no visible growth was considered to be the MIC. Each plate was read by two independent readers. The MIC result recorded by the first reader was the test result. The second reader's result was used to assess inter-reader agreement.

Antibiotic plasma concentration measurement

Blood collections to measure steady-state isoniazid, rifampicin, pyrazinamide, and ethambutol concentrations were undertaken on day 14 or 21 of treatment. Patients attended the study clinic after an overnight fast at 07:30 hours. Samples were collected pre-dose, then 2 and 6 hours after medications. To allow patients to return home before nightfall, 6 hours post-dose was the latest sampling timepoint. Similar field-based clinical studies in southern Africa[14] have deployed similar collection strategies. Plasma was separated by centrifugation and stored at -70^{o}C until batched analysis. Rifampicin, isoniazid and ethambutol concentrations were determined using previously published liquid chromatographic/tandem mass spectrometry methods[15,16] with appropriate internal standards validated to internationally recognised acceptance criteria as previously described.[17] Pyrazinamide concentrations were measured by HPLC using an Ultraviolet visible (UV-Vis) absorption detector.

Pharmacokinetics

Population pharmacokinetic models were developed using the Stochastic Approximation Expectation Method in the Fortran based software package NONMEM[18] with a g95 Fortran compiler. Empirical Bayes $AUC_{0\text{-}24}$ and C_{MAX} estimates were derived for each patient and evaluated as predictors on bacillary clearance and treatment outcome. Details on pharmacokinetic model building can be found in the supplementary materials.

Pharmacokinetics-pharmacodynamics

Bacillary clearance from sputum can be captured using mono-phasic (Fig. 2, profile A) or bi-phasic models (Fig. 2, profile B and C). Our PKPD model used the parameter LAM to represent the slope of a monophasic bacterial decay curve or the first phase of a bi-phasic bacterial decay curve (Fig. 2). For bi-phasic clearance the magnitude of decline in bacterial clearance was parameterised in this model by BETA and the time it takes to switch from fast killing to slow killing was

parameterised using the parameter $t_{1/2}$ (Fig. 2). Details on PKPD model building can be found in the supplementary materials.

P-values of 0.05 and 0.01 were used as cut-offs in the forward inclusion and backward elimination step of stepwise covariate model building. Covariates, to be evaluated on PKPD model parameters, were selected based on physiological plausibility and a correlation matrix with post-hoc pharmacodynamic parameter estimates from the baseline model. The following parameters were consequently evaluated on baseline sputum bacillary load using an exponential relationship: age, body mass index (BMI), percentage of abnormal lung-field on CXR , and baseline bilirubin and creatinine concentrations. Isoniazid and rifampicin $AUC_{0\text{-}24}$ and C_{MAX}, BMI, baseline serum bilirubin and creatinine concentrations, HIV infection, current alcohol consumption and treatment adherence were evaluated on θ_{LAM}. $AUC_{0\text{-}24}$ and C_{MAX} of all four study drugs, age, BMI, percentage of abnormal lung-field on CXR, baseline bilirubin and baseline creatinine were evaluated on $\theta_{T_{1/2}}$. $AUC_{0\text{-}24}$ and C_{MAX} of all four study drugs, age, BMI, percentage of abnormal lung-field on CXR, baseline bilirubin and creatinine concentrations were evaluated on θ_{β}. MIC adjusted isoniazid and rifampicin $AUC_{0\text{-}24}$ were evaluated on a subset of patients with available MIC data. $AUC_{0\text{-}24}$/MIC was substituted for $AUC_{0\text{-}24}$ equivalents in the final model and statistical significance was tested using a back-ward elimination approach.

Empirical Bayes estimates for parameters LAM, BETA and $T_{1/2}$ were derived for each patient and evaluated as predictors for treatment outcome.

Study outcome

Univariate logistic regression was used to investigate the role of clinical, pharmacokinetic and bacteriological factors as explanatory variables to predict stable cure and failure. A Cox-proportional hazard model was used to investigate the role of clinical, pharmacokinetic and bacteriological factors as explanatory variables to predict recurrence amongst patients that had stable cure at EOT. Bacteriological factors were categorised as static measurements (e.g. sputum

smear and culture [SSCC or MGIT] conversion to negative at end of month one or two) or dynamic PKPD model derived parameters (e.g. LAM, $T_{1/2}$ and BETA). Correlations between MIC adjusted isoniazid, rifampicin or ethambutol exposure (C_{MAX} or AUC_{0-24}) and outcome (failure or recurrence) were evaluated on patients with available MIC data.

Results

Plasma antibiotic concentration-time data were available from 154 patients (Table 1). 52 patients contributed fewer than 2 bacterial load samples leaving 102 for the PKPD analysis. Seven patients withdrew from the study before reaching EOT, 3 upon the patients' request, 3 according protocol and 1 patient was lost to follow-up, leaving 95 patients for the treatment failure outcome analysis. Five patients failed treatment. Amongst the 90 successfully treated patients one withdrew from the study immediately after EOT and consequently did not participate in the follow-up phase leaving 89 patients for recurrence outcome analysis. Amongst these patients 78 remained TB free and 4 had recurrent infection. Seven patients were censored as 5 were lost to follow-up, and 2 died of non-tuberculous causes during post-treatment follow-up (S1 Fig.).

87% of patients reported no missed treatment doses, 9% reported 1-2 missed doses and 7/143 (5%) reported ≥3 missed doses. All patients had rifampicin and isoniazid susceptible TB at baseline. MICs for isoniazid, rifampicin and ethambutol, available from 47 patients with clinical outcome data, confirmed that the *Mtb* isolates were extremely sensitive to the antibiotics used (Table 1). Of the 5 treatment failures, one had isoniazid and rifampicin resistance detected on LPA at EOT, suggesting acquisition of multi drug resistant (MDR-)TB during therapy. Of the four TB recurrences, one had isoniazid mono-resistance detected at symptomatic re-presentation, suggesting either acquisition of resistance on treatment or re-infection with an isoniazid mono-resistant strain. A baseline profile of the study cohort is shown in Table 1.

Pharmacokinetics

Visual predictive checks (VPCs) and goodness of fit diagnostics (GOFs) for the isoniazid, rifampicin, pyrazinamide and ethambutol models showed adequate predictive performance (Fig. 1; S2 Fig.; S3 Fig.; S1 Table). Model derived median C_{MAX} estimates and ranges for the study cohort were: isoniazid, 3.24 (2.19-5.50) mg/l; rifampicin, 4.35 (2.30-12.30) mg/l; pyrazinamide, 40 (25-63) mg/l; and ethambutol, 2.30 (1.43-4.40) mg/l. Median AUC_{0-24} estimates and ranges were: isoniazid, 18.83 (6.97-71.20) hxmg/l rifampicin, 29.10 (14.50-119.00) hxmg/l; pyrazinamide, 419 (210-2014) hxmg/l; and ethambutol 18.50 (12.80-38.50) hxmg/l.

Pharmacokinetics-pharmacodynamics

Isoniazid AUC_{0-24} at steady state and baseline serum bilirubin concentrations positively correlated ($p < 0.01$) in an exponential relation with LAM (Fig. 2) in the PKPD model (Fig. 3; S4 Fig.; S5 Fig.), resulting in a steeper bacillary kill-curve in sputum with higher isoniazid exposures and baseline bilirubin concentrations (Fig. 4; S2 Table). Rifampicin AUC_{0-24} at steady state in an exponential relation positively correlated ($p < 0.01$) with $t_{1/2}$ (Fig. 2), resulting in more pronounced bi-phasic kill-curves with lower rifampicin concentrations (Fig. 4; S2 Table). Alcohol consumption correlated with a slower LAM, resulting in a steeper bacillary kill-curve in sputum in non-alcohol consuming patients (Fig. 4; S2 Table).

Step-wise backward exclusion of isoniazid AUC_{0-24}/MIC, when replacing AUC_{0-24}, as covariate on LAM resulted in significant worsening of the model fit on a subset of the patients that had MICs reported (S6 Fig.; S3 Table). Step-wise backward exclusion of rifampicin AUC_{0-24}/MIC, when replacing AUC_{0-24}, as covariate on $t_{1/2}$ resulted in a model that did not converge on the same subset of patients (S6 Fig.; S3 Table). As per protocol, MIC adjusted PK parameters for ethambutol were not evaluated here because neither MIC unadjusted ethambutol AUC_{0-24} or C_{MAX} was significantly correlated with LAM, $t_{1/2}$ or BETA.

Study outcome

From the static bacteriological treatment response measurements, only SSCC negativity at month 2 correlated with the likelihood of treatment failure (Table 2). Amongst the pharmacokinetic variables, higher isoniazid C_{MAX}, C_{MAX}/MIC and AUC_{0-24}/MIC correlated with treatment success (Table 2 and 3). MIC adjusted correlations were stronger when compared to MIC unadjusted equivalents (Table 3).

From the dynamic measurements of treatment response lower model derived LAM and higher model derived BETA correlated with failure ($p < 0.05$) on univariate generalised logistic regression (Table 2). Lower LAM was also the only variable from any analysis which associated with TB recurrence ($p < 0.05$) (Table 2 and Table 3).

Discussion

C_{MAX} and AUC_{0-24} estimates for each drug from our PK models were consistent with prior studies in African populations.[14,19–21] Rifampicin exposure was low, supporting the ongoing case for increasing current rifampicin dosages for TB treatment.[22] Our PKPD model adequately captured the typical bacillary clearance profile and demonstrated that inter-individual variability in antibiotic exposure influences treatment response. Early bacterial clearance (characterised by the LAM parameter) is faster with higher isoniazid AUC_{0-24}, and bacillary clearance remains rapid, with later progression to biphasic decline, with higher rifampicin AUC_{0-24}. These findings are consistent with prior reports that the bactericidal effect of isoniazid is enhanced by co-administration with rifampicin.[6] Although the model structure was rather empirical in it's description of decreasing bactericidal effect over time we did not consider more complex mechanistic PKPD models in order to avoid over parameterisation.[23]

Elevated serum bilirubin levels have previously been reported with prolongation of isoniazid clearance[24] and may also correlate with higher exposure of the other two hepatically cleared drugs

rifampicin and pyrazinamide. This may be considered as a partial measure of the ability of the body to clear these antibiotics.

Static bacteriological measurements (e.g. sputum smear or culture conversion at month 1 or 2) are commonly used to monitor TB treatment response in clinical practice and early phase clinical trials and in this study SSCC at month 2 predicted final outcome. Some larger analyses of pooled individual patient data have described a similar relationship between month 2 culture results and outcome although it has also been noted that correlations are not predictive at individual patient level.[5,12]

MIC adjusted PK estimates, i.e. $AUC_{0\text{-}24}$/MIC or C_{MAX}/MIC, have been reported as marker of the sterilising effect of anti-TB drugs, in line with these findings our PKPD model displayed significant correlations in a subset of patients (S3 Table).[25] It showed significant correlations between isoniazid C_{MAX}, C_{MAX}/MIC and $AUC_{0\text{-}24/MIC}$ and treatment success, but not with remaining free of TB (Table 3).

Caution is required when interpreting the findings that isoniazid C_{MAX} correlates separately with LAM and with treatment failure; co-linearity between explanatory variables on univariate analyses could bias interpretation of the results where isoniazid drives early bacillary clearance from sputum, which subsequently correlates with treatment failure. Multivariate regression sometimes resolves such issues but was not feasible in this dataset of 95 patients with only 5 failures. Overall, delineation of a relationship between antibiotic PK and treatment response remains noteworthy. It underlines the role of PK data in explaining trial results and indicates that treatment shortening decisions for individual patients requires consideration of antibiotic exposure at steady state, e.g. 2 months. A large recent meta-analysis seeking to define an “easy-to-treat” patient phenotype explicitly commented on the lack of PK data for assessment.[26]

Our dynamic, model-derived, parameters of bacterial clearance over the first 8 weeks of treatment correlated with treatment failure (LAM and BETA) and recurrence (LAM). These results indicate

that the pattern of early bacillary clearance matters. The finding that LAM was the only predictor of relapse suggests that efficient use of quantitative longitudinal data at the level of individual patients may be preferable to reliance on traditional static measurements of bacteriological response.[27]

Our study has limitations. Isolates from recurrent TB infections were not sequenced and consequently it remained unclear whether these were re-infections or relapses. Moreover, the PKPD model remains descriptive rather than predictive, due to inability to split data into training and test batches. Antimicrobial concentration ranges were restricted by standard dosing guidelines, yet a concentration-effect relationship could be characterised. However, the model is not suitable for extrapolations and dose optimisations due to the absence of a concentration-effect relationship that was characterised over an extended concentration range. MIC data were not available for every patient and were not available at all for pyrazinamide, hindering efforts to account for the variability added to PKPD models by incorporating MIC measurements to indices such as $AUC_{0\text{-}24}$/MIC or C_{MAX}/MIC.[25] More comprehensive MIC data may be of particular importance in global settings where clinical *Mtb* isolates are less uniformly antibiotic sensitive than in Malawi.

A large pooled individual patient data analysis, ideally with dose escalating studies, would enable further characterisation of early bacillary clearance from sputum, and provide improved statistical power to correlate early bacillary clearance with treatment failure and recurrence. Nonetheless, the data presented here illustrates the value of PKPD and dynamic bacillary clearance modelling techniques which could be applicable beyond the drug combination used in this study. More extensive use of this approach may improve regimen evaluation in clinical trials. Moreover, it could help generate algorithms to assist with clinical decision making for individualised or stratified treatment strategies. These algorithms can take into account how well the patient is doing during early phases of treatment rather than just baseline measures of on how well the patients is before treatment starts.

Conclusion

In conclusion, isoniazid and rifampicin exposure correlate with bacillary clearance from sputum during the first 8 weeks of treatment for pulmonary TB and bacillary clearance correlates with clinical outcome. A pooled individual patient data analysis is needed to validate the range of early pharmacokinetic and sputum bacillary clearance effects which predict treatment failure and relapse.

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Transparency declarations

The authors have declared that no competing interests exist.

References

1. World Health Organization. Global tuberculosis report 2019. In: *Global tuberculosis report 2019*., 2019.

2. Gillespie SH, Crook AM, McHugh TD *et al.* Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. *New England Journal of Medicine* 2014; **371**: 1577–87.

3. Jindani A, Harrison TS, Nunn AJ *et al.* High-dose rifapentine with moxifloxacin for pulmonary tuberculosis. *New England Journal of Medicine* 2014; **371**: 1599–608.

4. Merle CS, Fielding K, Sow OB *et al.* A four-month gatifloxacin-containing regimen for treating tuberculosis. *New England Journal of Medicine* 2014; **371**: 1588–98.

5. Phillips PP, Fielding K, Nunn AJ. An evaluation of culture results during treatment for tuberculosis as surrogate endpoints for treatment failure and relapse. *PloS one* 2013; **8**: e63840.

6. Jindani A, Doré CJ, Mitchison DA. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. *American journal of respiratory and critical care medicine* 2003; **167**: 1348–54.

7. Rustomjee R, Lienhardt C, Kanyok T *et al.* A phase ii study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *The international journal of tuberculosis and lung disease* 2008; **12**: 128–38.

8. Clewe O, Aulin L, Hu Y, Coates AR, Simonsson US. A multistate tuberculosis pharmacometric model: A framework for studying anti-tubercular drug effects in vitro. *Journal of Antimicrobial Chemotherapy* 2015; **71**: 964–74.

9. Chen C, Ortega F, Rullas J *et al.* The multistate tuberculosis pharmacometric model: A semi-mechanistic pharmacokinetic-pharmacodynamic model for studying drug effects in an acute

tuberculosis mouse model. *Journal of pharmacokinetics and pharmacodynamics* 2017; **44**: 133–41.

10. Svensson RJ, Simonsson US. Application of the multistate tuberculosis pharmacometric model in patients with rifampicin-treated pulmonary tuberculosis. *CPT: pharmacometrics & systems pharmacology* 2016; **5**: 264–73.

11. Chen C, Wicha SG, Knegt GJ de *et al.* Assessing pharmacodynamic interactions in mice using the multistate tuberculosis pharmacometric and general pharmacodynamic interaction models. *CPT: pharmacometrics & systems pharmacology* 2017.

12. Phillips PP, Mendel CM, Burger DA *et al.* Limited role of culture conversion for decision-making in individual patient care and for advancing novel regimens to confirmatory clinical trials. *BMC medicine* 2016; **14**: 19.

13. Sloan DJ, Mwandumba HC, Garton NJ *et al.* Pharmacodynamic modeling of bacillary elimination rates and detection of bacterial lipid bodies in sputum to predict and understand outcomes in treatment of pulmonary tuberculosis. *Clinical infectious diseases* 2015; **61**: 1–8.

14. Denti P, Jeremiah K, Chigutsa E *et al.* Pharmacokinetics of isoniazid, pyrazinamide, and ethambutol in newly diagnosed pulmonary tb patients in tanzania. *PloS one* 2015; **10**: e0141002.

15. Srivastava A, Waterhouse D, Ardrey A, Ward SA. Quantification of rifampicin in human plasma and cerebrospinal fluid by a highly sensitive and rapid liquid chromatographic–tandem mass spectrometric method. *Journal of pharmaceutical and biomedical analysis* 2012; **70**: 523–8.

16. Chen X, Song B, Jiang H, Yu K, Zhong D. A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of isoniazid and ethambutol in human plasma. *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry* 2005; **19**: 2591–6.

17. Van Oosterhout J, Dzinjalamala F, Dimba A *et al.* Pharmacokinetics of anti-tuberculosis drugs in hiv-positive and hiv-negative adults in malawi. *Antimicrobial agents and chemotherapy* 2015: AAC–01193.

18. Beal S, Sheiner L, Boeckmann A, Bauer R. NONMEM user's guides.(1989–2013), icon development solutions, ellicott city, md, usa, 2013. 2013.

19. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrobial agents and chemotherapy* 2006; **50**: 1170–7.

20. Tappero JW, Bradford WZ, Agerton TB *et al.* Serum concentrations of antimycobacterial drugs in patients with pulmonary tuberculosis in botswana. *Clinical infectious diseases* 2005; **41**: 461–9.

21. Stott K, Pertinez H, Sturkenboom M *et al.* Pharmacokinetics of rifampicin in adult tb patients and healthy volunteers: A systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy* 2018; **73**: 2305–13.

22. Svensson EM, Svensson RJ, Te Brake LH *et al.* The potential for treatment shortening with higher rifampicin doses: Relating drug exposure to treatment response in patients with pulmonary tuberculosis. *Clinical Infectious Diseases* 2018; **67**: 34–41.

23. Jacobs M, Grégoire N, Couet W, Bulitta JB. Distinguishing antimicrobial models with different resistance mechanisms via population pharmacodynamic modeling. *PLoS computational biology* 2016; **12**: e1004782.

24. Weber WW, Hein DW. Clinical pharmacokinetics of isoniazid. *Clinical pharmacokinetics* 1979; **4**: 401–22.

25. Chigutsa E, Pasipanodya JG, Visser ME *et al.* Impact of nonlinear interactions of pharmacokinetics and mics on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. *Antimicrobial agents and chemotherapy* 2015; **59**: 38–45.

26. Imperial MZ, Nahid P, Phillips PP *et al.* A patient-level pooled analysis of treatment-shortening regimens for drug-susceptible pulmonary tuberculosis. *Nature medicine* 2018; **24**: 1708.

27. Mueller M, Pena A de la, Derendorf H. Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: Kill curves versus mic. *Antimicrobial agents and chemotherapy* 2004; **48**: 369–77.

Figure legends

Fig. 1: Simulation based (n=2,000) Visual Predictive Checks (VPCs) for isoniazid (top left), rifampicin (top right), pyrazinamide (bottom left) and ethambutol (bottom right). Open circles represent observations, solid and dashed black lines represent observed 2.5, 50th and 97.5 percentiles. Shaded areas represent the 90% confidence intervals around the simulated 2.5, 50th and 97.5 percentiles.

Fig. 2: Visualisation of PKPD model characteristics. The solid line (*A*) illustrates monophasic bacillary clearance from sputum with a clearance rate represented by LAM. The dashed (*B*) and dotted (*C*) lines illustrate bi-phasic bacillary clearance trajectories; LAM represents the initial clearance rate (faster in *B* than *C* line), BETA represents the magnitude of decreased bacillary clearance (larger in *C* than *B*) and $T_{1/2}$ represents time it takes to switch from fast to slow killing (earlier in *B* than *C*).

Fig. 3: Simulation based (n=2,000) VPCs for the PKPD model. Open circles in the upper panel represent observations, solid and dashed black lines represent observed 50th and 97.5 percentiles. Shaded areas in the upper panel represent the 90% confidence intervals around the simulated 2.5, 50th and 97.5 percentiles. The dots and solid line in the lower panel represent the observed proportion of samples below the limit of quantification and the shared area represent the corresponding 90% confidence interval of proportion samples below limit of quantification produced by the model. Bars at the bottom of the lower panel indicate the binning windows.

Fig. 4: Visualisation pharmacokinetic-pharmacodynamic covariate effects (baseline bilirubin - LAM, isoniazid - LAM, rifampicin - $_{t1/2}$ and alcohol consumption - LAM). High and low exposure refers to highest and lowest values in the study population: 1-32 μmol/l, 54.9-515 hrxμmol/l, and 19.9-145 hrxμmol/l for baseline bilirubin, isoniazid and rifampicin, respectively.

Figure 1

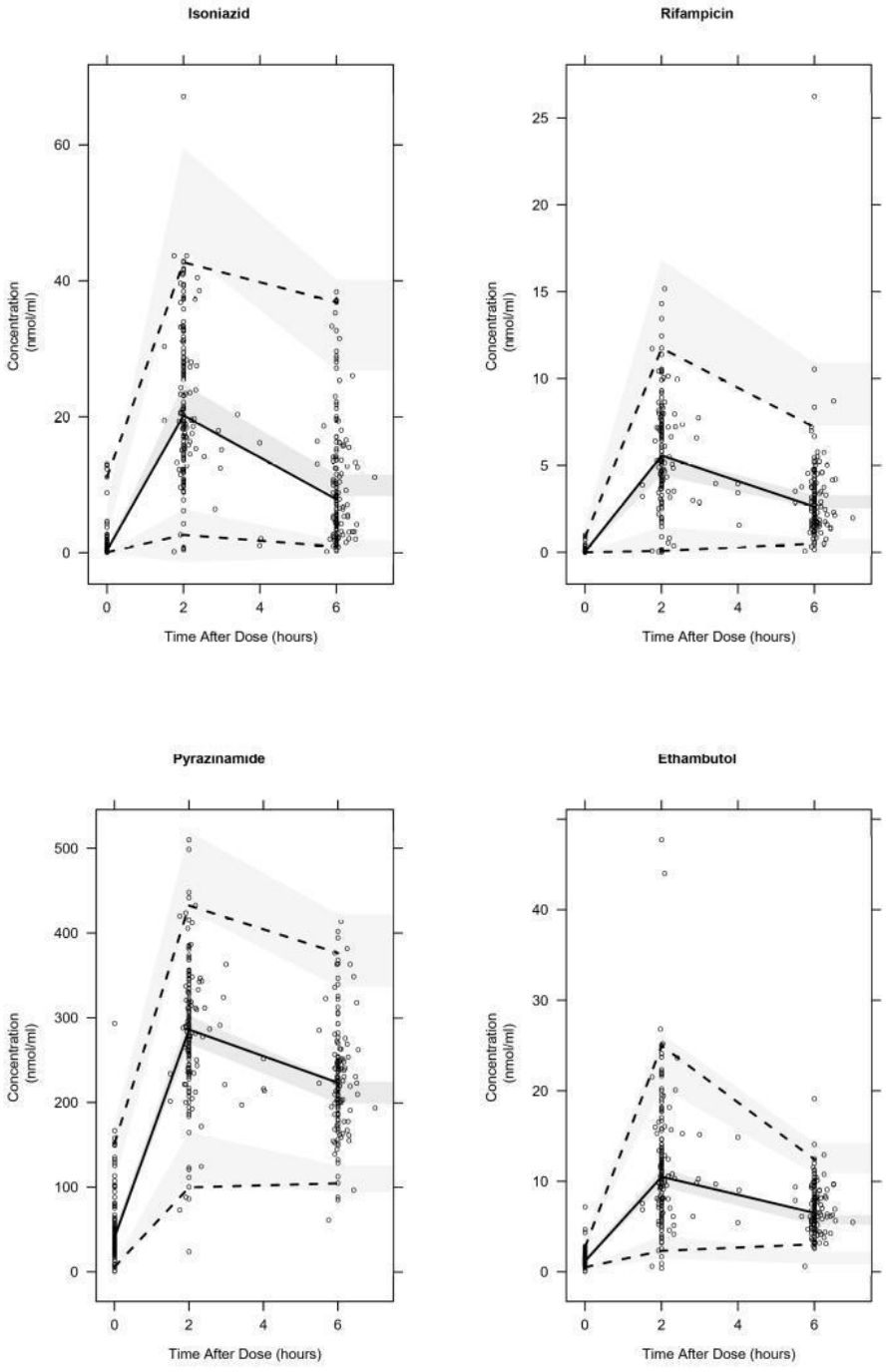

Figure 2

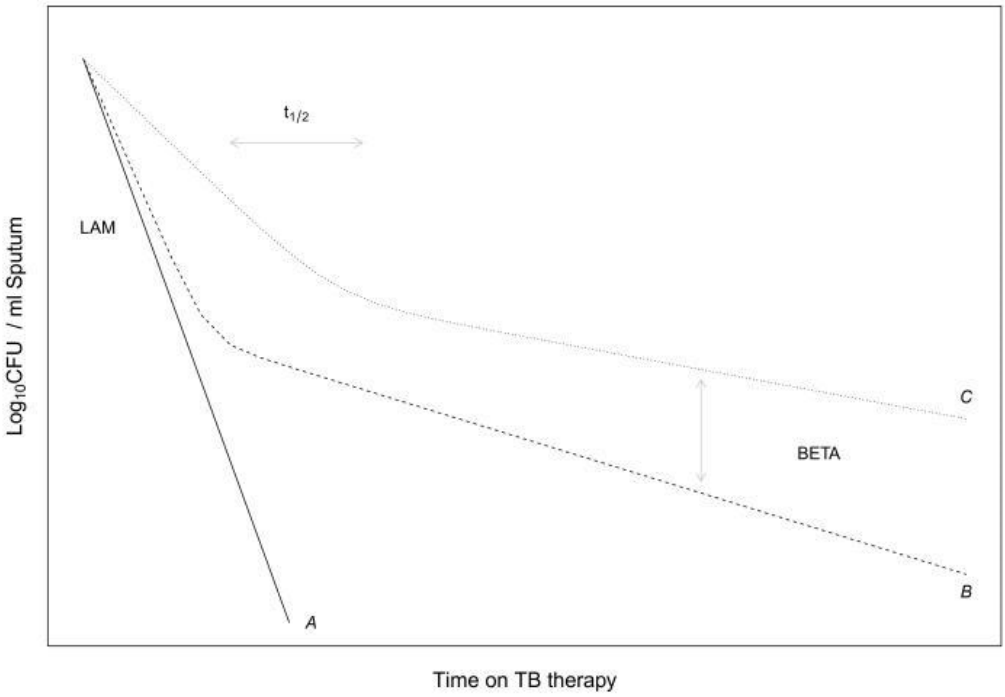

Figure 3

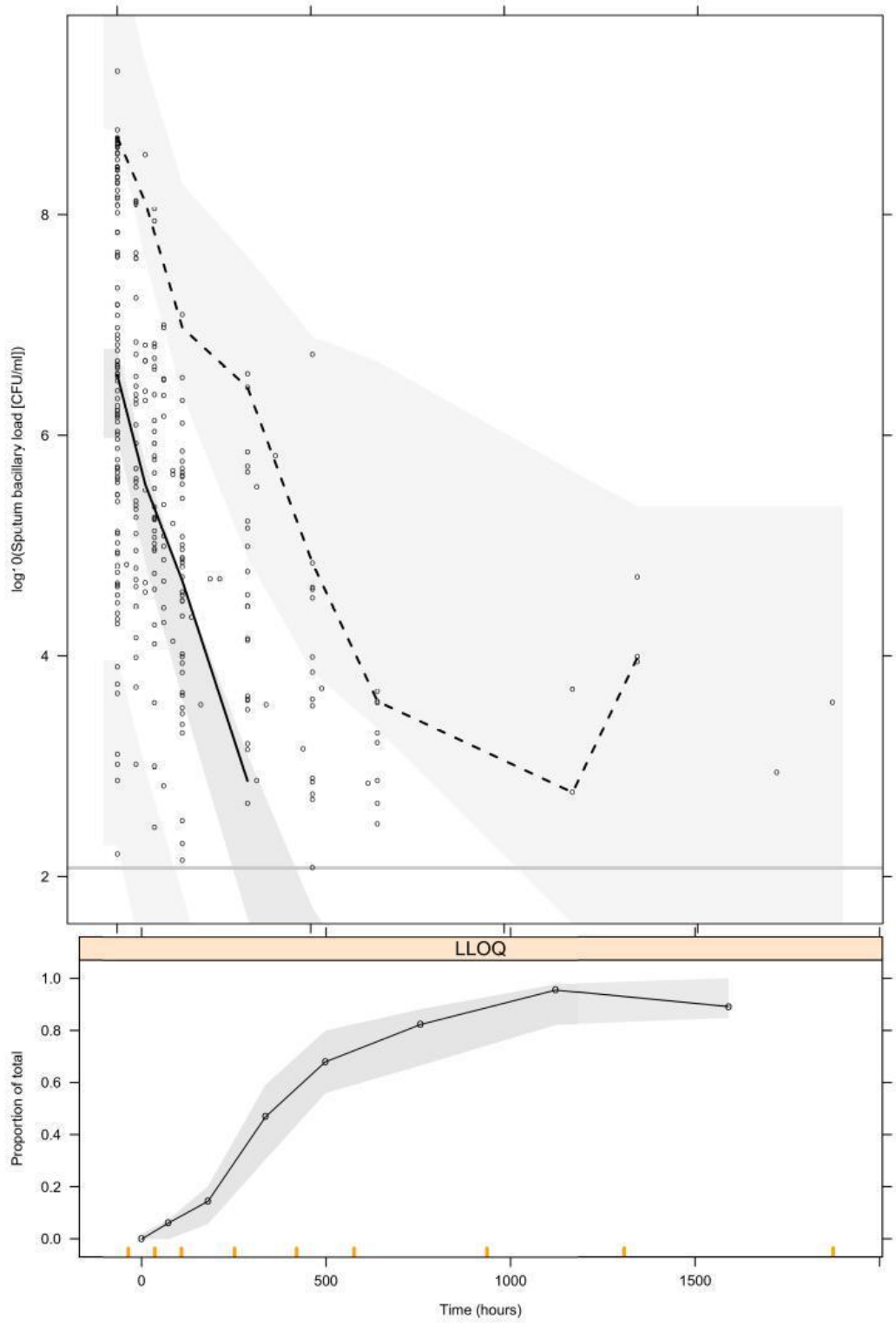

Figure 4