Clinical Infectious Diseases

MAJOR ARTICLE

Pharmacokinetic-Pharmacodynamic Determinants of Clinical Outcomes for Rifampin-Resistant Tuberculosis: A Multisite Prospective Cohort Study

Scott K. Heysell,[1,a] Stellah G. Mpagama,[2,3,a] Oleg B. Ogarkov,[4] Mark Conaway,[5] Shahriar Ahmed,[6] Svetlana Zhdanova,[4] Suporn Pholwat,[1] Mohammad H. Alshaer,[7] Anna M. Chongolo,[2] Buliga Mujaga,[3] Margaretha Sariko,[3] Sabrina Saba,[6] S. M. Mazidur Rahman,[6] Mohammad Khaja Mafij Uddin,[6] Alexey Suzdalnitsky,[8] Elena Moiseeva,[8] Elena Zorkaltseva,[9] Mikhail Koshcheyev,[8] Serhiy Vitko,[1] Blandina T. Mmbaga,[3] Gibson S. Kibiki,[3] Jotam G. Pasipanodya,[10] Charles A. Peloquin,[7] Sayera Banu,[6,b] and Eric R. Houpt[1,b]

[1]Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia, USA; [2]Kibong'oto Infectious Diseases Hospital, Sanya Juu, Tanzania; [3]Kilimanjaro Clinical Research Institute and Kilimanjaro Christian Medical University College, Moshi, Tanzania; [4]Department of Epidemiology and Microbiology, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russian Federation; [5]Department of Public Health Sciences, University of Virginia, Charlottesville, Virginia, USA; [6]International Center for Diarrheal Diseases Research, Bangladesh, Dhaka, Bangladesh; [7]Infectious Disease Pharmacokinetics Lab, College of Pharmacy, University of Florida, Gainesville, Florida, USA; [8]Irkutsk Regional Tuberculosis Referral Hospital, Irkutsk, Russian Federation; [9]Irkutsk State Medical Academy of Postgraduate Education–Branch of Russian Medical Academy of Continuing Professional Education, Irkutsk, Russian Federation; and [10]Quantitative Preclinical & Clinical Sciences Department, Praedicare Inc, Dallas, Texas, USA

Background. Rifampin-resistant and/or multidrug-resistant tuberculosis (RR/MDR-TB) treatment requires multiple drugs, and outcomes remain suboptimal. Some drugs are associated with improved outcome. It is unknown whether particular pharmacokinetic-pharmacodynamic relationships predict outcome.

Methods. Adults with pulmonary RR/MDR-TB in Tanzania, Bangladesh, and the Russian Federation receiving local regimens were enrolled from June 2016 to July 2018. Serum was collected after 2, 4, and 8 weeks for each drug's area under the concentration-time curve over 24 hours (AUC_{0-24}). Quantitative susceptibility of the *M. tuberculosis* isolate was measured by minimum inhibitory concentrations (MICs). Individual drug AUC_{0-24}/MIC targets were assessed by adjusted odds ratios (ORs) for favorable treatment outcome, and hazard ratios (HRs) for time to sputum culture conversion. K-means clustering algorithm separated the cohort of the most common multidrug regimen into 4 clusters by AUC_{0-24}/MIC exposures.

Results. Among 290 patients, 62 (21%) experienced treatment failure, including 30 deaths. Moxifloxacin AUC_{0-24}/MIC target of 58 was associated with favorable treatment outcome (OR, 3.75; 95% confidence interval, 1.21–11.56; $P = .022$); levofloxacin AUC_{0-24}/MIC of 118.3, clofazimine AUC_{0-24}/MIC of 50.5, and pyrazinamide AUC_{0-24} of 379 mg × h/L were associated with faster culture conversion (HR > 1.0, $P < .05$). Other individual drug exposures were not predictive. Clustering by AUC_{0-24}/MIC revealed that those with the lowest multidrug exposures had the slowest culture conversion.

Conclusions. Amidst multidrug regimens for RR/MDR-TB, serum pharmacokinetics and *M. tuberculosis* MICs were variable, yet defined parameters to certain drugs—fluoroquinolones, pyrazinamide, clofazimine—were predictive and should be optimized to improve clinical outcome.

Clinical Trials Registration. NCT03559582.

Keywords. multidrug-resistant tuberculosis; pharmacokinetics; pharmacodynamics; minimum inhibitory concentrations.

Globally, 9.9 million people were estimated to have tuberculosis (TB) disease in 2020, and approximately 500 000 had rifampin-resistant or multidrug-resistant (RR/MDR) TB [1]. RR/MDR-TB significantly reduces the likelihood of TB treatment success. For decades, antibiotic combinations for RR/MDR-TB used drugs of uncertain efficacy with long treatment durations [2]. Subsequently, an individual patient data meta-analysis of RR/MDR-TB treatment outcomes found certain medications of benefit, others of unclear benefit, and still others that may worsen outcomes [3]. This analysis prompted the World Health Organization (WHO) to reprioritize specific RR/MDR-TB drugs, and numerous trials are now underway to shorten the treatment duration or improve treatment efficacy with combinations of reprioritized drugs [4]. Nevertheless, the mechanisms have not been fully elucidated to explain why certain drugs proved of benefit in meta-analysis while other drugs, despite in vitro activity, did not.

Pharmacokinetic-pharmacodynamic (PK-PD) relationships are important drivers of *Mycobacterium tuberculosis* killing

Received 11 April 2022; editorial decision 15 June 2022; published online 22 June 2022

[a]S. K. H. and S. G. M. contributed equally to this work.

[b]S. B. and E. R. H. contributed equally to this work.

Correspondence: Scott K. Heysell, 345 Crispell Drive, MR-6; Charlottesville, VA 29908, USA (skh8r@virginia.edu).

Clinical Infectious Diseases® 2023;76(3):497–505

https://doi.org/10.1093/cid/ciac511

and prevention of acquired resistance in vitro [5]. Most anti-TB drugs exert concentration-dependent activity as measured by area under the concentration-time curve (AUC), often calculated from serum concentrations, relative to the infecting *M. tuberculosis* minimum inhibitory concentration (MIC) to that particular drug. While these relationships have been more convincingly demonstrated in humans for drugs used to treat rifampin-susceptible TB whereby higher AUC/MIC exposures improve drug efficacy [6], we and others have found complex relationships for individual drugs commonly used in RR/MDR-TB regimens. Certain drugs such as the fluoroquinolones (levofloxacin and moxifloxacin) exert more consistent exposure-dependent activity [7], yet for others such as ethionamide, activity is demonstrated only up to a threshold MIC [8]. Furthermore, drugs such as kanamycin or cycloserine may exert activity only at the highest exposures that risk serious toxicities [9]. Thus, understanding PK-PD relationships within multidrug combination regimens for RR/MDR-TB may better inform drug regimen composition and determine if certain drugs can be considered if dose and serum exposure are optimized or if other drugs warrant wholesale abandonment.

We present findings from a multicountry prospective cohort of adults with RR/MDR pulmonary TB to determine individual drug and combinatorial PK-PD relationships that predict treatment outcomes.

METHODS

Study Design

Patients were enrolled in a prospective cohort at initiation of RR/MDR-TB treatment from the following regional RR/MDR-TB referral sites: Dhaka, Bangladesh (National Institute of Diseases of the Chest and Hospital); Kilimanjaro region, Tanzania (Kibong'oto Infectious Diseases Hospital); and Irkutsk, Russian Federation (Irkutsk Regional Tuberculosis Referral Hospital). Eligible patients were aged ≥18 years with pulmonary TB and a respiratory specimen positive by Xpert Mycobacterium tuberculosis/rifampin with *rpoB* mutation (Cepheid, Sunnyvale, CA) or cultured for *M. tuberculosis* complex with phenotypic resistance to rifampin. Patients were excluded if pregnant, unable to undergo sample collection, or treated for RR/MDR-TB within 6 months of enrollment. The enrollment goal was 125 patients at each site for single site analyses based on a prior PK-PD study that defined individual drug AUC targets for predicting outcome in rifampin-susceptible TB [10] and an estimated proportion with treatment failure in RR/MDR-TB of 25%. The enrollment period covered June 2016 to July 2018. All patients provided written informed consent to a protocol following STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) criteria approved by all affiliated institutional review boards.

Procedures

RR/MDR-TB treatment regimens were initiated with WHO guidance for weight-based dosing by clinicians' discretion at the enrollment sites. Chest radiographs performed prior to treatment initiation were categorized as cavitary or noncavitary. Baseline laboratory data from hospital records were hemoglobin (g/dL), creatinine (mg/dL), human immunodeficiency virus (HIV) antibody testing, and CD4 count (cells/mm^3), while the study performed point-of-care glycosylated hemoglobin testing (HgbA1c %).

Sputa were collected at baseline and 4, 8, and 24 weeks after treatment initiation for culture on Lowenstein–Jensen slants or Middlebrook 7H11 media and with liquid culture in the Bactec MGIT 960 system (Becton, Dickinson, Franklin Lakes, NJ). Positive cultures were confirmed for *M. tuberculosis* complex using Gen-Probe (San Diego, CA). Other sputum mycobacterial cultures for routine care were documented by date of collection and whether positive or negative for *M. tuberculosis*. All positive cultures from baseline specimens and those at 8 or 24 weeks had MIC testing with a custom 96-well MYCOTB plate (Trek Diagnostic Systems, Independence, OH). Drugs on the custom plate were those most commonly prescribed at the time of study. MIC testing was performed as previously described post hoc and not available for alteration of patient regimen [11]. In analyses defining susceptibility or resistance, we used the MIC breakpoint closest to the WHO-endorsed critical concentration in liquid media [12]. Pyrazinamide was not available on the plate; therefore, binary susceptibility/resistance was determined genotypically for *pncA* mutation using Sanger sequencing.

Serum drug concentrations were measured at 2, 4, and 8 weeks after treatment initiation. Blood was collected at 1, 2, 6, and 12 hours after directly observed medication administration. Serum was stored at −80°C until shipment to the University of Florida Infectious Diseases Pharmacokinetics Laboratory. Concentrations were measured using validated liquid chromatography–tandem mass spectrometry assays. Estimated total exposures, that is, AUC over 24 hours (AUC_{0-24}), were determined by noncompartmental analysis using Phoenix WinNonlin version 8.0 (Certara USA, Princeton, NJ), using later hour concentrations in the elimination phase.

Outcomes Definitions

Clinical follow-up was performed at 2, 4, 8, 24, and 48 weeks after treatment initiation. Time to sputum culture conversion was from treatment initiation to the first negative sputum culture that was not followed by a positive culture. Death was defined as mortality from any cause up to 48 weeks. Favorable treatment outcome was defined as not having death, culture positivity at week 24 or beyond, acquired drug resistance (prespecified as a 4-fold or greater increase in MIC of any drug in the patient's regimen), or a lack of improvement of the major TB-related symptom at week 24 or beyond (clinical failure).

Statistical Analyses

For initial analyses that did not include PK-PD determinants, patients who had received a given drug and had *M. tuberculosis* isolates with MIC values known to be susceptible to that drug were compared to patients who had either received the drug and were found to be resistant or who did not receive the drug. Multivariable logistic regression models using Firth's penalized likelihood method defined the odds of favorable treatment outcome, and Weibull regression models were used to estimate the hazard ratios for time to sputum culture conversion. Given that some drugs were prescribed to low numbers of patients, regression models were adjusted for site of enrollment only.

To first assess the impact of PK exposure, we sought the individual drug AUC_{0-24}/MIC that yielded at least N = 20 patients with values above and below the target that generated the maximum odds ratios (ORs) and 95% confidence intervals (CIs) for treatment failure and hazard ratios (HRs) and 95% CIs for time to sputum culture conversion. Mean AUC_{0-24} across dosing intervals was used with the robustness of the PK sampling strategy determined by D-optimality design. AUC_{0-24} values that could not be calculated from measured concentrations from that dosing interval (2, 4, or 8 weeks) were imputed by random multiple imputation. Missing values for MIC were imputed using site-specific regression of median MIC values. These models were adjusted for site of enrollment, age, sex, diabetes, HIV, known cavitary disease, and body mass index.

Among patients with the most commonly prescribed medication regimens across sites, a K-means clustering algorithm with 4 clusters was applied to the AUC_{0-24}/MIC values at 2 weeks, the time point of earliest drug exposure measurement, whereby patients in each cluster had a similar pattern of values for each drug [13]. The difference in time to sputum conversion among clusters was estimated using the interval censored Weibull model, and a likelihood ratio test was used to assess differences.

RESULTS

A total of 340 patients were enrolled; 50 patients (15%) were unable to complete the first sampling procedures due to early death or loss to follow-up (n = 26 from Bangladesh, n = 17 from Tanzania, and n = 7 from the Russian Federation). The enrollment target was not met for the Russian Federation following temporary closure of the enrollment hospital. Thus, analyses included all sites, with adjustment for site at a minimum, for 290 patients. The median age was 35 years (interquartile range, 29–46), and 208 (72%) were male. There were expected differences in clinical characteristics among the sites (Table 1).

RR/MDR-TB Drug Regimen Composition

Supplementary Figure 1 displays the individual drug frequencies. There were 53 distinct multidrug regimens (11 from Tanzania, 14 from Bangladesh, and 28 from the Russian Federation). There were site-specific preferred regimens such as 116 (93.5%) of patients from Tanzania received 1 of 4 regimens that included 4–5 drugs and 124 (100%) received a regimen that contained pyrazinamide and a fluoroquinolone (levofloxacin or moxifloxacin). In Bangladesh, 101 (87.1%) received 1 of 3 regimens, 115 (99.1%) received a regimen containing a fluoroquinolone (levofloxacin or moxifloxacin), and 114 (98.2%) received pyrazinamide. Regimens were more varied in the Russian Federation.

Individual Drug Exposure Variability Over the 2- to 8-Week Treatment Interval

Figure 1 displays the individual drug mean AUC_{0-24} at 2, 4, and 8 weeks following treatment initiation, revealing drug-specific variability and further variability among sites. Complete site-to-site variability of AUC_{0-24}/MIC relative to milligram per kilogram dosing is presented in Supplementary Table 1.

Treatment Outcomes and Impact of Individual Drugs in the Multidrug Regimen

Of the 290 participants, 228 (79%) had a favorable treatment outcome. Of 62 (21%) failures, 30 died. There was no significant difference in the time to death among sites (Supplementary Figure 2). Of the remaining participants categorized as treatment failures, 13 (4.4%) were culture-positive at week 24 or beyond, 14 (4.8%) had acquired resistance, and 10 (3.4%) had clinical failure. Interval-censored time to culture conversion was significantly faster for patients from the Russian Federation compared with those from Tanzania ($P = .018$) and Bangladesh ($P = .038$; Supplementary Figure 3).

To quantify the impact of individual drugs without PK-PD influence, the odds of favorable treatment were compared in those who had received the drug and were known susceptible with those who had received the drug and were found to be resistant or did not receive the drug. Sequencing for *pncA* mutations was performed in 122 patients' isolates, with 88 (72%) determined susceptible to pyrazinamide; the proportion susceptible did not significantly vary across sites ($P = .164$). In adjusted regression models, patients who received pyrazinamide were significantly more likely to have favorable treatment (Supplementary Figure 4*A*). Pyrazinamide, moxifloxacin, and clofazimine were associated with a shorter time to sputum culture conversion (Supplementary Figure 4*B*).

PK-PD Relationship to Treatment Failure and Time to Sputum Culture Conversion for Individual Drugs

Next, we determined whether individual drug exposures and AUC_{0-24}/MIC refined or strengthened these associations with better treatment response. A moxifloxacin AUC_{0-24}/MIC target of 58 was associated with favorable treatment outcome (OR, 3.75; 95% CI, 1.21–11.56; $P = .022$; Table 2). The AUC_{0-24}/MIC for other drugs did not demonstrate significant associations. Table 3 shows the same analysis for time to culture conversion and indicates that AUC_{0-24}/MIC targets for levofloxacin, and

Table 1. Demographic and Clinical Characteristics by Site of Enrollment

Characteristic	Total N = 290	Tanzania N = 124	Bangladesh N = 116	Russian Federation N = 50	*P* Value
Age, median (IQR), years	35.5 (29–46)	42 (32–50)	32 (25–42)	35 (30–39)	<.001
Sex, n (%)					
Female	82 (28)	43 (35)	23 (20)	16 (32)	.031
Male	208 (72)	81 (65)	93(80)	34 (68)	
Duration of symptoms, median (IQR), days	90 (52–180)	91 (58–165)	90 (45–180)	126 (60–249)	.051
Prior history of TB, n (%)					
No	75(26)	32 (26)	19 (16)	24 (49)	<.001
Yes	211 (73)	89 (72)	97 (84)	25 (50)	
Unknown	4 (1)	3 (2)	0	1 (1)	
HIV infection, n (%)					
Negative	214 (74)	72(58)	116(100)	26 (52)	<.0010
Positive	72 (25)	48 (39)	0 (0)	24 (48)	
Unknown	4(1)	4 (3)	0	0	
CD4 count in HIV positive, median	227	224	…	237	.444
On antiretroviral therapy, n (%)					
Yes	44 (62)	41 (85)	…	3 (13)	< .001
No	28 (38)	7 (15)	…	21 (87)	
Diabetes mellitus, n (%)					
No	251 (87)	119 (96)	83 (72)	49 (98)	<.001
Yes	39 (13)	5 (4)	33 (28)	1 (2)	
Glycosylated hemoglobin in diabetes mellitus, median	9.2	9.1	9.5	6.6	
Smoking, n (%)					
No	140 (48)	80 (65)	52 (45)	8 (16)	<.001
Yes	145 (50)	39 (31)	64 (55)	42 (84)	
Unknown	5 (2)	5 (4)	0	0	
Alcohol use, n (%)					
No	205(71)	84 (68)	105 (91)	16 (32)	<.001
Yes	80 (27)	36 (29)	11 (9)	33 (66)	
Unknown	5 (2)	4 (3)	0	1 (2)	
Recreational injection drug use, n (%)					
No	262 (90)	116 (94)	107 (92)	39 (78)	.002
Yes	26 (9)	6 (5)	9 (8)	11 (22)	
Unknown	2 (1)	2 (1)	0	0	
Body mass index (kg/m^2), median (IQR)	19.0 (16.9–21·2)	17.8 (16.3·20.2)	19.0 (17.3–21.1)	20.6 (18.3–21.8)	<.001
Mean upper arm circumference (cm), median (IQR)	23.0 (20.5–25.1)	22.0 (19.5–25.0)	23.1 (21.0–25.0)	24.5 (22.0–27.0)	<.001
Hemoglobin (g/dL), median (IQR)	11.5 (10.1–12.7)	10.5 (9.1–11.8)	12.0 (11.3–12.8)	12.4 (9.9–14.0)	<.001
Creatinine (mg/dL), median (IQR)	0.90 (0.80–1.00)	0.90 (0.79–1.01)	0.88 (0.70–1.00)	0.90 (0.90–1.00)	.019
Cavitary disease, baseline chest X ray, n (%)					
No	82 (28)	61 (49)	13 (11)	8 (16)	<.001
Yes	118 (41)	49 (40)	31 (27)	38 (76)	
Unknown	90 (31)	14 (11)	72 (62)	4 (8)	
Extrapulmonary involvement of TB, n (%)					
No	278 (96)	121 (98)	116 (100)	41 (82)	<.001
Yes	12 (4)	3 (2)	0 (0)	9 (18)	

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; TB, tuberculosis.

clofazimine, and AUC0–24 for pyrazinamide, were significantly associated with faster time to culture conversion.

PK-PD Relationship and Time to Sputum Culture Conversion for Combinatorial Patterns

Since multiple drugs are used to treat TB, we examined the most commonly prescribed drug combination across sites, a 5-drug regimen prescribed in N = 95 patients. Patterns of pyrazinamide AUC_{0-24} and levofloxacin AUC_{0-24}/MIC most clearly separated clusters (Figure 2*A*). Figure 2*B* demonstrates the differences in time to culture conversion when separated by AUC_{0-24}/MIC pattern cluster, such that the cluster with the slowest time to culture conversion (HR = 0.59 ± standard error 0.29, nominal $P = .065$) had the lowest levofloxacin and

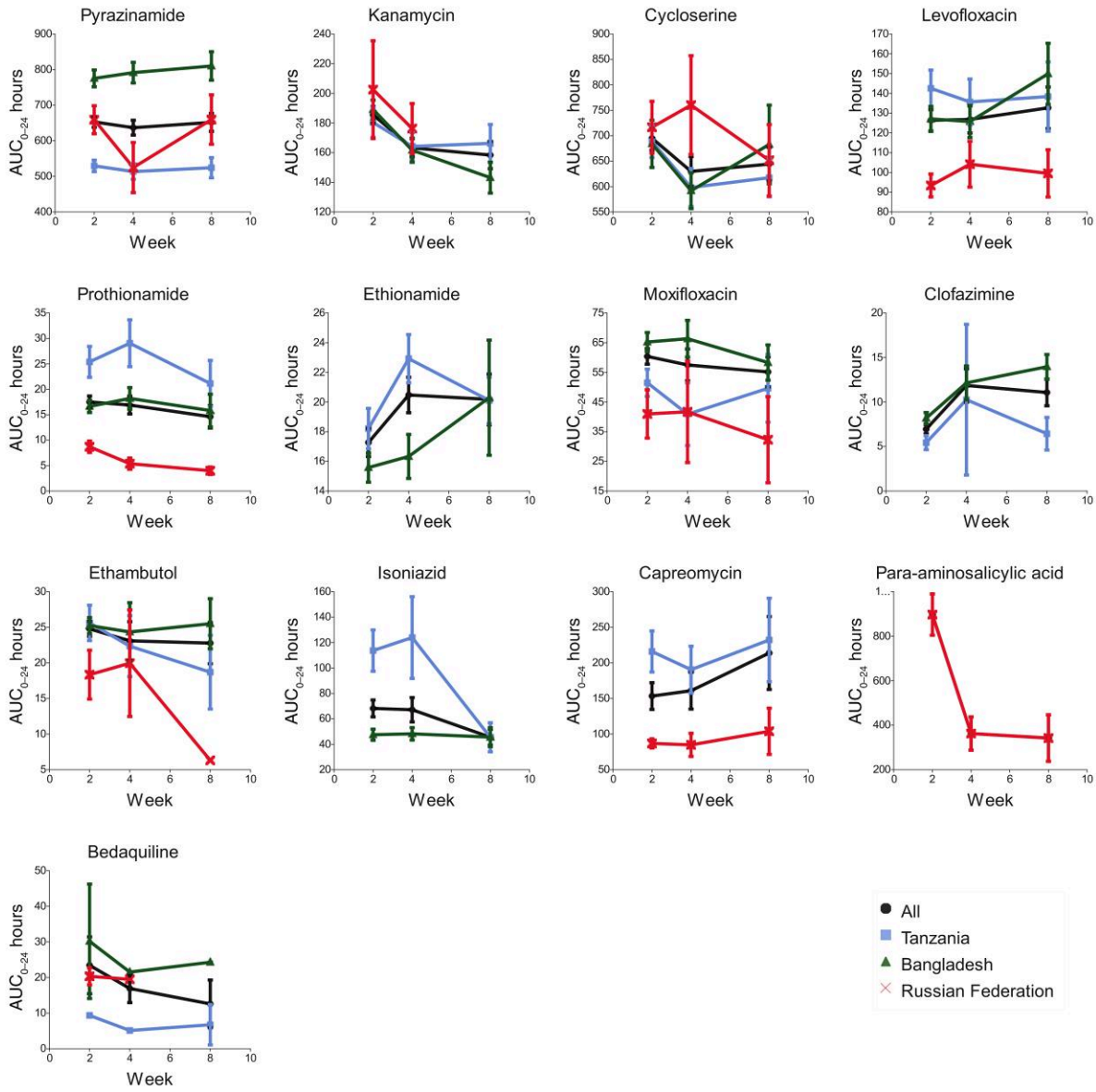

Figure 1. Change in total serum exposure. Change expressed as population mean and standard errors for the $AUC_{0–24}$ (mg × h/L) measured at 2, 4, and 8 weeks after treatment initiation as visualized for each drug at each site: Tanzania (square), Bangladesh (triangle), Russian Federation (x), and all sites averaged (circle). Total patients for each drug at 2-, 4-, and 8-week time points: pyrazinamide (266, 171, 127), kanamycin (193, 141, 88), cycloserine (163, 91, 79), levofloxacin (164, 101, 68), prothionamide (118, 69, 45), ethionamide (132, 105, 86), clofazimine (56, 13, 13), ethambutol (98, 28, 22), isoniazid (96, 64, 41), capreomycin (33, 25, 21), para-amino salicylic acid (20, 14, 8), and bedaquiline (6, 4, 3). Delamanid and linezolid are not displayed given infrequency of use. Abbreviation: $AUC_{0–24}$, area under the concentration-time curve during a 24-hour dosing interval.

pyrazinamide exposures. Proportions that reached PK-PD targets identified in Table 3 for drugs within each cluster are described in Supplementary Table 2.

DISCUSSION

In this multicountry, prospective cohort of adults with pulmonary RR/MDR-TB, individual drug and combinatorial PK-PD relationships significantly influenced clinical outcomes. It was already evident from prior meta-analyses that bedaquiline, moxifloxacin or levofloxacin, linezolid, and clofazimine were associated with better treatment outcomes [3]. Drugs such as pyrazinamide, ethambutol, ethionamide or prothionamide, para-aminosalicylic acid, delaminid, imipenem-cilastin, and amikacin have been recommended only when a prioritized medication is unavailable or if the *M. tuberculosis* isolate is resistant; kanamycin and capreomycin were no longer recommend due to association with poor

Table 2. Association of Favorable Treatment Outcome With Maximum Individual Drug Area Under the Concentration-Time Curve During a 24-Hour Dosing Interval/Minimum Inhibitory Concentration Cut Points Yielding at Least N = 20 Above and Below the Cut Point

Drug (Number of People Prescribed)	Number Below Cut Point (%)	Cut Point AUC_{0-24}/MIC	Odds Ratio (95% Confidence Interval)	*P* Value
Pyrazinamide (283)	46 (16)	443[a]	1.92 (.89–4.13)	.098
Kanamycin (199)	64 (32)	63	.92 (.43–1.98)	.834
Cycloserine (184)	148 (80)	83	2.14 (.75–6.06)	.153
Levofloxacin (178)	25 (14)	129	1.49 (.53–4.16)	.448
Prothionamide (141)	60 (43)	1.78	1.42 (.58–3.47)	.436
Ethionamide (140)	28 (20)	1.76	1.66 (.62–4.47)	.315
Moxifloxacin (112)	21 (19)	58	3.75 (1.21–11.56)	.022
Clofazimine (105)	50 (48)	62	2.01 (.74–5.43)	.168
Ethambutol (105)	48 (46)	2.58	0.76 (.28–2.07)	.594
Isoniazid (98)	37 (38)	11.1	1.22 (.41–3.54)	.719

Abbreviation: AUC_{0-24}/MIC, area under the concentration time curve over the 24 hours/minimum inhibitory concentration.

[a]Pyrazinamide MIC was not measured, cut point is AUC_{0-24} mg × h/L only. Other drugs (capreomycin, para-aminosalicylic acid, linezolid, bedaquiline, delaminid) were not prescribed in enough patients to generate cut points with at least 20 patients with AUC_{0-24}/MIC values above and below a maximum cut point. Adjusted for site of enrollment, age, sex, diabetes, human immunodeficiency virus, cavitary disease, and body mass index.

outcome [4]. Our PK-PD findings support this prioritization but with notable refinements.

First, we found pyrazinamide to be important. Inclusion of pyrazinamide in a regimen when the *M. tuberculosis* isolate was susceptible was significantly associated with favorable treatment outcome and a faster time to culture conversion.

Table 3. Impact on Time to Sputum Culture Conversion of Maximum Individual Drug Area Under the Concentration-Time Curve During a 24-Hour Dosing Interval/Minimum Inhibitory Concentration Cut Points Yielding at Least N = 20 Above and Below the Cut Point

Drug (Number of People Prescribed)	Number Below Cut Point (%)	Cut Point AUC_{0-24}/MIC	Hazard Ratio (95% Confidence Interval)	*P* Value
Pyrazinamide (283)	20	379[a]	1.83 (1.02–3.27)	.042
Kanamycin (199)	20	30.1	1.53 (.89–2.62)	.121
Cycloserine (184)	115	57.5	1.19 (.82–1.74)	.362
Levofloxacin (178)	23	118.3	2.0 (1.15–3.48)	.015
Prothionamide (141)	37	0.86	1.27 (.77–2.07)	.347
Ethionamide (140)	26	1.52	1.36 (.83–2.24)	.224
Moxifloxacin (112)	20	54.1	1.35 (.74–2.49)	.323
Clofazimine (105)	46	50.5	1.60 (1.01–2.55)	.046
Ethambutol (105)	22	1.63	1.65 (.95–2.86)	.073
Isoniazid (98)	59	18.7	0.95 (.58–1.53)	.825

Abbreviation: AUC_{0-24}/MIC, area under the concentration time curve over the 24 hours/minimum inhibitory concentration.

[a]Pyrazinamide MIC was not measured, cut point is AUC_{0-24} mg × h/L only. Other drugs (capreomycin, para-aminosalicylic acid, linezolid, bedaquiline, delaminid) were not prescribed in enough patients to generate cut points with at least 20 patients with AUC_{0-24}/MIC values above and below a maximum cut point. Adjusted for site of enrollment, age, sex, diabetes, human immunodeficiency virus, cavitary disease, and body mass index.

Among all patients who received pyrazinamide, an AUC_{0-24} target (379 mg × h/L) was found to be significantly associated with faster time to culture conversion. Furthermore, in adjusted cluster analyses of the most commonly prescribed drug regimens, a pattern of below-target pyrazinamide AUC_{0-24} (along with low levofloxacin AUC_{0-24}/MIC) identified patients with the slowest time to culture conversion. The AUC_{0-24} target identified in this study was similar to that in serum for predicting outcomes in rifampin-susceptible TB patients (363 mg × h/L) [10] and in a hollow fiber model that translated to exposure within lung tissue [14]. Pyrazinamide has enhanced the in vitro and animal model activity of new TB drugs and remains one of the few anti-TB agents with sterilizing activity against *M. tuberculosis* in different phases of growth [15]. Yet, most PK-PD studies in humans with pyrazinamide have been performed in rifampin-susceptible TB where modeling studies have found that only very high doses and exposures could further shorten treatment duration [16]. Our findings rekindle the importance of pyrazinamide as an effective drug in treating RR/MDR-TB, particularly when susceptibility can be determined and when a minimal target AUC_{0-24} is achieved.

We found benefits to fluoroquinolones as well. Use of moxifloxacin (vs not using moxifloxacin) was associated with shorter time to conversion. However, when the analysis was restricted to just those participants who received the drug (N = 112), a specific AUC_{0-24}/MIC target that was significantly associated with a faster time to culture conversion could not be found (Table 3). These findings may reflect the different background regimens as moxifloxacin was commonly prescribed in the 7-drug regimen studied in the Standard Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR-TB trial (STREAM trial) and included clofazimine [17]. Clofazimine, a drug whose inclusion was significantly associated with improved sputum culture conversion (Supplementary Figure 3*B*) and for which a target AUC_{0-24}/MIC could be derived that improved time to culture conversion (Table 3), may have masked individual moxifloxacin impact. Levofloxacin, which was less commonly prescribed with clofazimine, did yield an AUC_{0-24}/MIC target associated with a faster time to sputum culture conversion (Table 3) and provided important discrimination in cluster analyses. The levofloxacin AUC_{0-24}/MIC target identified was also similar to the AUC_{0-24}/MIC of 146 predictive of maximal *M. tuberculosis* kill in the hollow fiber model [7].

We did not find PK-PD targets predictive of outcomes for other drugs. Statistical associations were expectedly limited by the infrequent use of some drugs used in different countries, or for certain drugs, the more narrow ranges of observed PK exposures [9]. Nevertheless, not only were no PK-PD targets found for ethionamide and cycloserine, inclusion of these drugs in the regimen even when susceptible was significantly associated with slower culture conversion. We did not find PK-PD

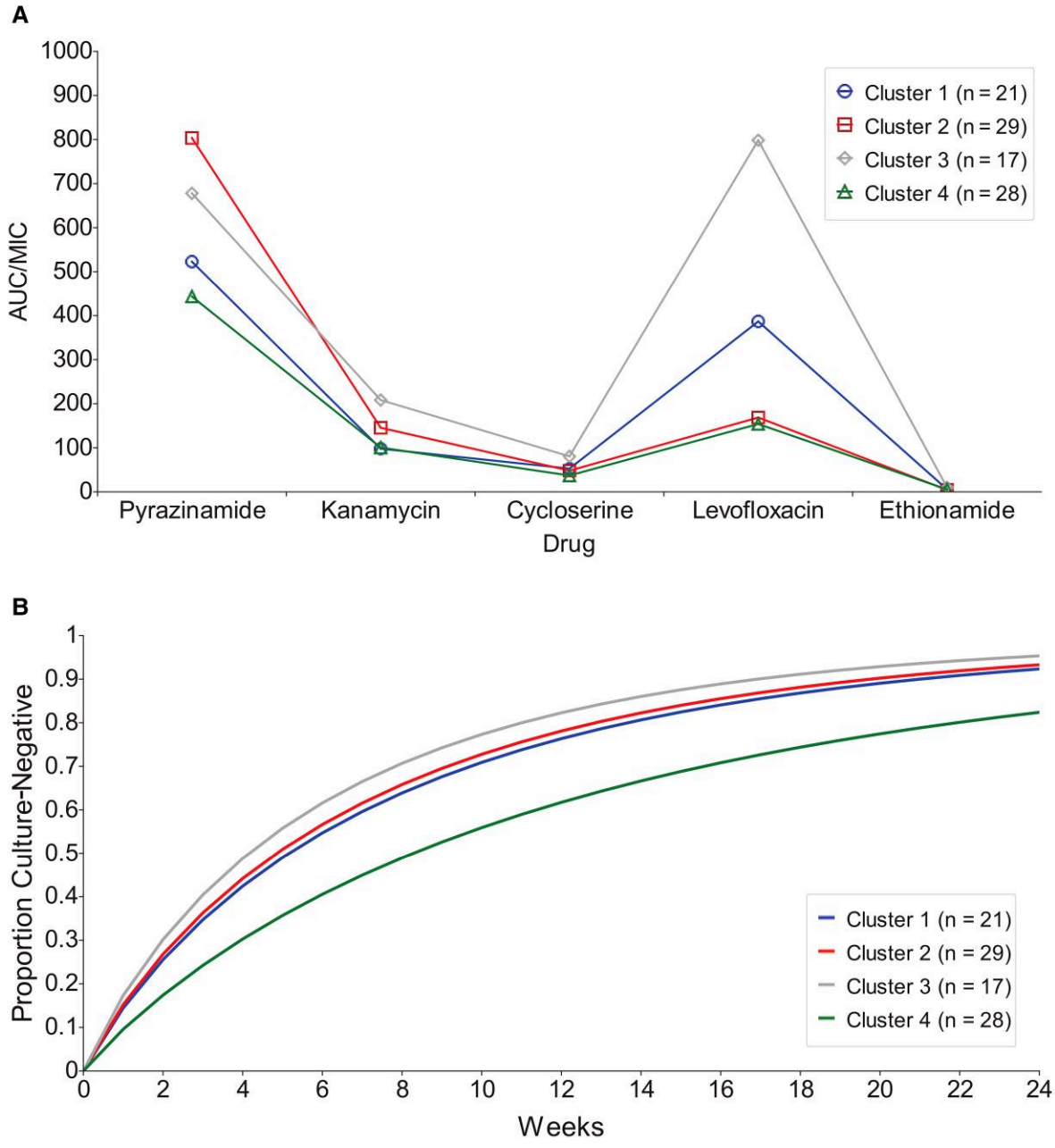

Figure 2. *A*, Pharmacokinetic-pharmacodynamic pattern for the most common multidrug regimen. Clusters identified by AUC_{0-24}/MIC pattern at 2 weeks after treatment initiation for the regimen of pyrazinamide, levofloxacin, kanamycin, cycloserine, and ethionamide, N = 95. Pyrazinamide values represent AUC_{0-24} only (pyrazinamide MIC not performed). *B*, Time to sputum culture conversion to negative for patients receiving the most common regimen as clustered by pharmacokinetic-pharmacodynamic pattern. Hazard ratio = 0.59 ± standard error 0.29, nominal $P = .065$, for difference between cluster 4 and other clusters. Abbreviations: AUC, area under the concentration-time curve during a 24-hour dosing interval; MIC, minimum inhibitory concentration.

justification for inclusion of kanamycin in the RR/MDR-TB regimen, but we did find serum exposures that may have placed patients at high risk of ototoxicity [18].

Along with site-specific trends in MIC distribution for certain drugs (Supplementary Table 1), we noted regional and temporal variation in AUC_{0-24} (Figure 1). Prior to this study, most anti-TB drug serum exposure was assumed to be stable after the first few weeks of therapy following induction of genes related to absorption and metabolism [19]. Globally, TB medicines are dosed using weight bands and not personalized to an individual's serum exposure or a PK-PD parameter such as AUC_{0-24}/MIC. Our findings conceptually support an expanded effort toward PK-PD interventions to facilitate individual exposure-based dosing or highly regionalized dosing informed

by local PK-PD studies [20]. For RR/MDR-TB, drugs of priority should include fluoroquinolones and pyrazinamide where in vitro and clinically identified targets now appear to align. Alternative sample matrices such as saliva [21] and urine [22] that use spectrophotometric methods may bring such personalized dosing closer to the point of care.

Limitations of this study include fewer patients treated with the currently prioritized drugs of bedaquiline and linezolid. Furthermore, for analyses that included pyrazinamide susceptibility, we used Sanger sequencing of the *pncA* gene and classified as resistant if a mutation was previously associated with phenotypic resistance or was likely to be associated with resistance but excluded mutations with insufficient evidence, documented neutrality, or likely neutrality [23]. Pyrazinamide resistance in rifampin-resistant TB has been documented higher proportions in other cohorts, yet likely represents an overestimation when using any *pncA* mutation. While the current study enrolled a majority of men, which is not atypical for TB studies, serum exposures for drugs such as pyrazinamide may differ by sex [22]. Additionally, the overall rate of favorable treatment outcomes in this study may be higher than experienced in other RR/MDR-B settings but is similar to contemporary trials with similar regimens [17]. We included the patient-centered outcome of clinical failure in the composite of favorable treatment outcome [24].While an uncommon cause of unfavorable treatment outcome alone, this may have modestly underestimated conventional treatment success, but did not factor into the analyses of time to sputum culture conversion. Last, early death or loss to follow-up prior to the first PK procedures may have been overrepresented among patients with more severely altered PK-PD, although baseline characteristics did not significantly differ among those with completed procedures.

In summary, this multicountry, real-world cohort of PK-PD and RR/MDR-TB treatment outcomes found that not only the use of certain anti-TB drugs but also the PK-PD targets of those drugs could be defined that were predictive of clinically important outcomes. Therefore, RR/MDR-TB treatment centers that use pyrazinamide, fluoroquinolones, and clofazimine should consider implementation studies of individual exposure-based dosing. Last, new RR/MDR-TB regimens that contain bedaquiline and pretomanid appear highly efficacious [25], yet as those drugs are used for RR/MDR-TB removed from the settings of the original clinical trials, our findings would predict that similar PK-PD principles will drive outcomes over time and result in another hierarchy of importance of certain drugs over others.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. S. K. H., S. G. M., O. O., G. K., S. B., and E. R. H. conceptualized and designed the study. S. G. M., O. B. O., S. B., S. A., S. Z., B. T. M., A. C., and E. Z. managed patient recruitment and oversaw the study sites. M. H. A. and C. A. P. performed pharmacokinetic analyses. A. S., M. K., E. M., B. M., M. S., S. S., S. M. M. R., M. K. M. U., and S. V. contributed to data collection. S. K. H., S. G. M., S. A., and S. Z. verified the source data. M. C., J. G. P., and S. K. H. performed statistical analyses. S. K. H., S. G. M., S. P., S. B., and E. R. H. interpreted the data. S. K. H. wrote the first draft of the manuscript, and M. C. produced the figures. All authors revised and edited the final version of the manuscript.

Acknowledgments. We are indebted to the participants for their generosity.

Data sharing. Metadata and standard operating procedures are available upon request to the corresponding author.

Financial support. This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (NIH; grant U01AI115594 to S. K. H. and E. R. H.).

Potential conflicts of interest. S. K. H., C. A. P., and E. R. H. report grants or contracts from the NIH outside of the submitted work. All remaining authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. World Health Organization. Global tuberculosis report. Geneva, Switzerland: World Health Organization, **2021**.
2. Schnippel K, Firnhaber C, Ndjeka N, et al. Persistently high early mortality despite rapid diagnostics for drug-resistant tuberculosis cases in South Africa. Int J Tuberc Lung Dis **2017**; 21:1106–11.
3. Collaborative Group for the Meta-Analysis of Individual Patient Data in MDR-TB treatment–2017, Ahmad N, Ahuja SD, et al. Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis. Lancet **2018**; 392:821–34.
4. Mirzayev F, Viney K, Linh NN, et al. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. Eur Respir J **2021**; 57:2003300.
5. Alffenaar JC, Gumbo T, Dooley KE, et al. Integrating pharmacokinetics and pharmacodynamics in operational research to end tuberculosis. Clin Infect Dis **2020**; 70:1774–80.
6. Zuur MA, Pasipanodya JG, van Soolingen D, et al. Intermediate susceptibility dose-dependent breakpoints for high-dose rifampin, isoniazid, and pyrazinamide treatment in multidrug-resistant tuberculosis programs. Clin Infect Dis **2018**; 67: 1743–9.
7. Deshpande D, Pasipanodya JG, Mpagama SG, et al. Levofloxacin pharmacokinetics/pharmacodynamics, dosing, susceptibility breakpoints, and artificial intelligence in the treatment of multidrug-resistant tuberculosis. Clin Infect Dis **2018**; 67:S293–302.
8. Deshpande D, Pasipanodya JG, Mpagama SG, et al. Ethionamide pharmacokinetics/pharmacodynamics-derived dose, the role of MICs in clinical outcome, and the resistance arrow of time in multidrug-resistant tuberculosis. Clin Infect Dis **2018**; 67:S317–26.
9. Alghamdi WA, Alsultan A, Al-Shaer MH, et al. Cycloserine population pharmacokinetics and pharmacodynamics in patients with tuberculosis. Antimicrob Agents Chemother **2019**; 63:e00055–19.
10. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis **2013**; 208:1464–73.
11. Lee J, Armstrong DT, Ssengooba W, et al. Sensititre MycoTB MIC plate for testing *Mycobacterium tuberculosis* susceptibility to first- and second-line drugs. Antimicrob Agents Chemother **2014**; 58:11–8.
12. World Health Organization. Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. Geneva, Switzerland: World Health Organization, **2018**.

13. Hastie T, Tibshirani R. Friedman J. The elements of statistical learning: data mining inference and prediction. 2nd ed. New York: Springer Series in Statistics, **2009**.
14. Gumbo T, Dona CS, Meek C, Leff R. Pharmacokinetics-pharmacodynamics of pyrazinamide in a novel in vitro model of tuberculosis for sterilizing effect: a paradigm for faster assessment of new antituberculosis drugs. Antimicrob Agents Chemother **2009**; 53:3197–204.
15. Mudde SE, Alsoud RA, van der Meijden A, et al. Predictive modeling to study the treatment-shortening potential of novel tuberculosis drug regimens, towards bundling of preclinical data. J Infect Dis **2022**; 225:1876–85.
16. Zhang N, Savic RM, Boeree MJ, et al; Tuberculosis Trials Consortium and Pan African Consortium for the Evaluation of Antituberculosis Antibiotics Networks. Optimising pyrazinamide for the treatment of tuberculosis. Eur Respir J **2021**; 58:2002013.
17. Nunn AJ, Phillips PPJ, Meredith SK, et al; STREAM Study Collaborators. A trial of a shorter regimen for rifampin-resistant tuberculosis. N Engl J Med **2019**; 380: 1201–13.
18. Ghafari N, Court R, Chirehwa MR, et al. Pharmacokinetics and other risk factors for kanamycin-induced hearing loss in patients with multi-drug resistant tuberculosis. Int J Audiol **2020**; 59:219–23.
19. Alsultan A, Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis: an update. Drugs **2014**; 74:839–54.
20. Antimycobacterial Susceptibility Testing Group. Updating the approaches to define susceptibility and resistance to anti-tuberculosis agents: implications for diagnosis and treatment. Euro Resp J **2022**; 59:2200166.
21. Mohamed S, Mvungi HC, Sariko M, et al. Levofloxacin pharmacokinetics in saliva as measured by a mobile microvolume UV spectrophotometer among people treated for rifampicin-resistant TB in Tanzania. J Antimicrob Chemother **2021**; 76:1547–52.
22. Zentner I, Modongo C, Zetola NM, et al. Urine colorimetry for therapeutic drug monitoring of pyrazinamide during tuberculosis treatment. Int J Infect Dis **2018**; 68:18–23.
23. Köser CU, Cirillo DM, Miotto P. How to optimally combine genotypic and phenotypic drug susceptibility testing methods for pyrazinamide. Antimicrob Agents Chemother **2020**; 64:e01003–20.
24. Min J, Chung C, Jung SS, et al. Understanding illness perception in pulmonary tuberculosis patients: one step towards patient-centered care. PLoS One **2019**; 14:e0218106.
25. Conradie F, Diacon AH, Ngubane N, et al. Treatment of highly drug-resistant pulmonary tuberculosis. N Engl J Med **2020**; 382:893–902.