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# PK/PD Support for a Phase 1 Study of TBA-7371

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**Background:** TBA-7371 is a potent, non-covalent inhibitor of DprE1, an essential enzyme for cell-wall synthesis. A phase 1 study for TBA-7371 was recently completed by TB Alliance. The study had three parts: 1) Single Ascending Dose (SAD), with a food-effect sub-part; 2) Multiple Ascending Dose (MAD); 3) Drug-Drug Interaction (DDI). This presentation will describe how PK/PD approaches were used to support decision making during the study and interpretation of study results.

The following topics will be covered:

1. Preclinical results had identified increased heart-rate as a potential safety liability, which might be due to PDE inhibition, and had identified exposure thresholds that were to be approached cautiously during dose escalation. Bayesian forecasting of exposures at possible next doses was undertaken to support dose-escalation decisions.

2. After completion of the SAD part, exposure-response modeling of heart-rate increases was used to support the decision to proceed to the MAD part and the doses to carry forward. Differences among measures of heart-rate by vital signs, safety ECGs, and Holter monitoring were examined.

3. Another adverse effect was visual disturbance. Exploratory graphics were used to characterize the relationship between the concentration-time profile and the onset/offset times of visual adverse events both in the SAD and across multiple days of the MAD.

4. The food effect and the impact of TBA-7371 on probe drugs metabolized by CYP2B6 and CYP3A4, including TBA-7371 itself via possible auto-induction, were assessed using non-compartmental methods.

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# Bedaquiline appears to antagonize its own main metabolite's QTcF interval prolonging effect

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**Background:** Bedaquiline (BDQ) has been the first anti-tuberculosis drug with a novel mechanism of action that received approval for the treatment of multidrug-resistant (MDR) tuberculosis (TB) in adults as part of combination therapy. BDQ can shorten the time to sputum culture conversion and increase the cure rate. On the other hand, it may also lead to prolongation of the heart's QT interval, which is a safety concern since it can cause sudden death.
This study was aimed to investigate potential relationships between concentrations of BDQ and/or its main metabolite (M2) and QTcF (corrected QT with Fridericia's coefficient) interval in MDR TB patients using the approved BDQ dose regimen.

**Methods:** Data were obtained from two phase IIb studies (C208 and C209) and were pooled to include a total of 335 patients treated with BDQ and 105 patients with placebo. Patients received BDQ or placebo for 24 weeks (or 8 weeks in C208 stage 1) in combination with a background regimen of 5-7 drugs. Pre-dose BDQ/M2 PK samples were drawn regularly in all patients while full PK profiles were performed at week 2 and 8 (stage 1) or week 2 and 24 (subset of patients in stage 2) for patients of the C208 study. Single ECGs were performed weekly while triplicate measurements at pre-dose and 5 hour post-dose were taken at week 2, 8, 12 (only C209) and 24.

Since a PK-model for BDQ and M2 was previously established for these trials, a sequential analysis approach was used. The individual model-predicted BDQ and M2 concentrations were evaluated as predictors (covariates) in the development of the pharmacodynamic model for QTcF interval. The effect of the presence/absence of background regimen also was explored.

**Results:** The baseline QTcF increased by 0.2% in the presence of background regimen in C208 study and by 1% in C209 study. After testing several drug effects for BDQ and M2 (on/off, linear, Emax, full and partial competitive agonist), the model that best described the data was a competitive antagonist model. Thus, BDQ appears to act as an antagonist of M2 effect and has no intrinsic activity (i.e. Emax,BDQ=0 and related estimated parameters are: Emax,M2:12.9 ms (RSE 5%), EC50,M2:14 ng/mL (RSE 6%), EA50,BDQ:229 ng/mL (RSE 8%)). This model performed better than Emax models of BDQ and M2 alone, and showed robustness of the results (pharmacologic mechanism and parameters estimates) while analysing each study data separately.
This finding implies that QT prolongation is driven exclusively by M2 concentrations while BDQ antagonizes the effect of M2 on QT prolongation. Interaction of BDQ and M2 at the same target is supported by preclinical studies, but the magnitude of each effect could not be quantified in vitro.

**Conclusion:** The QTcF interval prolongation observed in the phase IIb studies of BDQ was explained by an effect of the background regimen and M2 exposures, while BDQ antagonizes the effect of M2. This model can together with previously developed models for PK with drug-drug interactions and sputum conversion, inform an integrated dose-exposure-efficacy-safety analysis to optimize BDQ therapy.

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# Translational pharmacokinetic modelling & simulation of an experimental long-acting injectable formulation of bedaquiline

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**Background:** Bedaquiline (BDQ, Sirturo®) is currently marketed as an oral tablet formulation. A long-acting injectable (LAI) formulation for SC or IM administration would be a great asset to have for improving suboptimal adherence. In an ideal scenario, a patient could be treated with just a single injection, thus ensuring full adherence over at least one dosing interval. Potential applications are treatment of latent TB infection and post-exposure prophylaxis of family members of MDR TB patients.

**Methods**: A few aqueous suspension formulations were developed. As BDQ is very lipophilic (logP=6.37), its dissolution rate is extremely slow, which allows the development of nano- or microsuspensions, where particle size drives the release: the smaller the particle size, the faster the release is expected to be. Mice, rats and dogs received single SC and IM injections of a nano- or microsuspension formulation at doses of 80 and 160 mg/kg (mice, n=5/dose) or 40 mg/kg (rats and dogs, n=3/dose). Plasma samples were collected up to 3 (mice) or 6 months (rats and dogs) post dose, analysed for both unchanged BDQ and its active M2 metabolite, and subjected to noncompartmental analysis. Individual plasma concentration-time data of parent BDQ after IM administration of the microsuspension, selected for additional mice studies, were also analysed using population PK modelling with NONMEM v7.3. The resulting model was subsequently applied to simulate expected plasma concentration-time profiles in patients.

**Results:** After IM injection of the nano- and microsuspension, a biphasic plasma concentration-time profile of BDQ could be observed across species. In all species, peak plasma levels were reached earlier and were higher for the nano- than for the microsuspension. The apparent elimination half-life, which is reflecting the release rate of BDQ from the formulation (flip-flop kinetics), was shorter for the nanosuspension (20-48 days) as compared to the microsuspension (30-56 days). After SC administration, a more gradual increase of BDQ's concentration could be observed, peak plasma levels were lower, and the overall exposure was lower (microsuspension) or similar (nanosuspension) compared to the IM administration route. Plasma levels of the M2 metabolite declined in all species in parallel with BDQ levels.

A compartmental modelling analysis of the microsuspension data revealed that a fraction of the dose was rapidly absorbed in the systemic circulation, with clear between-species differences (rat and mouse more similar, 43-54%, compared to the dog, 4%). The remainder of the dose was absorbed at a much lower rate, which was remarkably similar across species (ka 0.0005-0.0008 hr-1). The developed PK model was subsequently used to predict the expected plasma concentration-time profile in patients after a single IM administration of the microsuspension formulation at 1000 mg, and showed that at this dose, sustained levels of 100 ng/mL can be achieved over a period of more than 1 month. The M2 metabolite was not included in the compartmental analysis, as it does not contribute to the antimicrobial activity in patients.

**Conclusion:** The current LAI formulations tested in mice, rats and dogs hold promise for further development.

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# Preclinical and Clinical Pharmacokinetics and Pharmacodynamics Analysis of Delamanid

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**Background:** Delamanid is a bicyclic nitroimidazooxazole that has demonstrated anti-tuberculosis activity. To understand the relationship between drug exposure and efficacy, and whether the current approved dose is optimal for delamanid, we conducted a pharmacokinetics and pharmacodynamics (PK-PD) analysis according to the published "Guideline on the use of pharmacokinetics and pharmacodynamics in the development of antimicrobial medicinal products, European Medicines Agency, 2016", using preclinical and clinical data.

**Methods and Results:** A mouse PK-PD study was conducted using a series of single dose administrations of delamanid from 0.625 to 40 mg/kg, followed by simulations to obtain multiple dosing PK parameters. The same multiple dosing regimens were then utilized to study the reduction of lung colony-forming-unit (CFU) in a chronic infection model, in which mice were infected with M. tuberculosis Kurono (delamanid MIC: 0.012 μg/mL) for 4 weeks followed by 4 weeks of treatment with delamanid. A nonlinear regression model was used to analyze the relationship between the net reduction of log10CFU by delamanid from the untreated controls and PK-PD parameters. Log10CFU reduction correlates strongly with AUC/MIC (correlation coefficient: 0.97; $p<0.0001$) and moderately with %T>MIC (correlation coefficient: 0.53; $p<0.01$), but not with Cmax/MIC. The nonclinical pharmacodynamic target (nPDT), defined as the AUC/MIC achieving 80% of maximum efficacy (Emax), was 252 in mice. Delamanid AUC/MIC and log10CFU reduction data from two Early Bactericidal Activity (EBA) trials in DS-TB patients were modeled using a nonlinear mixed effects approach. An inhibitory sigmoid Emax model with random effect on Imax provided the best fit to the data. The clinical PDT (cPDT) (AUC/MIC) was determined to be 171. Finally, the probability of target attainment (PTA) following the approved 100 mg BID dose in adult pulmonary MDR-TB patients enrolled in two clinical trials was estimated using delamanid MIC95 of 0.012 μg/mL, determined from 460 clinical isolates. The PTA was 94 % in the formal trial and 93 % in the latter when using the nPDT (PTA is even higher when using the cPTA since cPTA is lower). The primary significant safety concern for delamanid is the potential for QTcF interval prolongation. Based on nonlinear mixed effects modeling, the model-based QTcF interval prolongation for delamanid indicated a 50 % increase in QTcF interval prolongation when increasing the dose from 100 mg BID (12.7 msecs increase) to 200 mg BID (18.7 msecs increase). Hence, there is potential for an increased risk of QTcF interval prolongation when the dose is increased from the current 100 mg BID.

**Conclusion**: AUC/MIC is determined to be the pharmacodynamic driver for delamanid. Based on the PTA, the approved dose of 100 mg BID is expected to achieve near maximum efficacy, thus is the appropriate dose for the treatment of adult, pulmonary MDR-TB. Increasing the dose from the 100 mg BID level is likely to increase cardiac risk with minimal corresponding increase in efficacy.

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# Higher rifampicin and isoniazid concentrations in epithelial lining fluid are associated with improved response to treatment in pulmonary TB

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**Background:** Detailed interrogation of site of infection pharmacokinetic-pharmacodynamic relationships may facilitate optimisation or shortening of treatment for pulmonary TB. We investigated whether antibiotic exposure in epithelial lining fluid (ELF) or alveolar cells determines the rate of bacterial clearance and clinical treatment response in pulmonary TB.

**Methods:** Malawian adults with pulmonary TB on first-line treatment were recruited to a longitudinal cohort study. Plasma and intrapulmonary samples were collected at 8 and 16 weeks into treatment, and drug concentrations measured by liquid chromatography-tandem mass spectrometry. Population PK modelling generated estimates of drug exposure in plasma, ELF, and alveolar cells. Participants supplied serial sputum samples over the intensive phase of therapy. Treatment response was measured by rates of 2-month sputum culture conversion, modelled time to sputum negativity, sputum bacillary elimination rates from mixed-effects modelling, and relapse-free cure to 18-months.

**Results:** 157 participants were recruited. Despite weight-based dosing, plasma Cmax concentrations of first-line drugs were low relative to therapeutic drug monitoring (TDM) targets. All 4 drugs achieved higher concentrations in ELF and alveolar cells, with isoniazid and pyrazinamide 20 and 50-fold higher in ELF than plasma respectively. Ethambutol concentrations were highest in alveolar cells.

Higher Cmax for rifampicin or isoniazid in plasma was associated with a shorter time to sputum culture negativity (estimate -110 days, SE 49, p=0.032; -168 days, SE 59, p=0.006 respectively for each 1-log increase in Cmax), and more favourable late outcomes (p=0.031 and p=0.029 respectively).

Higher AUC or Cmax for rifampicin or isoniazid in ELF was associated with more rapid bacillary elimination (by improved PK-PD model fit), shorter time to sputum culture negativity (rifampicin ELF Cmax: estimate -68 days, SE 27, p=0.022; isoniazid ELF AUC: estimate -31 days, SE 15, p=0.049 for each 1-log increase), and for rifampicin Cmax in ELF, more favourable late outcomes (p=0.048).

**Conclusion:** Despite plasma Cmax concentrations below TDM targets, first-line TB drugs concentrated at the pulmonary site of infection. This may reflect increased perfusion and greater capillary permeability in the inflamed lung. Higher plasma and ELF rifampicin and isoniazid concentrations in pulmonary TB are associated with improved treatment response.

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# Rifampicin, isoniazid and pyrazinamide exposures in children with DS-TB on WHO-recommended FDCs in the SHINE trial

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**Background:** Rifampicin, isoniazid and pyrazinamide comprise the core standard
WHO-recommended regimen for drug-susceptible tuberculosis. The development of
50/75/150mg isoniazid/rifampicin/pyrazinamide and 50/75mg isoniazid/rifampicin dispersible fixed-dose-paediatric combinations (FDCs) has facilitated uptake of WHO 2010 dosing guidelines for childhood tuberculosis.

**Methods:** We evaluated rifampicin, isoniaizd and pyrazinamide pharmacokinetics in 39 South African and Zambian children receiving WHO-recommended doses in the SHINE shortening trial. Children 4-7.9, 8-11.9,12-15.9 and 16-24.9 kg, respectively, received 1, 2, 3 and 4 paediatric FDCs (Macleods Pharmaceuticals Ltd) per dose. Children >25 kg received adult doses, using 75/150/400/275 FDCs of rifampicin/isoniazid/pyrazinamide/ethambutol (2 tablets for children 25-36.9 kg). Plasma rifampicin and pyrazinamide concentrations were measured in samples drawn pre-dose and at 1, 2, 4, 6, 8 and 12 hours after an observed dose, after at least 2 weeks of treatment. Noncompartmental analysis was used to compute the pharmacokinetic measures.

**Results:** Interim analysis includes 39 children aged 3 months to 11.8 years. Five children had HIV infection. Thirty-three were sampled during the intensive phase and 6 during the continuation phase of treatment. Median (IQR) peak concentrations and 0-24 hour area-under-the-curve (AUC) were 7.5 (4.9, 11.8) mg/L and 26.6 (17.8, 41.8) mg.h/L for rifampicin (n=39), 6.39 (4.29, 8.62) mg/L and 21.06 mg.h/L for isoniazid (n=38) and 39.4 (29.3, 46.4) mg/L and 322.3 (263, 4,454.4) mg.h/L for pyrazinamide (n=33), respectively. Exposures differed by weight band in those receiving 1 (n=7), 2 (n=11) ,3 (n=9), 4 (n=8) paediatric FDCs and adult doses (n=4), respectively: median AUC 17.8, 26.8, 38.8, 40.2 and 15.5 mg.h/L for rifampicin (p=0.011); 19.03, 20.31, 25.26, 27.33 and 7.75 mg.h/L for isoniazid (p=0.075); and 244.3, 322.3, 385.2, 434.2 and 302.6 mg.h/L, for pyrazinamide (p=0.015).

**Conclusion:** While median rifampicin and pyrazinamide exposures in children 12-24.9 kg were similar to adults, for rifampicin they remain well below recommended exposures. For isoniazid, children 12-24.9 kg had higher exposures than those typically documented in adults on standard doses. Children in the lowest 2 weight bands and those over 25 kg on adult doses had low drug exposures. While the findings need confirmation with further data, current weight band-based doses need adjustment.

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# Nevirapine pharmacokinetics in HIV-infected persons receiving rifapentine and isoniazid for TB prevention

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**Background:** AIDS Clinical Trials Group (ACTG) Study A5279 (BRIEF-TB, NCT 01404312) was a phase 3 clinical trial (N=3000) comparing 4 weeks of daily rifapentine + isoniazid (RPT/INH; 1HP) to 36 weeks of daily INH for the prevention of active tuberculosis in HIV-infected individuals. RPT induces cytochrome P450 (CYP) and nevirapine (NVP) is a CYP substrate leading to concern that co-administration could result in decreased NVP exposure and increased risk of virologic failure. We evaluated the effect of daily 1HP on NVP PK in the first 90 participants who enrolled in A5279 while on a stable NVP-containing antiretroviral (ART) regimen.

**Methods:** Ninety participants receiving ART containing NVP (200mg PO BID) randomized to daily 1HP (weight-based RPT ≈ 10mg/kg; INH 300mg) treatment in A5279 were included. Trough plasma samples were collected at week 0 (pre-1HP) and weeks 2 and 4 during concomitant 1HP. NVP concentrations were determined using a validated HPLC assay. NVP apparent oral clearance (CL/F) was modeled using Bayesian estimation (ADAPT). Week 2 and 4 NVP concentrations were used to estimate NVP CL/F while taking 1HP. The geometric mean ratio ((GMR) (90% confidence interval (CI)) of the during/pre 1HP NVP CL/F values were calculated. The protocol specified that NVP exposure would be judged acceptable if there is evidence that the proportion with NVP trough concentrations ≥3 mg/L exceeds >80% at both week 2 and 4.

**Results:** Demographic and baseline data for the 78 evaluable participants were: female, 61 (78%); Black Non-Hispanic, 51 (65%); median (range) age, 40y (13-66); median (IQR) weight, 57.9kg (47.1-66.8); 70 (90%) had undetectable HIV-1 RNA at entry; median (IQR) CD4+ count, 548 cells/mm3 (91-1233). Median (IQR) post-dose NVP sampling times were: week 0, 13.2 (12.6-13.5) hours, week 2, 13.2 (12.4-13.6) hours, and week 4, 13.2 (12.2-13.5) hours. Median (IQR) NVP trough concentrations were: week 0: 7322 (5266-9302) ng/mL, week 2: 5537 (3552-8462) ng/mL and week 4: 5388 (3516-8243) ng/mL. Median (IQR) NVP CL/F values were: week 0 pre-1HP 2.03 (1.58-2.58) (L/hr) and during 1HP 2.62 (1.81-3.42) L/hr. The GMR (90% CI) for NVP CL/F was 1.30 (1.26-1.33). The numbers (%, 90% CI) of participants with NVP concentrations ≥ 3 mg/L were: week 0, 78 (100%); week 2, 64 (82,73.2-88.6%); week 4, 63 (81, 71.7-87.6%); both weeks 2 and 4, 60 (77, 67.6-84.3%). Week 8 HIV-1 RNA was <40 copies/mL in 24 of 26 participants with results.

**Conclusions:** Overall, the oral clearance of NVP significantly increased while taking 1HP, as judged by the GMR and 90% CI. A decrease in the percentage of participants with NVP trough concentrations ≥ 3 mg/L during 1HP therapy suggests induction of NVP clearance, presumably from RPT. The proportion of participants with NVP trough concentrations ≥ 3 mg/L crossed below the pre-specified threshold of >80%. These drug-drug interaction data suggest 1HP should not be co-administered with NVP-containing ART.

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# Fluoroquinolones in the treatment of multidrug-resistant tuberculosis: Experience from three US TB treatment centers

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**Background:** Fluoroquinolones (FQs) are used to treat multidrug-resistant tuberculosis (MDR-TB). Initially, ciprofloxacin (CIP) and ofloxacin (OFL) were used, then levofloxacin (LVX) and moxifloxacin (MOX). We present accumulated USA experience with FQs in the treatment of TB.

**Methods:** A multi-center, retrospective study included three TB centers in the USA: A.G. Holley Hospital (AGH), Texas Center for Infectious Diseases (TCID), and University of Texas Health Science at Tyler. We included patients admitted between 1984 and 2015, infected with MDR-TB, who received a FQ for at least 28 days. Patients demographics, sputum cultures, susceptibility data, duration of treatment, treatment outcomes, and FQ serum concentrations were collected. Treatment outcome was defined as cured if there was at least 1 negative culture after 6 months of therapy, with no subsequent positive cultures. Failure was defined as positive culture after 6 months of treatment. A time-to-event (TTE) analysis was conducted to compare the time to culture conversion among FQs. The time was defined as the number of weeks from the start of treatment to culture conversion. Population pharmacokinetic (PK) models, established based on both sparse PK data in the current study and rich PK data from other studies, were used to generate the Empirical Bayes Estimates (EBEs) for maximum concentration (Cmax) and area under the concentration-time curve from 0-24 hours (AUC0-24). For the pharmacokinetic/ pharmacodynamic (PK/PD) analysis, we used the epidemiological cut-off values (ECOFF) in liquid medium of 1.0 mg/L for LVX and 0.25 mg/L for MOX as the minimum inhibitory concentration (MIC). Unbound drug fraction was estimated at 70% LVX and 60% MOX. Statistical tests were performed using JMP Pro v13.2 (SAS Institute). PK modelling was done using Monolix v2018R1 (Lixoft).

**Results:** 106 MDR-TB patients received FQs. The median (range) age was 39.5 years (15.0-92.0) and weight was 59.2 kg (38.2-105.0). Fifty-one patients (48.1%) received CIP or OFL, while 55 received LVX or MOX. In the TTE analysis, LVX/MOX showed faster time to culture conversion in MDR-TB patients compared to CIP/OFL (median 16 vs 40 weeks, log-rank p=0.0577). The median (range) of LVX and MOX serum concentrations were 9.2 mg/L (1.2-19.0) and 3.8 mg/L (0.9-10.4), respectively. EBEs were generated for 25 LVX and 26 MOX patients. The median (range) for LVX Cmax and AUC0-24 were 9.9 mg/L (6.4-16.1) and 118.8 mg.hr/L (76.7-287.6), while for MOX were 4.0 mg/L (2.9-8.3) and 46.1 mg.hr/L (28.7-90.9). The numbers of patients who achieved free Cmax/MIC>10 were 1 for LVX (4%) and 12 (46%) for MOX. For free AUC0-24/MIC>100, the numbers were 8 (32%) for LVX and 19 (73%) for MOX. A total of 8 patients had evaluable treatment outcome, hence statistical tests were not performed due to the small sample size.

**Conclusion:** In MDR-TB patients, LVX and MOX showed faster time to culture conversion compared to CIP and OFL. Higher percentage of patients achieved the PK/PD target in MOX compared to LVX, which may indicate that higher doses of LVX are needed.

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# Clofazimine pharmacokinetics in South African patients with drug-resistant tuberculosis

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**Background:** Because of its treatment-shortening potential clofazimine has become a key component of new treatment regimens for drug-resistant tuberculosis (TB). As a repurposed drug, there are very limited data on clofazimine pharmacokinetics (PK) in patients with TB. We aimed to describe the PK of clofazimine and explore the effect of covariates on clofazimine exposure in South African patients with drug-resistant TB.

**Methods:** We enrolled adult patients receiving clofazimine 100 mg daily as part of a multi-drug regimen for XDR and pre-XDR TB at a public-sector TB hospital in Cape Town. Participants underwent plasma PK sampling on a single occasion pre-dose and at 1, 2, 3, 4, 5, 6, 8 and 24 hours after a standardized meal and observed clofazimine administration. Non-compartmental analysis was performed to estimate clofazimine PK parameters, and linear regression was used to explore associations between clinically relevant covariates and log-transformed AUC0-24. Covariates included age, sex, percentage body fat, ethnicity, HIV status, serum creatinine concentration, and concurrent use of ritonavir-boosted lopinavir.

**Results:** Twenty-two participants were included, nine (41%) of whom were HIV-infected. Median age was 29 years (interquartile range [IQR] 25 to 44) and there were equal numbers of men and women. The median weight was 55.2 kg (IQR 48.8 to 64.8) with a median percentage body fat of 22.9% (IQR 12.4 to 37.2), which was significantly higher amongst women ($P < 0.0001$). Participants were on clofazimine therapy for a median of 71 days (IQR 62 to 80; range 18 to 97) at the time of PK sampling. Plasma clofazimine exposures were low (median AUC0-24 7.673 µg.h/mL (IQR 6.015 to 10.858)) with substantial interpatient variability (coefficient of variation for AUC0-24 41.7%). The time to maximum concentration was 4 hours (IQR 3 to 5), with a median Cmax of 0.401 µg/mL (IQR 0.337 to 0.548). There was no correlation between duration of clofazimine therapy and exposure ($R2 = 0.005$). On univariate analysis there was a significant association between percentage body fat and clofazimine exposure, with an estimated 19.4% (95% CI, 8.7 to 28.8) reduction in clofazimine AUC0-24 for every 10% increase in body fat. This is the likely explanation for the positive univariable correlation between male sex and exposure (65.5% increase in AUC0-24 compared to females) as body fat was significantly higher in females. Percentage body fat remained a significant predictor of clofazimine AUC0-24 in the multivariate model ($P = 0.037$).

**Conclusion:** In this population of TB patients with a high prevalence of HIV, clofazimine exposures were influenced by body fat, in keeping with the lipophilic properties of the drug and its extensive tissue distribution. This has implications for dose optimization. Additional studies are required to evaluate steady state PK and to characterize PK-pharmacodynamic relationships.

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## Pharmacokinetic-Pharmacodynamic Target Attainment Analysis of Cycloserine in TB Patients

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**Background:** There are limited pharmacokinetic/pharmacodynamic (PK/PD) data for cycloserine (CS) in tuberculosis (TB) patients. We estimated population PK parameters and performed Monte Carlo simulation and target attainment analyses to optimize dosing.

**Methods:** Our previous model was expanded, including data from healthy subjects and TB patients. The latter came from 6 sites: Georgia, Bangladesh, and four US sites. Monolix (2018R1) was used to build the population PK model. The final PK estimates were used in mlxR package (v3.3.0) in R software to simulate 1000 TB patients (steady state) for each regimen. We used PKPD targets of time above MIC ≥30% and ≥64%, representing bactericidal activity and EC80 (from Deshpande et al. and Dr. Tawanda Gumbo). We assumed 100% unbound drug. A range of MICs was studied (4 to 64 mg/L). Probability of target attainment (PTA) was calculated as the fraction of simulated patients who achieved the PKPD target at each MIC for each regimen. We selected a PTA of at least 90% for the highest MIC as the PKPD breakpoint.

**Results:** We included 1069 CS plasma concentrations, from 247 subjects (83% of patients had drug-resistant TB). The average age and weight were 42 years and 61 kg. About three-quarters of the patients were males. We selected a one-compartment model, with first-order absorption and lag phase. The PK parameters were estimated (CV): Tlag 0.326 h (0.43), ka 6.61 h-1 (2.9), V/F 24.9 L (0.17), and CL/F 2.00 (0.36) for healthy subjects and 1.03 L/h for TB patients. Weight and CrCL were found to have a significant effect on V and CL, respectively; and were included in the model. High PTA was achieved with dose increases when we compared 250 mg, 500 mg, and 750 mg given once daily (QD). Dividing the daily dose modestly increased the PTA, reflecting the long half-life of CS, 16.8 h. However, dividing the 750 mg dose to 250 and 500 mg twice daily (BID) had an MIC PKPD breakpoint of 16 mg/L, compared to 8 mg/L in the 750 mg QD regimen (PTA of 92 vs 84%, respectively). Dividing the dose reduced the Cmax significantly; for example, Cmax for 500 mg QD vs 250 mg BID were 33 vs 26 mg/L, respectively. The 250 mg regimens failed to achieve PTA >90% for MIC >16 mg/L. The 500 mg TID regimen achieved the targets for MIC of 32 mg/L. The 500 TID regimen and higher produced Cmax >55 mg/L, which may not be tolerable.

**Conclusion:** A PK model for CS was established and used for target attainment analysis. Although dividing the dose resulted in a slight increase in the PTA, it resulted in a significant decrease in Cmax, which might reduce the adverse CNS effects.

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# High peak rifampicin concentrations accelerate the slow phase of bacterial elimination in tuberculosis patients

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**Background:** Antibiotic treatments are often associated with a slowdown in elimination of bacteria, and this complicates treatments and contribute to relapse. Furthermore, low bacterial numbers and their reduced culturability limit the estimation of the rate of slow bacterial killing. This makes it problematic to extrapolate when the bacterial population is expected to be cleared and to empirically assess when it is safe to discontinue treatment. Mathematical models and predictions can aid in the design of trials by identifying possible strategies with higher success rates. Currently, it is unclear whether or how the slow phase of bacterial killing is affected by pharmacokinetic measures (such as peak drug concentration (Cmax) and total drug exposure (AUC)). Here, we used measurements from early bactericidal activity trials in tuberculosis (TB) patients to show that the slow phase of the elimination can be decreased with increased peak drug concentrations.

**Methods:** First, we used multi-scale pharmacodynamic models that incorporate drug target binding and differential bacterial susceptibility to antibiotics (Abel zur Wiesch & al. 2015 & 2017, Martinecz & Abel zur Wiesch 2018) in order to assess how different pharmacokinetic measures (Cmax and AUC) affect the slow phase of bacterial decline. Then, we tested our model predictions with data from a clinical trial. Bacterial counts measured in TB patients receiving a range of rifampicin doses from 10 to 35 mg/kg daily for two weeks (Boeree et al, 2015) were fit to biexponential bacterial count versus time curves. Next, we investigated the relationship between the slope of the slow phase of the biexponential curves and the corresponding pharmacokinetic measures for rifampicin.

**Results:** Our mathematical modeling predicted that the mechanism underlying the slow decline of bacterial counts determines whether higher drug exposure accelerates bacterial killing. It is commonly thought that a distinct bacterial state (e.g. different metabolic state) in which bacteria are completely unaffected by antibiotics is responsible for the slow phase of decline. In this case, bacterial decline would be independent of drug exposure. If the slow decline is due to bacteria that are not completely unaffected but less susceptible, higher drug concentrations and specifically high peak concentrations are expected to be successful. We performed both univariable and multivariable regressions of the slope of late bacterial decline (day4-14) in sputum samples of tuberculosis patients versus AUC and Cmax. We found a strong relationship between peak drug concentrations ($p=0.002$) and total drug exposure ($p=0.0036$) of rifampicin and late bacterial killing of M. tuberculosis in a univariable analysis. In multivariable analyses, we showed that peak drug concentrations are better predictors than AUC for the slow phase of bacterial eliminations.

**Conclusion:** As peak drug concentrations strongly affect the slow phase of bacterial killing in TB patients treated with rifampicin and thereby likely treatment length, our results indicate that antibiotic formulations with different pharmacokinetic profiles can affect treatment success rates. Furthermore, using different antibiotic formulations can allow us to increase the efficiency of treatments without increasing drug exposure and going outside the therapeutic window of the given antibiotic.

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# The Clinical value of Model-based Therapeutic Drug Monitoring in Tuberculosis Treatment – Optimized Target for Rifampicin

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**Background:** Therapeutic drug monitoring (TDM) coupled with Bayesian forecasting relies on a population pharmacokinetic approach, allowing accurate dose prediction using few pharmacokinetic samples, accounts for multiple predictors and is advantageous for drugs with time- or dose-varying pharmacokinetics or high inter-occasional variability (IOV). Existing rifampicin TDM target(s) are based on expected concentrations following 10 mg/kg rifampicin. In light of the completed clinical trials with high-dose rifampicin (35 mg/kg), it is necessary to update the rifampicin TDM target.
We aimed to propose new Bayesian TDM targets for rifampicin and to apply them using a model-based TDM approach well-suited for high-dose rifampicin, considering the saturable pharmacokinetics and auto-induction of rifampicin.

**Methods:** Rifampicin Bayesian TDM targets were defined using simulations with a rifampicin pharmacokinetic model including saturable pharmacokinetics plus auto-induction and informed by pharmacokinetic-pharmacodynamic models of rifampicin anti-mycobacterial activity based on one and 12 weeks data and clinical literature data on safety. Influence of including minimum inhibitory concentration (MIC) was explored. The targets were based on the predicted highest plasma concentration during the dosing interval of 24 hours (Cmax) or the predicted area under the plasma concentration-time curve during 24 hours (AUC0-24h). For the MIC-based target, a MGIT-determined MIC (MICMGIT) of 0.125 mg/L was applied as an expected mode in the target population. Sensitivity analyses were performed assuming another target MICMGIT and differences in the observed MICMGIT in the patient dataset reflecting uncertainty in MIC measurements.
The targets were applied to a patient dataset (n=24) by estimating individual pharmacokinetic parameters (including IOV) using sparse pharmacokinetic sampling at weeks two, four and twelve. Individual optimal doses were predicted by comparing the predicted individual Cmax or AUC0-24h without IOV, with or without including MIC, to the Bayesian TDM target for a dose range of 10-50 mg. The individual rifampicin MICMGIT was measured before starting treatment.

**Results**: The Cmax Bayesian TDM target range was 37-42 mg/L at day 1 and 33-38 mg/L at day 14. Predicted optimal doses in the patient dataset for the day 14 target ranged from 20-50 mg/kg (mode 25 mg/kg) which was lower than the Bayesian TDM target. The approach allows for computation of Bayesian TDM target ranges for any day after start of treatment. The Cmax/MIC Bayesian TDM target using a target of MICMGIT=0.125 mg/L predicted lower optimal individual doses of 10-40 mg/kg (mode 10 mg/kg). The sensitivity analyses involving MICs gave predictions of substantially different optimal doses.

**Conclusion:** A new optimized Bayesian TDM target was proposed in light of accumulated clinical evidence of a higher rifampicin dose being more effective than the recommended 10 mg/kg without safety issues in clinical studies so far. Targets involving MICs are theoretically superior compared to only measuring pharmacokinetics but the predicted doses were shown to be highly dependent on the assumed target MIC and the known uncertainty in MIC measurements. This work demonstrates a Bayesian forecasting approach for TDM that is optimal for personalized medicine of rifampicin by accurately handling dose- and time-dependent pharmacokinetics and IOV.

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# Model-based meta-analysis of rifampicin exposure and mortality in phase II tuberculosis meningitis trials

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**Background:** The mortality of tuberculosis meningitis (TBM) is frightfully high (often >40%). Increased rifampicin doses during the first critical days of treatment may improve outcomes. Our main objective was to characterize the population pharmacokinetics of high-dose rifampicin in plasma and cerebrospinal fluid (CSF) of TBM patients and to evaluate the relationship between individual exposures and mortality. We then used the developed model to investigate the possibility of abandoning weight-banded dosing of rifampicin for simpler flat dosing regimens.

**Methods:** Data originated from three randomized controlled phase II trials (Bandung, Indonesia) comparing oral rifampicin 450mg to intensified 14 or 30-days regimens including 750, 900 or 1350mg orally, or a 600mg intravenous infusion (1.5h). Intensive pharmacokinetic sampling was performed at day 2±1, and for two of the studies also at day 12±3. Single CSF samples were taken 3-9h after dose. Nonlinear mixed-effects modeling was used for pharmacokinetic data analysis. 6-month survival was described with parametric time-to-event models. Analyses were performed with NONMEM.

Using a virtual population of 10,000 African and Indonesian TBM patients, variability in exposure (single day and weekly average AUC0-24h, the latter included to mitigate stochastic day-to-day variability) was evaluated under four dosing scenarios: WHO-recommended weight-banded dosing 10 mg/kg, flat dosing with 450mg in Indonesian and 600mg in African patients, weight-banded dosing 35 mg/kg (bands as defined in the PanACEA-MAMS-TB-01 study) and lastly flat high dosing with 1500mg in Indonesian and 1800mg in African patients.

**Results:** Pharmacokinetic analyses included 133 individuals and 1150 rifampicin concentrations (170 from CSF). The final model featured two disposition compartments, a well-stirred liver model, saturable clearance and autoinduction. Typical oral bioavailability was estimated at 78% (95%CI 71-84); volume of distribution was 19% (12-26) lower at later sampling days. Rifampicin CSF concentrations were described by a partition coefficient (5.5% [4.4-6.4]) and half-life (2.1 h [1.3-2.9]) for the distribution between plasma and CSF. Higher CSF protein concentration increased the partition coefficient.

The survival analysis included 148 individuals of whom 58 died and 15 dropped out. An exponentially declining hazard described the survival well. Lower age, higher baseline Glasgow Coma Scale score and higher individual rifampicin plasma AUC0-24h reduced the hazard. Simulations predicted a reduction in mortality from around 50% to around 30% with the exposures generated from the highest evaluated rifampicin dose (1350mg, corresponding to ~30 mg/kg).

The simulations evaluating flat dosing demonstrated that inter-individual variability in exposure is not expected to increase without weight banding. The coefficient of variation in weekly average AUC0-24h using flat dosing was 25.6% and 29.8% for standard and high doses, respectively, compared to 24.5% and 33.3% for weight-banded dosing.

**Conclusion:** Higher rifampicin exposure early during treatment seems to substantially decrease the risk of death. Maximal effect was not reached within the studied range of exposures. The optimal dose of rifampicin in treatment of TBM should be further investigated in phase III type trials, for which flat dosing of rifampicin seems feasible.

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# Pharmacokinetic-pharmacodynamic modeling of pretomanid in pulmonary tuberculosis patients

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**Background:** The requirement of combination chemo-therapy for tuberculosis (TB) complicates the development of new TB drug regimens and emphasizes the importance of pharmacometric methods for efficient clinical testing. While quantitative dose-exposure-response relationships provide the basis for such methods, only certain aspects have been established for the several novel pretomanid-containing regimens that are currently in phase 3 testing.

**Methods:** A population pharmacokinetic/ pharmacodynamic (PK/PD) model of pretomanid was constructed by extension of our earlier described pretomanid PK model to include bacterial killing kinetics determined from solid culture colony forming unit (CFU)/mL sputum counts, and corresponding liquid culture time to positivity (TTP) measurements obtained from serial sputum samples. The PD model parameters were estimated from a Bayesian hierarchical analysis of individual patient data from two published phase 2 single-drug early bactericidal activity (EBA) studies, PA-824-CL-007 and PA-824-CL-010, conducted in Cape Town, SA. These studies included 122 adult male and female patients with newly diagnosed pulmonary TB, administered pretomanid oral doses of 50, 100, 150, 200, 600, 1000, or 1,200 mg/day for 14 days. A previously observed drug-independent proportionality between EBA calculated using log(CFU)/mL and TTP values was used to develop a separate kinetic equation for TTP in terms of the PD model parameters for CFU counts. Prior distributions for the PD model parameters were informed by preclinical animal model results and older EBA studies that included untreated patients. Previously determined individual PK parameter sets were used as measured covariates, with the posterior distribution conditioned on the corresponding individual CFU and TTP data and sampled using Markov chain Monte Carlo simulation. Individual patient profiles and population distributions for CFU/mL and TTP measurements were simultaneously simulated using the posterior distribution for all tested doses and compared with observed values.

**Results:** Pretomanid PD was described by capacity limited bacterial growth and single-site receptor binding kinetics for bacterial killing, and accounted for the observed CFU/mL and TTP profiles for all tested doses at both the population and individual patient levels. The individual patient PD parameters were specified as lognormal distributions sampled from population mean and population variance distributions. Preliminary values for the population geometric means and percent coefficients of variation describing interindividual variability were, respectively; 0.15±0.01 log10(CFU)/mL/day and 54%±6% for the maximum rate of pretomanid-induced bacterial killing, and 600±100 ng/mL and 48%±17% for pretomanid concentration at half-maximum effect. The proportionality constant between EBA[log10(CFU)/mL] and EBA(TTP) was lognormally distributed with population geometric mean equal to 47±2 hr, and population coefficient of variation equal to 26%±7%.

**Conclusion:** This population PK/PD model extends previous statistical analyses of pretomanid EBA studies to a predictive dynamic model that can be used as a starting point for modeling pretomanid in combination with other drugs, and for extended treatment duration. Also, the quantitative relationship between TTP and CFU/mL can be used as an informative prior distribution for other TB PK/PD models, and provides a possible mechanism to reduce the number of needed solid culture measurements in future TB clinical studies.

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# Clinical Validation of Intracellular Pharmacodynamic (PDi) based modelling for the prediction of Fluoroquinolone activity against TB

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**Background:** Clinical studies of new anti-tubercular drugs are costly and time consuming. Owing to the extensive TB treatment periods, the ability to predict drug activity in the early development stage is vital. Recent failures of pre-clinical models in predicting the activity of fluoroquinolones in the clinic underlines the importance of developing new predictive tools that will optimise the design of future trials.

**Methods:** Previously, we have developed an in vitro screening assay and shown that pharmacodynamic intracellular modelling (PDi) can be a powerful tool at predicting the activity of first line anti-TB drugs in patients.

**Results:** Here we show moxifloxacin (MXF) to be the superior fluoroquinolone, and PDi modelling based Monte-carlo simulations have accurately predicted clinical outcomes when validated against 8 independent human trials. Additionally, our simulations may explain why patients had a higher rate of relapse in the 4-month MXF trial compared to the standard regime.

**Conclusion:** Based on our results, we suggest that a 5-month MXF treatment period would be superior to the standard regime with a lower relapse rate.

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# PK-PD arguments for bedaquiline and delamanid replacement of aminoglycosides in MDR-TB regimens

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**Background:** Multi-drug resistant tuberculosis (MDR-TB) patients receive an injectable aminoglycoside, such as kanamycin (KAN) or amikacin (AMI), as part of their regimen for at least 6 months. Streptomycin (STR), although no longer considered a first-line drug in the US due to the increased prevalence of resistance, is still administered when other aminoglycosides cannot be used. Sadly, this drug class is notorious for causing ototoxicity and nephrotoxicity in 8 - 37% of recipients. The incidence of cochlear damage ranges from 7 - 90% in aminoglycoside-treated patients, depending on the study design and methodology. This damage is permanent and significantly impairs one's quality of life. In mature TB lesions, Mycobacterium tuberculosis (Mtb) bacilli are found mostly intracellularly in macrophages and extracellularly in the necrotic foci or caseum. Several studies have reported that aminoglycosides are poorly active or inactive against intracellular pathogens, and this has been attributed to both poor intracellular accumulation and their almost complete loss of activity at the acidic pH of lysosomes. Furthermore, there is limited clinical and pre-clinical evidence of the advantages of aminoglycoside inclusion or substitution in TB chemotherapy.
With the recent approval of bedaquiline (BDQ) and delamanid (DLM) for MDR-TB, we set out to compare the lesion-centric pharmacokinetics and pharmaco-dynamics (PK-PD) of the aminoglycosides versus the newly approved drugs, with the objective of determining whether the latter have the potential to replace aminoglycosides in conventional, WHO-recommended, MDR-TB drug regimens.

**Methods**: In previous studies, we have shown that drug exposure at the site of infection and drug activity against the resident bacterial populations are determinants of efficacy. We have developed in vitro assays and in vivo models to measure and/or predict site-of-disease PK-PD for TB drugs. These assays and in vivo models were used here to characterize the penetration of aminoglycosides, BDQ and DLM in TB lesions, and their activity against the major bacterial populations present in these lesions.

**Results:** Here we have applied these tools to (i) compare the in vitro lesion PK parameters of aminoglycosides, BDQ and DLM, as predictors of lesion penetration in vivo (ii) test the predictions for KAN, BDQ and DLM in clinical research samples (KAN) and in the mouse (BDQ) and rabbit (DLM) model of active TB, and (iii) measure the activity of aminoglycosides, BDQ and DLM against extracellular persisters in caseum and intracellular Mtb bacilli in macrophages. The penetration of KAN in pulmonary lesions of TB patients is presented for the first time, as is the lesion penetration of DLM in the rabbit model.

**Conclusion:** Our in vitro and in vivo lesion PK-PD data indicate that BDQ and DLM exhibit a number of attractive lesion-centric PK-PD properties compared to the aminoglycosides, which suffer from permanent side effects in a significant proportion of the patients with no clear evidence of a pharmacological advantage. These results suggest that BDQ and DLM may be considered as substitutes of the aminoglycosides in future MDR-TB trials.

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# Pharmacodynamic Correlates of Linezolid Activity and Toxicity in a Mouse TB Model

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**Background:** Linezolid (LZD) is clinically effective against multidrug-resistant (MDR) tuberculosis (TB). However, dose- and duration-dependent toxicity (myelosuppression and neuropathy), which appears to correlate with trough concentrations, is common. Differences in the pharmacokinetic-pharmacodynamic (PK/PD) drivers of LZD efficacy under different growth conditions could inform new dosing strategies to maximize efficacy and minimize toxicity. Similarly, dosing and contribution to treatment may depend on the potency of companion drugs. Here, we report an updated analysis of in vivo dose fractionation studies of LZD alone and in combination with pretomanid (PMD) and a new toxicodynamic analysis.

**Methods:** PK data were gathered after 5 d of single daily oral doses of LZD 10, 30, 100 or 335 mg/kg to BALB/c mice. Blood was collected 0, 0.5, 1, 2, 4, 8, and 24 hours post-dose. Plasma LZD concentrations were measured by LC/MS-MS assay. A 2-compartment PK model with oral absorption with varying bioavailability and Michaelis-Menten saturable clearance from the central compartment was derived and used to simulate PK of doses used in PD experiments. PD data were obtained in acute and chronic infection models in which mice were dosed with LZD once daily, 5 d/wk, either alone or with PMD 12.5 (bacteriostatic) or 50 mg/kg (bactericidal). LZD doses ranged up to the maximum tolerated dose. Lung colony forming unit (CFU) counts and complete blood counts were determined after 28d of treatment; correlations between these data and calculated PK/PD parameters were assessed.

**Results:** Time>MIC was the parameter that correlated best with CFU counts in each model. However, in models with greater growth constraints (i.e., chronic model with immune system contributions to killing and high-dose PMD model), AUC/MIC had similar explanatory power. Clear LZD exposure-dependent reductions of red blood cell indices (e.g., hematocrit) were observed, which correlated with Cmin (R2=0.75) but not Cmax (R2=0.22) or AUC (R2=0.37). Dose-dependent decreases in platelet and white blood cell counts were not observed.

**Conclusion:** The results indicate that LZD in vivo activity against Mtb tends to be more time-dependent. However, when Mtb growth is restrained, AUC/MIC correlates just as well as Time>MIC with bacterial killing (equivalent R2 values). Therefore, higher, less frequent doses should be as effective as more frequent, lower doses. Furthermore, our toxicology results were consistent with clinical data from the recent Nix-TB trial in which anemia was the most frequent cause of LZD discontinuation. Our toxicodynamic analysis supports other preclinical and clinical observations that LZD myelotoxicity is time-dependent. Thus, while daily (or even twice daily) LZD dosing may be preferred at treatment onset or in the absence of effective companion drugs, larger doses given less frequently (e.g., q48h) may be preferred after an initial phase of daily treatment (or even earlier in potent regimens), to preserve efficacy but minimize toxicity. Additional murine and human studies in the context of multidrug therapy given over prolonged periods are required to test this hypothesis. These results also suggest that mice may serve as suitable models for both pharmacodynamic and toxicodynamic evaluations of other oxazolidinones in development.

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# Delamanid Central Nervous System Pharmacokinetics in Tuberculous Meningitis

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**Background:** Central nervous system (CNS) tuberculosis (TB) is highly fatal and causes severe neurologic sequelae in survivors. Multidrug-resistant (MDR-) CNS TB (caused by strains resistant to isoniazid and rifampicin) is particularly devastating. MDR TB meningitis (TBM) is associated with 12-fold higher mortality than drug susceptible TBM, approaching 100% in some series. Delamanid, a nitro-dihydro-imidazooxazole, is a new TB drug. It is oral, well-tolerated, and displays activity against Mycobacterium tuberculosis (M.tb). It is commonly used in second-line treatment regimens, especially in patients with extensively drug resistant (XDR) TB. Data from rats suggest that delamanid crosses the blood-brain barrier (BBB) and concentrates in brain tissue, making it a potential candidate for MDR-TBM treatment. Here we present initial pharmacokinetic data from the CNS compartments of rabbits administered delamanid, plus two patients with XDR TBM.

**Methods:** Rabbits with and without experimentally-induced TBM (subarachnoid injection of H37Rv M.tb) underwent plasma (0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 24, 36, 48h), and/or terminal CSF and brain sampling (8, 10, or 24h) following a single 5 mg/kg dose of delamanid. Two patients (one from India and one from the Philippines) with XDR-TBM receiving second-line treatment that included delamanid underwent steady-state plasma (0, 2, 4, 7h) and cerebrospinal fluid (CSF) (4h) sampling for therapeutic drug monitoring following standard 100mg (twice-daily) dosing. Delamanid concentrations were measured by mass spectrometry (limits of quantification 10-2000 ng).

**Results:** In healthy rabbits, total plasma, CSF, and brain delamanid concentrations at 8 hours were 65, 0.50, and 488 ng/mL and at 24 hours were 9.6, 0.50, and 66 ng/mL (lesion concentrations in diseased rabbits pending). Brain DM-6705 metabolite levels at 8 and 24 hours were 160 and 134 ng/mL, respectively, CSF DM-6705 undetectable. Mean maximum plasma concentration (Cmax) value was 188.5 ng/mL (Tmax ~9.5). In patients, Cmax values were 750 and 500 ng/mL (Tmax ~4h); 4h CSF values were 1.9 (BLQ; DM-6705 0.9 ng/mL) and 33 ng/mL, respectively. Both patients tolerated delamanid well, with early clinical improvement demonstrated.

**Conclusion:** Delamanid, a highly protein-bound drug demonstrated high brain:plasma ratios in rabbits. CSF:plasma ratios were low in both rabbits and humans, but the relevance of this finding is unclear, as free drug concentrations in CSF may exceed the minimal inhibitory concentration (MIC95 on agar media with 0.5% albumin is 12 ng/mL) of the infecting organism for many patients (especially early in treatment when BBB may be more leaky), the number of M.tb bacilli in CSF are relatively few, and their viability in that matrix is unknown. Other antimicrobials display similar characteristics (e.g. anti-fungals) and are still highly effective despite low CSF concentrations. Delamanid may be a useful drug for CNS TB, but a fuller understanding of its distribution over time with treatment is needed to determine the right dose and duration to test in trials, its potential role in multidrug therapy, and the patient population (drug-sensitive TB, drug-resistant TB) that may benefit from its use.

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## Protein binding of rifampicin is not saturated when using high-dose rifampicin

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**Background:** A higher dose of rifampicin (35 mg/kg instead of 10 mg/kg daily) results in a strong, more than proportional increase in total (protein-unbound plus bound) rifampicin exposure in plasma, which is associated with a shorter time to culture conversion [Boeree et al., 2017]. Although concentration-response evaluations are ideally based on protein-unbound (active) exposures, total exposures are often used for this purpose, assuming a good relationship between total and protein-unbound exposure measures. It is currently unknown if strongly elevated rifampicin exposures cause a saturation in protein-binding, resulting in an increased rifampicin free fraction, a situation in which total exposures would be misrepresentative for the relevant protein-unbound exposures.

**Methods:** Protein-unbound and total rifampicin concentrations were measured in human serum spiked with different total concentrations (up to 64 mg/L) of rifampicin (in vitro study), and in a subset of samples obtained after intensive pharmacokinetic sampling in pulmonary TB patients who used standard (10 mg/kg daily, n=10) or high-dose rifampicin (35 mg/kg, n=10) in the PanACEA-MAMS-TB-01 study (in vivo study). Concentrations were measured using validated ultrafiltration and UPLC techniques. In the in vivo study, pharmacokinetic parameters were assessed using standard non-compartmental analysis and compared between the two dosing groups using independent-sample t-tests on log-transformed pharmacokinetic parameters. Total AUC0-24 values of all individuals (n=20) were correlated with the corresponding unbound AUC0-24 measures and with the AUC0-24 free fraction, using Spearman's rho. The performance of total AUC0-24 to predict unbound AUC0-24 was evaluated using the jackknife method as resampling technique. Ratios of unbound over total AUC0-24 were calculated and the median of these ratios was used as conversion factor to predict unbound from total AUC0-24. Bias was assessed using the median percentage prediction error (MPPE) and imprecision was determined by the median absolute percentage prediction error (MAPE), which both should be <15%.

**Results:** The in vitro free fraction of rifampicin remained unaltered (~9%) up to 20 mg/L and increased up to 13% at 41 mg/L and 17% at 64 mg/L rifampicin. In the in vivo study, rifampicin total and unbound AUC0-24 values showed a large and more than proportional (up to eight-fold) increase upon increasing the dose from 10 to 35 mg/kg. The geometric mean peak concentration (Cmax) in the 35 mg/kg dosing group was 26.1 mg/L (range: 20.4-38.9 mg/L). The arithmetic mean percent unbound to total AUC0-24 in vivo (free fraction) was 13.3% (range: 8.1-24.9%) and 11.1% (range: 8.6-13.6%) for the standard and high-dose group (p=0.214), respectively. A high and significant correlation was found between total and unbound AUC0-24 (Spearman's rho, 0.920; p<0.001). There was no significant correlation between total AUC0-24 and the AUC0-24 free fraction (Spearman's rho, -0.280; p=0.232). Prediction of unbound AUC0-24 based on total AUC0-24 resulted in a MPPE of -0.05% and a MAPE of 13.2%.

**Conclusion:** High-dose rifampicin (35 mg/kg) does not result in an increased free fraction and, thus, plasma protein saturation. Unbound exposures to rifampicin were well predicted from total exposures, supporting the use of total rifampicin concentrations in exposure-response evaluations of this pivotal TB drug.

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# Using Mycobacterium tuberculosis single nucleotide polymorphisms to predict fluoroquinolone treatment response

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**Background:** Clinical phenotypic drug susceptibility testing (DST) for fluoroquinolone resistance in Mycobacterium tuberculosis (Mtb) is currently based on empirically detecting Mtb growth at a single "critical concentration" of the fluoroquinolone of interest. This method of resistance detection is time-consuming, resource intensive, and provides limited information for a nuanced treatment response. Recently developed molecular diagnostics allow for rapid detection of gyrA Mtb resistance-conferring single nucleotide polymorphism (SNPs). We propose that SNPs can be used to predict a strain's approximate minimum inhibitory concentration (MIC) and, based on population pharmacokinetic and pharmacodynamic (PKPD) modeling, can be used to make informed decisions for more effective individualized fluoroquinolone dosing for TB.

**Methods:** We sequenced the resistance determining region of the gyrA gene of 138 clinical Mtb isolates collected from India, Moldova, Philippines and South Africa. After determining each strain's ofloxacin, moxifloxacin, levofloxacin, and gatifloxacin MIC, we grouped specific gyrA SNPs by fluoroquinolone specific MICs into high or low resistance categories. Using published population pharmacokinetic models and Monte Carlo simulations we explored the probability of reaching therapeutic targets defined in the literature of the area under the concentration time curve (AUC)/MIC ratio by dose for each fluoroquinolone relative to MIC category.

**Results:** Among simulated patients harboring Mtb isolates with SNPs associated with low level gatifloxacin MICs, we estimated only 5% treated with 400 mg/day of gatifloxacin would achieve an AUC/MIC target ratio of 184. However, if dosing were increased to 800 mg/day or 1200 mg/day, approximately 40% and 60% of patients would attain the therapeutic target, respectively. Among simulated patients with SNPs associated with low level moxifloxacin MICs, only 5% of would achieve a target AUC/MIC ratio of 106 with standard dosing of 400 mg/day of moxifloxacin. Increased moxifloxacin dosing of 800 mg/day would result in 44% of the patient population attaining therapeutic targets. Among patients harboring low level levofloxacin or ofloxacin MICs, increased dosing of either drug would not meaningfully increase the proportion of patients likely to attain therapeutic targets. In contrast, among patients with isolates harboring SNPs associated with high level fluoroquinolone resistance, increased dosing of levofloxacin, moxifloxacin, gatifloxacin, or ofloxacin did not increase the probability of therapeutic target attainment.

**Conclusion:** We demonstrated using a limited dataset that SNPs in the gyrA gene of Mtb can be used to rapidly predict approximate MICs of individual fluoroquinolones, allowing for potentially clinically relevant MIC categorization of Mtb strains. This MIC categorization, together with Monte Carlo simulations can be used to predict the probability a patient will achieve therapeutic targets for individual fluoroquinolones based on the presence of a specific gyrA SNP. This novel approach to interpreting molecular based DSTs could potentially provide a template for evaluation of anti-tuberculosis drugs in real time using currently available tools, and move closer to precision medicine for treatment of drug resistant TB.

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## Moxifloxacin population pharmacokinetics and exposure/MIC target attainment in patients on treatment for MDR-TB

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**Background:** The WHO recommends moxifloxacin at a dose of 400 mg for the treatment of multidrug-resistant TB (MDR-TB). There is limited data characterising the pharmacokinetics of moxifloxacin, including descriptions of moxifloxacin exposure in relation to the described ratio of the area under the unbound drug concentration-time curve to minimum inhibitory concentration ($fAUC_{0-24}$/MIC) targets in patients on treatment for MDR-TB.

**Methods:** We performed a pharmacokinetic study in adult patients over the age of 18 years on treatment for MDR-TB at two TB hospitals in Cape Town. All participants received a moxifloxacin dose of 400mg as per national guidelines during the study period. We measured total plasma concentration just before the dose and at 2, 4, 6, 8, and 10 hours post-dose. We interpreted the pharmacokinetic data using nonlinear mixed-effects modelling in NONMEM and evaluated one- and two-compartment disposition models. We included allometric scaling on all disposition parameters using either total body weight (TBW) or fat-free mass (FFM). Baseline sputum MICs were determined using Sensititre MYCOTB MIC plates. Parameter estimates of the final model were used to derive the steady-state $fAUC_{0-24}$ for all patients. We determined the proportion of patients achieving a $fAUC_{0-24}$/MIC ratio above the recommended target of 53 (assuming fu = 50%). Using demographic data of TB patients from South Africa and West Africa, we described the probability of achieving the expected $fAUC_{0-24}$/MIC ratio at each MIC level using the current 400-mg-dose-fits-all approach. We also explored the effect of the dosing approach on patients in the difference weight-bands currently recommended by WHO for other drugs in the treatment regimen for MDR-TB (<33 kg, 33 - 50 kg, 51- 70 kg and >70 kg).

**Results:** Plasma samples were available from 131 patients, including 155 pharmacokinetic profiles. The median weight and FFM of the patients were 47 kg (range: 30 – 85) and 40.1 kg (24.1 – 58.9), respectively. Moxifloxacin pharmacokinetics was best described using a two-compartment disposition model, first-order elimination and delayed first-order absorption. Allometric scaling using FFM resulted in a better fit compared to TBW. Between-subject variability was supported on apparent oral clearance (CL/F) and bioavailability parameters while between-occasion variability was included on absorption rate constant (Ka), Tlag and bioavailability. Patients with higher weight achieved lower exposures than low weight patients. Baseline MIC data were available for 101 patients and MICs for 72/101 patients were less than or equal to 0.25 mg/L, the breakpoint associated with resistance. The probability of target attainment at each MIC was: 0.06 (100%), 0.12 (86%), 0.25 (27%), 0.5 (1%), and <0.01% for MICs above 0.5. Our simulations show that the proportion of patients achieving target $fAUC_{0-24}$/MIC decreases exponentially with weight, ranging from 51% in individuals weighing 30 kg down to 24% for 90 kg individuals.

**Conclusion:** 92% of our patients with moxifloxacin MIC's < the 0.25 mg/L breakpoint attained the $fAUC_{0-24}$/MIC target ratio ≥53. Our study adds to the growing body of evidence that higher doses of moxifloxacin are required in patients in higher weight bands.

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## Population pharmacokinetics of cycloserine dosed as terizidone in relation to minimum inhibitory concentrations in patients on treatment for multidrug-resistant tuberculosis

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**Background:** Cycloserine is a key drug recommended by WHO in long treatment regimens for multidrug-resistant tuberculosis (MDR-TB). There is limited data on the pharmacokinetics of cycloserine dosed as terizidone, which consists of two molecules of cycloserine. Considering the neurotoxicity associated with the use of cycloserine, and the relative weakness of many of the second-line anti-TB drugs, data defining target cycloserine $fAUC_{0-24}$/minimum inhibitory concentrations (MIC) ratios require rapid accumulation.

**Methods:** We performed a pharmacokinetic study in adult patients over the age of 18 years on treatment for MDR-TB at two hospitals in South Africa. During the study period, the standard MDR-TB treatment regimen consisted of moxifloxacin, kanamycin, pyrazinamide, cycloserine dosed as terizidone (dosed by weight band), ethambutol and ethionamide or high-dose isoniazid. Blood samples were collected pre-dose and at 2, 4, 6, 8, and 10 hours post-dose, and in nine patients additional samples were collected at 12, 24, and 26 hours post-dose. Plasma concentrations of cycloserine were quantified using a validated LC/MS/MS assay. Concentration-time data were interpreted using nonlinear mixed-effects modelling in NONMEM software. The dose of terizidone was converted to an equivalent dose of cycloserine by assuming two molecules of cycloserine for each molecule of terizidone and adjusting for the molecular weight. We evaluated one- and two-compartment disposition models with first-order absorption (with or without a delay) and elimination. Allometric scaling was included in the base model and the effect of other physiologically plausible covariates including creatinine clearance (calculated using Cockroft-Gault formula) was investigated. Baseline sputum MICs were determined using Sensititre MYCOTB MIC plates.

**Results:** 927 plasma samples were available from 166 profiles contributed by 133 patients. The median weight and fat-free mass (FFM) were 47 kg (range: 30 – 85) and 40.7 kg (24.1 – 58.9), respectively. A one-compartment disposition model with first-order absorption (delay described by a chain of transit compartments) and elimination best described the pharmacokinetics of cycloserine. The parameter estimates of the final model were: apparent oral clearance (CL/F = 0.832 L/h), apparent volume of distribution (V/F = 23.3 L), absorption rate constant (Ka= 0.836 h-1), mean transit time (MTT = 0.565 h). The model could detect two clearance pathways, non-renal and renal, with the latter being modulated by creatinine clearance and accounting on average for 50% of the total elimination. Allometric scaling was included on both pathways and on the volume of distribution using FFM. Between-subject variability was supported on CL/F, and between-occasion variability was included on Ka, MTT, and F. MIC values (median[range]: 16 [2, 32] mg/L) were available for 103 patients. The cumulative percentage of time the concentration is above MIC in the lungs (assuming lung cavity-to-serum penetration ratio of 0.09) was 100% in 2/103 patients and zero in the rest of the cohort.

**Conclusion:** To our knowledge, this is the largest population pharmacokinetic study describing cycloserine dosed as terizidone. Cycloserine pharmacokinetics was described by a one-compartment disposition model with first-order absorption and elimination. The average elimination half-life estimated by our model (19 hours) is longer than previously reported values.

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## Correlation between saliva and plasma levofloxacin concentrations in MDR-TB patients.

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**Background:** In a move towards individualized TB treatment, a non-invasive alternative sampling matrix such as saliva has gained significant attention for its advantages over venous blood sampling. Therapeutic Drug Monitoring (TDM) has gained momentum, and using saliva sampling might become a game changer in TB treatment.

The aims of our study were: a) to evaluate the correlation between plasma and salivary levofloxacin concentrations; and b) to gauge the possibility of using saliva as an alternative sampling matrix for TDM of levofloxacin in TB endemic areas.

**Methods:** This was a prospective pharmacokinetic study that enrolled MDR-TB patients receiving levofloxacin (Lfx; 750-1000mg once daily dosing) as a part of standardized treatment regimen at German Nepal TB Project Clinic, Nepal. Paired blood and saliva samples were collected at steady state; before and at 1,2,4,8 hours after intake of Lfx at two-time periods (first and second month of treatment). Lfx concentrations were quantified using liquid chromatography- tandem mass spectrometry (LC-MS/MS).

**Results:** Lfx had a good salivary exposure in 23 MDR-TB patients. During the first month, the median (IQR) area under the concentration-time curve (AUC0-24) was 58.60 (47.60-87.81) mg*h/L in saliva and 99.91 (76.80-129.70) mg*h/L in plasma. A moderate positive correlation (rs= 0.59; p=0.004) was demonstrated between the saliva and plasma AUC0-24. Similarly, during the second month, the median (IQR) AUC0-24 was 75.63 (61.45-95.70) mg*h/L in saliva and 102.7 (84.46-131.9) mg*h/L in plasma. This time, saliva and plasma AUC0-24 showed a strong positive linear relationship (rs =0.772; p=0.0001) compared to the first month. Furthermore, a trend towards moderate positive correlation (r=0.455; p=0.004) was observed when Lfx Cmin in saliva was evaluated to predict its AUC0-24 in plasma (r= 0.395; estimated regression equation). The Cmin levels below 2 mg/L corresponded to low plasma and saliva exposures.

**Conclusion:** Our findings suggest that salivary Lfx concentrations can be a valuable pre-selection tool to identify patients with a LFX concentration below the Cmin cut-off of 2 mg/L. Despite a good Lfx penetration in saliva, the large inter- and intra- individual variability might limit the accurate quantitative determination of plasma levels. Despite this limitation, saliva could contribute in early semi-quantitative TDM of Lfx to optimize dosing.

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## Levofloxacin pharmacokinetics in MDR-TB patients at month one and after two months of treatment.

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**Background:** Levofloxacin belongs to the group A drugs for treating multi-drug resistant tuberculosis (MDR-TB). The aims of this study were to explore the inter- and intra-individual pharmacokinetic variability of levofloxacin exposure in MDR-TB patients and to assess the probability of target attainment of levofloxacin in these patients.

**Methods:** This was a prospective pharmacokinetic study that enrolled MDR-TB patients receiving levofloxacin (750-1000mg once daily dosing) as a part of standardized treatment regimen at German Nepal TB Project Clinic, Nepal. The plasma samples were collected at steady state; before and at 1,2,4,8 hours after intake of levofloxacin at two-time periods (first and second month of treatment). Levofloxacin concentrations were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Non-compartmental analysis was utilized to compute the pharmacokinetic parameters.

**Results:** The median age and body mass index of 23 enrolled patients were 32 (28-47) years and 17.96 (16.23-18.83) kg/m2 respectively; 16 (70%) were male. During the first month, maximum plasma concentration (Cmax) of 10.09 (9.1-11.27) mg/L was achieved at corresponding tmax of 2 (1.08-3.92) hours. The median area under concentration time curve (AUC0-24) was 99.9 (76.80-129.7) mg*h/L with an elimination half-life (t1/2) of 9.0 (6.5-10.7) hours. In the second month, median Cmax was 10.56 (9.18-11.58) mg/L and tmax was 2.0 (1.0-2.0) hours. Similarly, the median AUC0-24 was 102.7 (84.46-131.9) and t1/2 was 8 (6.3-10.11) hours. We found that levofloxacin AUC0-24 did not change significantly (p= 0.90) over time comparing between these two-time periods. However, large inter-individual variability in AUC0-24 was observed. Furthermore, 83% (in first month) and 91% (in second month) of patients attained the target value of 146 (hollow fiber study on maximum M. tuberculosis kill) when MIC was 0.5 mg/L. As the MIC increased to 1 mg/L, the proportion of patients attaining the PK/PD target decreased to 17% and 20%. This is worrisome, as the median MIC in our patient population was 1mg/L.

**Conclusion:** The findings suggest that currently prescribed levofloxacin dosages for most patients don't seem sufficient to meet the PK/PD target.

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## Bioavailability of pyrazinamide, moxifloxacin, isoniazid, ethambutol, and terizidone when tablets are crushed in the treatment of multidrug-resistant tuberculosis

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**Background:** Treatment outcomes in multidrug-resistant tuberculosis (MDR-TB) are poor. Due to the toxicity and long duration of the treatment regimen, only approximately half of patients are successfully treated. A heavy pill burden together with nausea and vomiting, which are common adverse effects, contribute to poor regimen tolerability. It is regular practice in some centers to crush medication to ease ingestion in a belief that this will reduce gastrointestinal upset, and in children where suitable formulations are frequently not available for those unable to swallow whole tablets. Considering that subtherapeutic plasma concentrations of some TB drugs have been associated with poor clinical outcomes including acquisition of drug resistance, it is important to investigate whether crushing affects second line drug exposure in the treatment of MDR-TB.

**Methods:** We performed a sequential pharmacokinetic study in patients over 18 years of age on treatment for MDR-TB at two TB hospitals in Cape Town. We evaluated the bioavailability of second line anti-TB drugs including pyrazinamide, moxifloxacin, isoniazid, ethambutol, and cycloserine dosed as terizidone when crushed and mixed together in water, with reference to whole tablets at the same dose. Intensive pharmacokinetic sampling was conducted on two occasions approximately two weeks apart once patients were established on treatment. We sampled blood pre-dose and at 2,4,6,8 and 10 hours post-dose on both occasions and used liquid chromatography tandem mass spectrometry to perform the drug concentrations in plasma. The following pharmacokinetic measures were assessed using non-compartmental analysis: area under the concentration-time curve to 10 hours ($AUC_{10}$) and peak concentration (Cmax). We assessed bioequivalence of crushed versus whole tablets using geometric mean ratios (GMR).

**Results:** Twenty participants completed the study: 15 men, median age 31.5 years and BMI 17.3 kg/m². Ten of the 20 participants (50%) were HIV-infected. There was a 42% reduction in $AUC_{10}$ and a 46% reduction in Cmax of isoniazid when the tablets were crushed ($AUC_{10}$ GMR: 58%; 90% CI: 47% to 73%; Cmax GMR: 54%; 90% CI: 40% to 73%). Crushed pyrazinamide, moxifloxacin, ethambutol and terizidone were bioequivalent compared to the whole tablet reference.

**Conclusion:** We recommend that crushing of isoniazid tablets be avoided if possible when administering isoniazid together with other second line anti-TB drugs. Paediatric isoniazid formulations may be a viable alternative for adults in clinical settings where the crushing of isoniazid is indicated.

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## Linezolid pharmacokinetics in South African patients with drug resistant tuberculosis and high rates of HIV co-infection

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**Background:** Despite the widespread use of linezolid for drug-resistant tuberculosis (TB), its pharmacokinetics (PK) are poorly defined in patients with TB, particularly at the most commonly used dose of 600 mg daily and amongst HIV-infected patients in sub-Saharan Africa. We aimed to describe the PK of linezolid, explore the effect of covariates on linezolid exposure, and estimate the probability of PK/pharmacodynamic (PD) target attainment in South African patients with drug-resistant TB.

**Methods:** Consecutive adult patients on linezolid-based regimens were recruited from a public-sector TB hospital in Cape Town. Participants underwent plasma PK sampling on a single occasion pre-dose and at 1, 2, 3, 4, 5, 6, and 24 hours after a standardized meal and observed linezolid administration. Non-compartmental analysis was performed to estimate linezolid PK parameters, and linear regression was done to explore associations between clinically-relevant covariates and log-transformed area under the concentration-time curve (AUC0-24) and trough concentration (Cmin). Covariates included age, sex, ethnicity, HIV status, linezolid dose/kg, serum creatinine concentration, and concurrent use of ritonavir-boosted lopinavir. The PK/PD target for efficacy was defined as free AUC0-24/MIC (fAUC/MIC) of 119 mg.L/hr; the parameter for toxicity was Cmin of 2 mg/L.

**Results:** Thirty participants were included: 26 were on the initial dose of 600 mg daily at the time of sampling, and 4 participants were on 300 mg daily after undergoing dose reduction for suspected linezolid-related toxicity. Median age was 33 years (interquartile range [IQR] 27 to 44); 19 participants (63%) were male, and the median weight was 55.5 kg (IQR 49.8 to 67.6). Half the participants were HIV-infected, all of whom were on concurrent antiretroviral therapy at the time of the PK sampling visit. The median duration on linezolid was 59 days (range 20 to 95). The multivariate model described 38% of the variability associated with AUC0-24. There was a correlation between linezolid dose and exposure (13.7% (95% confidence interval [CI], 1.5 to 27.6) increase in AUC0-24 per 1 mg/kg dose increment) after adjustment for other covariates. Age was an independent predictor of Cmin, with an estimated 39.1% (95% CI, 3.4 to 86.9) increase in trough concentrations per 10-year increment in age. The standard 600 mg dose was predicted to achieve the efficacy target at wild type minimum inhibitory concentration values, but the probability of target attainment dropped to 61.5% (95% CI, 40.6 to 79.8) at the critical concentration of 1 mg/L. Trough concentrations exceeded the toxicity threshold in 57.7% of those on 600 mg daily, and in 75% of those who had undergone dose reduction to 300 mg daily.

**Conclusion:** Much of the PK variability was unexplained, but age and dose were identified as predictors of trough concentrations and exposure, respectively. Although the standard 600 mg dose is likely to achieve efficacy targets for wild type M tuberculosis, the suboptimal target attainment at the critical concentration is concerning. It is a priority to perform additional studies to evaluate the use of therapeutic drug monitoring and alternative dosing strategies in order to optimize the use of this important antituberculosis agent.

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## Development of a Therapeutic Drug Monitoring Platform for Patients with MDR-TB

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**Background:** The pharmacokinetics and pharmacodynamics (PK/PD) of antibiotics used in the treatment of tuberculosis (TB) become increasingly important, especially in the therapy of multi-drug resistant (MDR) TB. Better understanding of the PK/PD properties of anti-TB drugs not only may help prevent adverse events and therapy discontinuation. It also has the potential to predict and stop the development of acquired drug resistance in the targeted mycobacteria. However, the assessment of PK/PD in TB and its clinical application in the form of therapeutic drug monitoring (TDM) are hampered by the large number of antibiotics used for treatment of TB: Therapy regimens regularly comprise at least four antibiotics. Measuring them one by one at several time points as needed for PK/PD analyses is resource- and time consuming. Therefore, we aimed to develop a high-performance liquid chromatography-mass spectrometry (HPLC-MS2)-based multi-analyte assay to extract and measure all essential anti-TB drugs from human plasma in a single-instrument/single-run setting.

**Methods:** The panel of drugs included amikacin, amoxicillin, bedaquiline, capreomycin, clavulanic acid, clofazimine, cycloserine/terizidone, delamanid, ethambutol, isoniazid, kanamycin, levofloxacin, linezolid, meropenem, moxifloxacin, PAS, prothionamide, pyrazinamide, rifabutin, and rifampicin. Target ranges for the assay performance were defined based on published PK/PD properties of the assessed drugs. The analytes were extracted by liquid-liquid extraction with acetonitrile (ACN) and 1% formic acid in water (FA). Analysis was performed on an Agilent 1100

HPLC system featuring a Merck Milipore SeQuant ZIC-HILIC (2.1x100mm, 5µm, 200 Å) column and a Waters Micromass Quattro Premier XE triple quadrupole MS using electro spray ionization (ESI). A 20-minute gradient of ACN and 1% FA was used for LC separation. We applied multiple reaction monitoring (MRM) to quantify several compounds during one HPLC-MS2 analysis. Dihydrostreptomycin, gentamicin, muscimol, reserpine, and trovafloxacin were evaluated as potential internal standards (IS).

**Results:** We identified specific MRM-transitions and retention times for all 20 antibiotics and five IS. Clavulanic acid was the only compound to be measured in negative ion mode. All 20 antibiotics fulfilled FDA/EMA calibration criteria and met the required lower limit of quantification (LLOQ). The target upper limit of quantification (ULOQ) was not achieved in amikacin, ethambutol, kanamycin, levofloxacin, linezolid, and streptomycin. However, these antibiotics were calibrated at least up to 1/10 ULOQ so that highly concentrated samples could be diluted 10-fold prior to analysis. $1/X^2$-weighted, $1/Y^2$-weighted, and robust linear regressions yielded the best calibration results. Gentamicin was selected as IS for amikacin and streptomycin, muscimol for cycloserine and ethambutol, trovafloxacin for moxifloxacin, and reserpine for the remaining substances.

**Conclusion:** We developed a single-instrument single-run multi-analyte assay to extract and measure 20 anti-TB drugs from human plasma. The simple extraction procedure and the runtime of 20 minutes allow at least for a mid-throughput application. When integrating a dilution step for highly concentrated samples, the assay performed well in the previously defined clinically relevant concentration range. Yet, full validity of the assay still has to be proven.

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## Sampling Strategies for Therapeutic Drug Monitoring of Moxifloxacin with and without Rifampicin in Tuberculosis Patients

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**Background:** Therapeutic drug monitoring (TDM) of moxifloxacin has been recommended to increase treatment outcomes, since moxifloxacin exposure shows large interindividual variability and is influenced by a clinically relevant drug-drug interaction with rifampicin. To reduce the burden of TDM, area under the concentration curve for the 24 h dosing interval (AUC0-24) can be adequately estimated using a small number of appropriately timed samples. This study aimed to develop a population pharmacokinetic model in tuberculosis patients for moxifloxacin alone (MFX) and together with rifampicin (MFX+RIF) along with sampling strategies using a Bayesian approach as well as multiple linear regression.

**Methods:** Three datasets of moxifloxacin pharmacokinetic curves in tuberculosis patients were included in this study (n=101). A population pharmacokinetic model and sampling strategies using a Bayesian approach were developed for MFX (n=77) and MFX+RIF (n=24). Sampling strategies using multiple linear regression were developed as well for MFX and MFX+RIF. Only clinically suitable sampling strategies (1-3 samples, maximum timespan 8 h) were tested. Jackknife analyses were used for validation of the models and sampling strategies. AUC0-24 estimated by the population pharmacokinetic model or sampling strategy was compared with AUC0-24 calculated with the trapezoidal rule using Bland-Altman plots and Passing Bablok regression.

**Results:** Moxifloxacin AUC0-24 was non-clinically relevant underestimated in the models of MFX (-5.1%, SE 0.8%) and MFX+RIF (-10.2, SE 2.5%). AUC0-24 was adequately estimated with the Bayesian approach using 0 and 5 h post-dose samples for MFX (-0.4%, SE 1.3%) and t=0 and 6 h samples for MFX+RIF (-5.5%, SE 3.1%). The sampling strategies using multiple linear regression used 0 and 4 h post-dose samples for MFX (n=66) and t=1 and 6 h samples for MFX+RIF (n=14), showing a mean AUC0-24 underestimation of -0.2% (SE 1.3%) and mean AUC0-24 overestimation of 0.9% (SE 2.1%), respectively.

**Conclusion:** In this study, we successfully developed and validated four sampling strategies, i.e. Bayesian approach (MFX t=0 and 5 h, MFX+RIF t=0 and 6 h) and with multiple linear regression (MOX t=0 and 4 h, MFX+RIF t=1 and 6 h). All described sampling strategies are suitable to be implemented in clinical practice to facilitate TDM of moxifloxacin in tuberculosis patients.

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## Quality Assessment of Dried Blood Spots from Tuberculosis Patients from Four Countries

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**Background:** Dried blood spot (DBS) sampling is a blood collection tool that uses a finger prick to obtain a blood drop on a dried blood spot card. It can be used for therapeutic drug monitoring (TDM), a method that uses blood drug concentrations to optimize individual treatment. DBS sampling is thought to be a simpler way of blood collection compared to venous sampling. The aim of this study was to evaluate the quality of dried blood spots from tuberculosis (TB) patients all around the world based on quality indicators in a structured assessment procedure.

**Methods:** In total 464 DBS cards were obtained from four countries: Bangladesh, Belarus, Indonesia and Paraguay. The quality of the dried blood spots DBS cards was assessed using a checklist consisting of 19 questions divided in four categories: the integrity of the DBS materials, appropriate drying time, blood volume and blood spot collection.

**Results:**
After examination, 859 of 1856 (46 %) blood spots did not comply with present quality criteria. In 625 cases (34%) this was due to incorrect blood spot collection. The DBS cards from Bangladesh, Indonesia and Paraguay seemed to be affected by air humidity, causing the blood spots not to dry appropriately. New tools to help obtain DBS of sufficient quality are necessary as well as environmental specific recommendations. Additionally, 3% of the blood spots were rejected because of the integrity of the materials suggesting that the quality of plastic zip lock bags currently used to protect the DBS against contamination and humidity may not be sufficient.

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## Optimal sampling strategies for therapeutic drug monitoring of first-line tuberculosis drugs in patients with tuberculosis

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**Background:** The 24-hour area under the concentration-time curve (AUC0-24) over minimal inhibitory concentration (MIC) ratio is best predictive pharmacokinetic/pharmacodynamic parameter of efficacy of first-line anti-tuberculosis (TB) drugs. An

optimal sampling strategy (OSS) is useful to accurately estimate AUC0-24. OSS has not been developed in fed state or in early phase of treatment for the first-line anti-TB drugs.

**Methods:** An OSS for the prediction of AUC0-24 of isoniazid, rifampicin, ethambutol and pyrazinamide was developed for patients with tuberculosis. A prospective randomized crossover trial was performed during the first three days of treatment in which first-line anti-TB drugs were administered either intravenously, in fasting or fed conditions. The pharmacokinetic data were used to develop OSS with best subset selection multiple linear regression. The OSS was internally validated using jackknife analysis.

**Results:** OSS using time points 2, 4 and 8h post-dose performed best. Bias < 5% and imprecision were <15% for all drugs, except for ethambutol in fed condition.

**Conclusion:** OSS at 2, 4 and 8h post-dose enabled an accurate and precise prediction of AUC0-24 values of first-line anti-TB drugs.

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## Assessment of rifampicin exposure using Dried Blood Spot Analysis; a pilot study

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**Background:** The objective of our study was to assess the effect of pharmacokinetic variability of rifampicin on sputum conversion rate, using Dried Blood Spot (DBS) as method of bioanalysis for Therapeutic Drug Monitoring (TDM).

**Methods:** Rates of sputum culture conversion were determined at weeks 2, 4, 8, 12, 16 after initiating therapy in patients with newly diagnosed drug susceptible sputum smear- and culture-positive tuberculosis, hospitalized in the Regional Clinical Center ,,Ftisiatria" Grodno, Belarus between January 1, 2014 and March 1, 2016. DBS samples obtained at steady state 1, 3, 6 hours after drug administration were measured by validated liquid chromatography-tandem mass spectrometry. The MICs of rifampicin were determined by the MGIT960 system in the range of 0.125mg/L-2 mg/L. The AUC of rifampicin were calculated using MWPharm® version 3.82; Medi-ware, Netherlands, by the log-linear trapezoidal rule.

**Results:** Altogether, 18 patients - 16 males and 2 females - were included. The median age was 44,5 (interquartile range (IQR) 48-87) years and body mass index 22.8 (IQR 19.4-31.4) kg/m2 . All patient isolates had MIC<0.125mg/L; median AUC0–24 36.06mg*h/L (IQR 24.14-59.55) and AUC/MIC 576.88 (IQR 386.24-952.8). Six (33%) patients had sputum culture conversion at the end of the first month and 14 (78%) at the end of the second month of treatment. Two out of 4 still culture positive patients died during the treatment due to tuberculosis, one patient died because of co-morbidity and one had treatment failure. The median AUC/MIC ratio of patients with negative culture at the end of 8-th week (775.2 (IQR 381.92-952.8) was nearly 2 times higher compared to patients with still positive culture - 406.32 (IQR 410.08-611.68;P=.373).

**Conclusion:** a trend was observed in the association between rifampicin drug exposure, culture conversation rate and treatment outcome. Limited sampling by DBS was conveniently used for TDM.

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## Effect of Moxifloxacin plus Pretomanid against Mycobacterium tuberculosis in Log-phase, Acid-phase and Non-Replicating-Persister (NRP)-phase in an in vitro Assay

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**Background:** Tuberculosis is the ninth-leading cause of death worldwide. Treatment success is approximately 80% for susceptible strains and decreases to 30% for extensively resistant strains. Pretomanid (PA824) and moxifloxacin (MXF) are promising options currently in evaluation in clinical trials for the treatment of susceptible and resistant Mtb. However, an even more promising option might arise from the combination of those two drugs.

Methods and Results: Our objective was to characterize the interaction between PA824 and MXF towards Mtb killing in logarithmic growth phase (Log-phase), slowly replicating Acid-phase and Non-Replicating Persister (NRP) phase. The combination was evaluated using our recently developed strategy: H37Rv was used to simulate Log and Acid-phase growth while Mtb strain 18b was used to simulate NRP-phase growth. The studied metabolic states were evaluated in a microdilution plate system containing a 9 x 8 matrix with concentrations of both drugs alone and in combination. We characterized the interaction as antagonistic, additive, or synergistic using the Greco Universal Response Surface Approach model. The interaction between MXF and PA824 is additive, but with a tendency to synergy with acid and NRP-phase organisms, while it is strictly additive for Log-phase organisms.

**Conclusion:** This combination is a promising option for possible shortening treatment of tuberculosis. It will be further tested in the hollow fiber infection model and in animal studies.

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## Predicting antibiotic pharmacodynamics from molecular mechanisms of resistance

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**Background:** Tuberculosis (TB) is the leading cause of death by an infectious agent. Despite the magnitude of the problem, we have limited knowledge how to employ antibiotics in a way that maximizes treatment efficacy. Identifying optimal dosing strategies can be difficult and expensive. Previously, we demonstrated that a drug target binding pharmacodynamic model that links bacterial population biology with chemical reaction kinetics has high predictive and explanatory power for antibiotic pharmacodynamics. Here, we extend the model to incorporate several distinct molecular mechanisms of resistance such as drug-target affinity, target molecule content, or bacterial susceptibility downstream of drug-target binding to explore the how these mechanisms may affect the risk of acquired resistance during treatment.

**Methods:** We introduce a pharmacodynamic model based on a system of partial and ordinary differential equations. The central assumption encoded in the model is that the bacterial growth declines (bacterial killing increases) as the fraction of bound target molecules within the bacterium increases. The model describes the binding and unbinding of antibiotics to their target and how this affects bacterial growth and death.

**Results:** We first set out to validate that our model can predict bacterial susceptibility based on drug-target binding characteristics. Based on reaction kinetics alone, we predict that bacterial MICs should be directly proportional to KD, i.e. that the antibacterial effect is linear with drug-target affinity. We are able to verify this prediction with clinical isolates of 11 bacterial species exposed to 6 different quinolones. We went on to fit our model to time-kill curves of the model organism E. coli exposed to beta-lactams. We are able to predict both MIC and target occupancy at MIC based on drug-target binding and enzymatic degradation rates. We then further test our model with ciprofloxacin time-kill curves of experimentally manipulated bacteria expressing increasing amounts of target molecules. Based on kill curves alone, we can predict the amount of target overexpression in the experimentally manipulated strains. The predictions match target molecule quantifications by Western blot, such that we were able to experimentally verify a hypothesis generated by our theoretical work.
After validating our model, we explore how different molecular mechanisms of resistance are expected to affect bacterial growth and show how to predict the susceptibility of resistant mutants across drug concentrations, thereby predicting concentration ranges at which resistance is selected for.

**Discussion:** Here, we establish a quantitative link between biochemical mechanisms of drug action and bacterial susceptibility. Our novel pharmacodynamic model that allows silico quantitative predictions of

ranges of antibiotic concentrations that should be avoided to minimize resistance. Since our work improves quantitative and mechanistic understanding of drug action, it can also be used as a tool for designing new, improved antibiotics.

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## The Activity of Moxifloxacin against Acid-Phase and Nonreplicative Persister Phenotype Phase Mycobacterium tuberculosis in a Hollow Fiber Infection Model

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**Background:** A major goal for improving tuberculosis therapy is to identify drug regimens with improved efficacy and shorter treatment durations. Shorter therapies improve patient adherence to the antibiotic regimens which, in turn, decreases resistance emergence. M. tuberculosis exists in multiple metabolic states. At the initiation of therapy, the bulk of the population is in Log-Phase growth. Consequently, it is logical to focus initial therapy on these organisms. Moxifloxacin has good early bactericidal activity against Log-Phase growth bacteria and is a logical component of initial therapy. It would be optimal if this agent also possessed activity against Acid-Phase and Non-Replicative Persister (NRP) Phenotype organisms.

**Methods and Results:** We studied multiple exposures to moxifloxacin (equivalent to 200 mg-800 mg) in our Hollow Fiber Infection Model against strain H37Rv in Acid-Phase and against strain 18b in streptomycin starvation, which is a model for NRP-Phase organisms. Moxifloxacin possesses good activity against Acid-Phase organisms, generating from 3.75 Log10(CFU/ml) cell kill (200 mg daily) to 5.16 Log10(CFU/ml) cell kill (800 mg daily) over the 28 days of the experiment. Moxifloxacin also has activity against the streptomycin-starved strain 18b. The 400-800 mg daily regimens achieved extinction at day 28, while the no-treatment control still had 1.96 Log10(CFU/ml) culturable. The lowest dose (200 mg daily) still had 0.7 Log10(CFU/ml) cell measurable at day 28.

**Conclusion:** Moxifloxacin is an attractive agent for early therapy, as it possesses activity against three metabolic states of M. tuberculosis.

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## The Hollow Fibre Infection Model to further fill our preclinical drug development pipeline; focus on rifampin dosing.

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**Background:** The treatment duration of tuberculosis is prolonged, causing poor treatment adherence and the emergence of drug resistance. Therefore, a shorter treatment duration is highly required. To this end, increasing the standard rifampicin dose has been assessed. In vitro and in vivo studies showed that increasing the dose increased therapeutic activity and efficacy. Besides, recent clinical studies showed that doses up to 50 mg/kg rifampicin were well tolerated. In our novel hollow fibre infection model we assessed whether a simulated high dose rifampicin of 50 mg/kg resulted in increased bactericidal activity compared to the standard dose.

**Methods:** A Mycobacterium tuberculosis strain of the H37Rv genotype was exposed to either a standard rifampicin simulated dose of 10 mg/kg (Cmax 1.8 mg/L, half-life time 3 hours) or a high dose of 50 mg/kg (Cmax 50.2 mg/L) during 14 days in our hollow fibre infection model. Cmax and half-life time were based on human pharmacokinetics. Pharmacokinetic exposure was assessed using a bio-activity assay. Therapeutic activity was assessed by counting colony forming units.

**Results:**
Pharmacokinetics:
Linear regression analyses of the rifampicin observed and targeted concentration resulted in $R^2$=0.934 and $R^2$=0.948 for a simulated dose of 10 mg/kg and 50 mg/kg rifampicin, respectively.

Pharmacodynamics:

The mycobacterial load decreased in 8 days from an average of Log 6.8 CFU/ml to Log 1.8 CFU/ml and Log 1.5 CFU/ml upon exposure to standard and high dose rifampicin, respectively. The emergence of rifampicin resistance was observed from day 10 at both rifampicin concentrations.

**Conclusion:**
In our newly designed hollow fibre infection model, higher dosing of rifampicin did not result in increased bactericidal activity after 14 days of exposure and neither did it prevent the emergence of drug resistance.

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## Population pharmacokinetic model and limited sampling strategies for personalized dosing of levofloxacin in tuberculosis patients

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**Background:** Levofloxacin is an anti-tuberculosis drug with substantial interindividual pharmacokinetic variability; therapeutic drug monitoring (TDM) is therefore recommended to improve treatment results. TDM would be more feasible with limited sampling strategies (LSSs). A population pharmacokinetic (popPK) model is needed to estimate drug exposure by using a limited number of samples while capturing the relevant pharmacokinetic parameter associated with microbial kill, i.e. area under the concentration curve for the 24 h dosing interval (AUC0-24). This study aimed to develop a population pharmacokinetic (popPK) model of levofloxacin in tuberculosis patients, along with LSSs using a Bayesian and multiple linear regression approach.

**Methods:** The popPK model and Bayesian LSS were developed using data of 30 patients and externally validated with a separate dataset of 20 patients. The LSS based on multiple linear regression was internally validated using jackknife analysis. AUC0-24 estimated by the popPK model or LSS was compared with AUC0-24 calculated with the trapezoidal rule using Bland-Altman plots and Passing Bablok regression. Only clinically suitable LSSs (maximum timespan 8 h, minimum interval 1 h, 1 to 3 samples) were tested. Performance criteria were root mean squared error (RMSE)<15%, mean prediction error (MPE)<5%, and $r^2$>0.95.

**Results:** A one compartment model with lag time best described the data while only slightly underestimating the AUC0-24 (mean -7.9%, SE 1.7%). The Bayesian LSS using 0 and 5 h post-dose samples (RMSE 8.8%, MPE 0.42%, $r^2$ 0.957) adequately estimated the AUC0-24 with a mean underestimation of -4.4% (SE 2.7%). The multiple linear regression LSS using 0 and 4 h post-dose samples (RMSE 7.0%, MPE 5.5%, $r^2$ 0.977) was internally validated with a mean underestimation of -0.46% (SE 2.0%).

**Conclusion:** In this study, we successfully developed a popPK model and two LSSs that could be implemented in clinical practice to facilitate TDM of levofloxacin.

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## Efficacy of chemotherapy infusions in patients with failed treatment of tuberculosis and malabsorption syndrome

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**Background:** The aim of our study was to learn about the efficacy of chemotherapy infusions in patients with failed treatment of tuberculosis and malabsorption syndrome.

**Methods**: 49 patients with failed treatment of tuberculosis, malabsorption syndrome (MS) and disruption of indicators of intestinal penetration (IIP) and 20 relatively healthy donors (3d group) were under our surveillance. Patients were divided into two groups: The first group (main) included 20 patients that were administered ethambutol (E) through and rifampicin (R) through and IV and pyrazinamide (Z), isoniazid (H) orally; the second group (control) included 29 patients that were administered standard chemotherapy orally. Standard chemotherapy included: H, R, Z and E. All patients had drug-sensitive tuberculosis. The severity grade of MS was defined by the disruption of indicators of intestinal penetration (IIP). IIP was determined by the concentration of lactulose and mannitol (lactulose-mannitol test) in the urea. Indicators of H,R,E concentration in the blood serum was was determined by liquid chromatography on a chromatograph (Perkin Elmer (USA)). The concentration of anti-tuberculosis drugs (ATD) was determined after 2, 4, 6 hours after drug administration.

**Results:** After assessing the results of the study we found a disruption of IIP in all the observed patients; however, we did not see any changes in the third group. During the study of ATD concentration in the second group, we found that the ATD concentration was significantly lower than the average therapeutic concentration in the first group ($p<0.05$). The indices regarding the number of patients who took H were not significant between the first and second group ($p> 0.05$).

After establishing the failure of treatment, repeated chemotherapy of ATD was performed. Patients from the first group after the intensive treatment phase had significantly higher indicators of terapy efficacy in: The quantity of clinical symptoms disappearance - 18 (90.00±6.88%) and 16 (55.17±9.40%) ($p<0.05$), cessation of mycobacteria secretion- 16 (80.00±9.18%) and 13 (44.83±9.40%) ($p<0.05$) and resorption of infiltrative changes and closure of cavities of decay in the lungs.

Moreover, after the finish of the intensive treatment phase various forms of tuberculosis resistance against ATD were found in patients with marked manifestations of MS and a low concentration of ATD in their serum. First group - 2 (10.00±6.88%) and second group (37.93±9.17%) of patients ($p<0.05$).

**Conclusion:** In patients with failed treatment of tuberculosis, disruption of IIP is found, which leads to a lower concentration of the main ATD in the blood serum and ineffective treatment, especially with R and E. In order to increase the effectiveness of chemotherapy in patients with failed treatment, disruption of IIP and malabsorption syndrome and drug-sensitive tuberculosis, it is recommended to adminsiter R and E through an IV during the intensive treatment phase to increase the treatment efficacy in this cohort of patients. With severe manifestations of MS, a decrease in the concentration of ATD in the blood and insufficient intensive anti-tuberculosis treatment, tuberculosis resistance to ATD may develop.

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## Moxifloxacin efficacy in patients with co-infections pulmonary tuberculosis/HIV with concominant hepatitis B and/or C in intensive anti-tuberculosis treatment phase

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**Background:** The aim of our study was researching moxifloxacin efficacy in patients with co-infections pulmonary TB/HIV with concominant hepatitis B and/or C in intensive anti-TB treatment phase.

**Methods:** Our research included 63 patients with co-infections, first diagnosed pulmonary TB/HIV with a positive sputum smear and with concomitant viral hepatitis B and/or C. Patients were divided into the second groups: the first group- 30 patients that were administered the second anti-tuberculosis treatment according to a scheme (Anti - TB treatment: Moxifloxacin - 0.4, Izoniazid - 0.3, Ryfampicin - 0.3 and Etambutol (E) - 1.2) and the second group- 33 patients that were administered a standard anti-tuberculosis therapy (Anti - TB treatment: Izoniazid - 0.3, Ryfampicin - 0.3 and Etambutol and Pyrazinimid - 2.0) during 2 months. Patients in comparison groups did not differ in forms of disease and in grade of immunodeficiency, they had similar prevalence of the process in lungs ($p>0,05$). All the patients were administered a standard antiretroviral therapy. Treatment of viral hepatitis B and C was not performed during this stage.

**Results:** Due to the use of the modified chemotherapy regimen in patients from Group 1, the efficacy of treatment after completing the intensive phase of

chemotherapy was significantly higher than the incidence of clinical symptoms– 23 (76.67 ± 7.72%) and 16 (48.48 ± 8.70 %) ($p<0.05$), termination of mycobacterium secretion— 20 (66.67 ± 8.61 %) and 13 (39.39 ± 8.51 %) ($p<0.05$) and resorption of infiltrative changes in the lungs - 19 (63.33 ± 8.80 %) and 11 (33.33 ± 8.21 %) ($p<0.05$), than standard treatment in patients from group 2. Quantity of adverse events in anti-tuberculosis and antiretroviral drugs was credibly lower in 7 (23.33 ± 7.72%) patients from group 1 compared to 16 (48.48 ± 8.70) group 2.

**Conclusion:** The use of Moxifloxacin in of Izoniazid, Ryfampicin, Etambutol in patients with co-infection of TB/HIV with concomitant viral hepatitis B and/or C in the intensive phase of anti-TB treatment allows to achieve an increase in the frequency of termination of bacterial secretion, to reduce the incidence of hepatotoxic adverse reactions, and to reduce the severity of their manifestations, which provides continuous treatment compared to patients that are taking standard anti - TB treatment.

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## Low anti-tuberculosis drug concentrations in HIV-Tuberculosis co-infected adults with low body weight

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**Background:** Anti-tuberculosis (TB) drugs display large pharmacokinetic (PK) variability, which may be influenced by several factors including age, body weight, sex, genetic polymorphisms, co-morbidities and drug-drug interactions. We set out to determine the factors that contribute to PK variability of anti-TB drugs and determine the dose necessary for optimal drug concentrations.

**Methods:** HIV-infected Ugandan adults diagnosed with pulmonary TB were enrolled and underwent pharmacokinetic sampling (1, 2, and 4 hours after drug intake) at 2, 8, and 24 weeks after initiation of TB treatment. High-performance liquid chromatography was used to quantify drug concentrations. Population PK modelling was implemented in Monolix (version 2016R). We tested one- and two-compartment disposition models with first-order elimination. Allometric scaling was applied to clearance and volume. Single nucleotide polymorphisms were analyzed through real-time PCR.

**Results:** Between May 2013 and November 2015, we enrolled 268 patients (148 males) with median weight 53.5 (IQR: 47.5 - 59) kg and age 35 (IQR: 29 - 40) years.

Rifampicin and pyrazinamide demonstrated a one-compartmental disposition, while isoniazid and ethambutol demonstrated a two-compartment disposition. Both drugs had first-order elimination and delayed first-order absorption. Allometric scaling by fat-free mass (FFM) (for rifampicin and ethambutol) and total body weight (for isoniazid and pyrazinamide) was applied to the clearance and volume.
In a typical individual (54 kg body weight and 43 kg FFM), the values for apparent clearance and volume of distribution were 12.6 L/h and 58 L for rifampicin, 2.97L/h and 33L for pyrazinamide, 36.3L/h and 76.4L for ethambutol and 22.8 L/hr and 64.1L for NAT2 fast acetylators on isoniazid, with intermediate and fast acetylators demonstrating a 26.3% and 74.6% reduction in clearance. Concomitant use of efavirenz increased isoniazid clearance by 24.1%. Rifampicin clearance was 20 % higher at week 8 and 24 compared to week 2. There was no association between SLOCOB1 and rifampicin clearance ($p>0.05$).

The bioavailability and absorption of rifampicin and isoniazid varied in patients on different formulations (up to 30%), in particular when comparing intensive and continuation phase of treatment.
When the final models were used to simulate exposures achieved with the current WHO dosing guidelines, participants in the WHO weight band category <55kg had a median exposure 12 to 17% lower (in terms of AUC(0-24h) and Cmax) for all four TB drugs, compared to those in the higher weight band categories.
A dose increment of one fixed-dose combination (FDC) tablet (150 mg for rifampicin, 75 mg for isoniazid, 400 mg for ethambutol and 275 mg for pyrazinamide) for weight band categories 30-37 kg and 38-54 kg led to target median Cmax values and comparable AUC values to those in higher weight bands.

**Conclusion:** TB-HIV co-infected patients with weight less than 55kg are at risk of sub-optimal anti-TB drug exposure compared to higher weight bands. In our simulations, addition of one FDC tablet to the current

weight-band based dosing evened out the differences in exposure; however, these findings need to be confirmed in a dose-adjustment clinical trial.

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## Pharmacokinetics of Pyrazinamide

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**Background:** The objectives of our study were to evaluate whether low drug exposure of pyrazinamide (PZA) is associated with delayed sputum conversion and to assess the feasibility usefulness of Dried Blood Spot (DBS) as method of Therapeutic Drug Monitoring (TDM).

**Methods:** Rates of sputum smear and culture conversion were determined at weeks 2, 4, 8, 12, 16 after initiating therapy of 35 patients with newly diagnosed drug susceptible sputum smear- and culture-positive tuberculosis (TB), hospitalized in the Regional Clinical Center ,,Ftisiatria" Grodno, Republic of Belarus. DBS samples obtained at steady state 1, 3, 6 hours after drug administration were measured by validated liquid chromatography-tandem mass spectrometry. The MIC values of PZA were determined by the MGIT960 system. The average value of AUC/MIC is used as reference of TDM.

**Results:** 35 patients (30 (14%) men and 5 (86%) woman) with a median age of 56 years (interquartile range (IQR) 47 -90) were enrolled between January 1, 2014 and March 1, 2016. The median AUC0–24, Cmax, MIC and AUC/MIC were 401.8mg*h/L (IQR 905.3-330.1), 36mg/L (IQR 65-30.2), 12.5mg/L (IQR 50-12) and 22.2 (IQR 55.2-14.4) respectively. 26 patients had and 6 had not sputum culture conversion at the end of 8 weeks. The median AUC/MIC of,,converted patient'' was 26.1 (IQR 55.2-16.2) and not converted 15.3 (IQR 32.1-9.6). The rates of sputum conversion were lower in the group with AUC/MIC under the average value of 24.1. At the end of the treatment three patients are died, 2 of them from the consequences of TB and one had treatment failure.

**Conclusions:** The low PZA drug exposure together with high MIC values associated with worse treatment outcome. Limited sampling by DBS is feasible and allows for TDM to be performed at a larger scale. The execution of the DBS test requires certain training

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## Clinical significance of plasma concentrations of first-line anti-tuberculosis drugs

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**Background:** Low concentrations of one or more anti-tuberculosis drugs are reported in developed and developing countries. Despite the magnitude of the TB epidemic, appropriately designed studies investigating the clinical significance of low drug concentrations are extremely limited.

**Method:** We conducted a prospective pharmacokinetic study nested within the control arm of the Improving Treatment Success (IMPRESS) open-label randomized controlled trial (NCT 02114684) in Durban, South Africa. Patients were >18 years of age, had previous pulmonary TB, and were now newly diagnosed with drug-susceptible pulmonary TB. During the first two months of treatment, participants received weight-based dosing of first-line TB drugs (rifampicin, isoniazid, pyrazinamide and ethambutol), and had serum drug concentrations measured at 2 and 6 hours after drug administration during the eighth week of treatment. Intermediate (8 weeks), end of treatment (6 months), and follow-up TB outcomes were assessed using WHO criteria.

**Results:** We assessed serum drug concentrations on available samples in 43 participants. Peak serum drug

concentrations were below the therapeutic range in 39/43(90.7%) for rifampicin, 32/43 (74.4%) for isoniazid, 27/42 (64.3%) for pyrazinamide, and 5/41 (12.2%) for ethambutol. Serum concentrations were very low (less than half of the lower limit of the therapeutic range) in 19/43 (44.2%) of participants for rifampicin, 11/43 (25.6%) for isoniazid, and 1/42 (2.38%) for pyrazinamide. Similarly, the overall median peak concentrations for rifampicin, isoniazid, and pyrazinamide were below the therapeutic interval. At the end of the intensive phase of treatment (week 8), 20.9% (9/43) participants remained culture positive. All participants were cured at the end of treatment and there were no relapses in the 12-month follow-up period. Overall, we did not find a relationship between the concentrations of first-line drugs and treatment outcomes at week 8, end of treatment outcome, or following 12-months of follow-up.

**Conclusion:** Low serum drug concentrations occurred commonly, but treatment outcomes were favourable. Unfavourable treatment outcomes were not associated with low drug concentrations. The impact of drug concentrations, including those achieved by higher drug dosages, should be evaluated in further prospective studies.

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## Antituberculosis drug-induced liver injury in children: Incidence and risk factors during the two-month intensive phase of therapy

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**Background:** As one of the most frequent and serious adverse reactions during tuberculosis treatment, antituberculosis drug-induced liver injury (ATLI) in children has been studied insufficiently compared with adults. We aimed to determine the incidence and risk factors of ATLI in children during the first two months of TB-therapy.

**Methods:** A total of 41 children with tuberculosis and treated with first-line antituberculosis drugs were prospectively followed-up for the development of ATLI. Liver function tests were performed at baseline and after two weeks of therapy. Subsequent tests were conducted at 4, 6 and eight weeks if the initial 2-week measurement was abnormal, or if symptoms of hepatotoxicity were reported.

**Results:** ATLI was detected in 11 (27%) patients within 14 to 42 days from the start of therapy, with most of them (54%) occurred after two weeks. TB treatment was stopped immediately in 6 out of 11 patients who developed ATLI, and no recurrent hepatotoxicity after drug reintroductions in these patients. Univariate analysis showed that ATLI was significantly associated with TB meningitis ($p<0.01$), hypoalbuminemia ($p<0.05$) and hepatotoxic co-medications ($p<0.01$). Age, gender, nutritional status, HIV status, and baseline liver function abnormalities were not associated with ATLI. Multivariate analysis identified hypoalbuminemia and hepatotoxic co-medications (both $p<0.1$) tend to be independently associated with ATLI.

**Conclusions:** Children with hypoalbuminemia and use of hepatotoxic co-medications are suggested to be monitored closely for the development of ATLI. Further pharmacokinetic and pharmacogenetic studies would provide a clearer picture of factors associated with susceptibility to ATLI in children.

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## Tuberculosis Treatment Outcomes from Three TB Centers in the US

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**Background:** Three tuberculosis (TB) treatment centers in the US accumulated decades of experience, including MDR-TB. Our objective was to analyze their experience to better understand and predict treatment outcomes for difficult cases.

**Methods:** Data from 1984 to 2015 were collected from three TB centers: A.G. Holley Hospital, Texas Center for Infectious Diseases (TCID), and University of Texas Health Science at Tyler (TYLER). Data included patient demographics, comorbidities, TB treatment, sputum cultures, and drug concentrations. Patients were stratified to drug-susceptible (DS-TB) or rifampin/multidrug-resistant (RR/MDR-TB). TB drugs were included in the patients' regimens if dosed for at least 28 days. Treatment outcomes were determined using WHO definitions for the primary analysis. The secondary analysis used modified definitions adapted from Günther et al., where cured was extended to at least 1 negative culture/smear after 2 months of therapy for DS-TB, and 6 months of therapy for RR/MDR-TB, with no subsequent positive cultures. Favorable outcomes included cured, while unfavorable outcomes included failed and dead. Individual bivariate (using p-value < 0.05 to conclude significance) and stepwise multiple logistic regressions using backward-mixed procedure were performed. Inclusion and exclusion criteria in the model were set to p-value ≤ 0.15 for DS-TB and ≤ 0.20 for RR/MDR-TB cohort. Statistical analyses were performed using JMP (v13.0).

**Results:** A total of 356 TB patients were included. The majority were males (77%) with a mean (SD) age of 47.0 (15.9) years. About 41% had RR/MDR-TB, 19% had diabetes, and 17% were co-infected with HIV. The treatment outcomes were determined for 250 patients (70%), the remaining were classified as indeterminate. In the individual logistic regressions, site was a significant covariate for both DS-TB and RR/MDR-TB groups, along with other covariates. For DS-TB patients, individual predictors for favorable outcomes were length of stay, liver disease, and drug monitoring, while age, recurrent TB, tobacco use, autoimmune disease, cancer, and complications were associated with unfavorable outcomes (primary and secondary analyses). In the multiple regression models, final covariates were length of stay and liver disease (both favorable) and recurrent TB, tobacco use and complications (unfavorable, in the secondary analysis). Among TB drugs, only injectables were associated with unfavorable outcomes, after fixing the other covariates. For RR/MDR-TB patients, psychiatric disease was associated with unfavorable outcomes consistently (primary and secondary analyses). In the multiple regression models, final covariates were site (TCID was favorable) and race (Black or Hispanic was favorable, secondary analysis). Fluoroquinolones were associated with favorable outcomes in the primary analysis, while pyrazinamide and drug monitoring for at least 2 drugs were associated with unfavorable outcomes in the secondary analysis.

**Conclusion:** The TYLER site included the earliest cases (1980s) and older fluoroquinolones (ciprofloxacin and ofloxacin), thus contributing to differences by site. Recurrent disease, tobacco abuse, and complications during treatment were covariates associated with more difficult-to-treat cases. The use of fluoroquinolones was associated with favorable outcomes in RR/MDR-TB patients.

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