Optimising pyrazinamide for the treatment of tuberculosis

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The activity of pyrazinamide, a critical drug for tuberculosis treatment, increases as drug concentrations go up, but optimising this drug alone is unlikely to result in treatment shortening. Rather, rifampicin dosing must increase in parallel. https://bit.ly/2KenbHW

Cite this article as: Zhang N, Savic RM, Boeree MJ, *et al*. Optimising pyrazinamide for the treatment of tuberculosis. *Eur Respir J* 2021; 58: 2002013 [DOI: 10.1183/13993003.02013-2020].

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This article has supplementary material available from erj.ersjournals.com

Received: 29 May 2020
Accepted: 11 Dec 2020

Abstract

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

Pharmacokinetic data, sputum culture, and safety laboratory results were compiled from Tuberculosis Trials Consortium (TBTC) studies 27 and 28 and Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA) multi-arm multi-stage tuberculosis (MAMS-TB), multi-centre phase 2 trials in which participants received rifampicin (range 10–35 $mg \cdot kg^{-1}$), pyrazinamide (range 20–30 $mg \cdot kg^{-1}$), plus two companion drugs. Pyrazinamide pharmacokinetic–pharmacodynamic (PK–PD) and pharmacokinetic-toxicity analyses were performed.

In TBTC studies (n=77), higher pyrazinamide maximum concentration (C_{max}) was associated with shorter time to culture conversion (TTCC) and higher probability of 2-month culture conversion (p-value<0.001). Parametric survival analyses showed that relationships varied geographically, with steeper PK–PD relationships seen among non-African than African participants. In PanACEA MAMS-TB (n=363), TTCC decreased as pyrazinamide C_{max} increased and varied by rifampicin area under the curve (p-value<0.01). Modelling and simulation suggested that very high doses of pyrazinamide (>4500 mg) or increasing both pyrazinamide and rifampicin would be required to reach targets associated with treatment shortening. Combining all trials, liver toxicity was rare (3.9% with grade 3 or higher liver function tests (LFT)), and no relationship was seen between pyrazinamide C_{max} and LFT levels.

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

Introduction

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

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

In the World Health Organization (WHO) 1984 treatment guidelines, the recommended daily dose of pyrazinamide was 35 $mg \cdot kg^{-1}$ (in keeping with studies that showed its treatment shortening benefit). In 2003, WHO reduced the recommended daily dose to 25 $mg \cdot kg^{-1}$; the rationale was left unstated [5]. In some studies, low pyrazinamide exposures have been associated with worse outcomes [6, 7]. However, pyrazinamide can cause liver injury at high doses given for prolonged periods [8, 9]. The relationship between pyrazinamide exposures and either efficacy or, on the flip side, hepatotoxicity is not firmly established [10].

The Tuberculosis Trials Consortium (TBTC) is a multinational trials network funded by the US Centers for Disease Control and Prevention (CDC). TBTC conducted two clinical trials assessing the substitution of moxifloxacin for a first-line agent: Study 27 (S27 (NCT00140309); moxifloxacin substituted for ethambutol) and Study 28 (S28 (NCT00144417); moxifloxacin substituted for isoniazid) [11, 12]. The Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA), funded by the European & Developing Countries Clinical Trial Partnership (EDCTP), conducted a multi-arm multi-stage tuberculosis (MAMS-TB) trial (NCT01785186) assessing combinations that included higher-dose rifampicin, moxifloxacin, and an investigational drug, SQ-109 [13]. We used pharmacokinetic–pharmacodynamic (PK–PD) modelling to assess the relationships between pyrazinamide exposures and efficacy or hepatotoxicity using data from these trials.

Material and methods

Study design

We included pharmacokinetic, safety and efficacy data from participants in S27, S28 and MAMS-TB [11–13], all randomised phase 2 clinical trials involving adults with sputum smear-positive, drug-susceptible, pulmonary TB, to establish the relationship between exposure and efficacy as well as exposure and toxicity.

In S27, during the intensive phase of treatment, all participants received isoniazid (H), rifampicin (R) and pyrazinamide (Z), and they were randomised to receive either moxifloxacin (M) (HRZM) or ethambutol (E) (HRZE) [11]. Dosing was daily for the first 2 weeks, followed by three- or five-times per week. Pharmacokinetic sampling was conducted during initial daily dosing. In S28 the arms were HRZE *versus* MRZE [12], and dosing was five times per week. In both trials, pyrazinamide was 20–25 $mg \cdot kg^{-1}$ (1000–2000 mg), and rifampicin was at standard dose, 10 $mg \cdot kg^{-1}$, for 24 weeks (supplementary table S1). In S27 and S28, after the intensive phase, participants transitioned to standard continuation-phase treatment with rifampicin and isoniazid. Sputum cultures and safety testing, including liver function tests (LFT) (aspartate aminotransferase (AST) and total bilirubin) were collected at weeks 12, 16, and 24. Over the studies' duration, 850 sputum samples were inoculated on liquid media (two participants in S27, all participants in S28) or on both solid and liquid media (seven participants in S27).

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

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

Pyrazinamide pharmacokinetic and minimal inhibitory concentration assessments

Intensive pharmacokinetic sampling was performed in a subset of participants in S27 and S28 in Uganda, South Africa, or the USA (n=72). Pharmacokinetic sampling was performed pre-dose and 1, 2, 6, 12

and 24 h post-dose, and so that pharmacokinetic values would reflect steady-state measures, these were collected at least 10 days after beginning treatment. Pyrazinamide pharmacokinetic analysis was performed using a validated gas chromatography assay with mass selective detection [14]. For minimal inhibitory concentration (MIC) determinations, isolates were stored at baseline. The pyrazinamide MIC of participants' isolates were determined using the BD BACTEC MGIT 320 system (BD Diagnostics, Sparks, MD, USA). Pyrazinamide MIC testing was performed using the standard method described in the package insert, except that, in addition to the standard test concentration of 100 $\mu g \cdot mL^{-1}$, three additional concentrations were tested (25, 50 and 75 $\mu g \cdot mL^{-1}$) [15].

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

Population PK–PD modelling

Pyrazinamide with standard-dose rifampicin

Using pharmacokinetic data from S27 and S28, a nonlinear mixed effects (NLME) model was previously developed to characterise pyrazinamide population pharmacokinetic (supplementary table S3) [14]. The existing model was used to generate *post hoc* Bayesian estimates of secondary pharmacokinetic parameter values ($AUC_{0-24\,h;}$ and maximum concentration (C_{max})) in NONMEM (version 7.4.3; ICON, Gaithersburg, MD, USA) for each participant, taking into account an individual's pharmacokinetic data and characteristics (*e.g.* dose, weight, sex, age). Pharmacodynamic indices were calculated using MIC data (*e.g.* $AUC_{0-24\,h}$/ MIC or C_{max}/MIC). Cox proportional hazards regression analysis in R program (version: 3.6.1; package: survival 2.44) was performed to assess the relationship between pyrazinamide PK–PD indices *versus* outcomes (time to sputum culture conversion, probability of culture conversion by 8 weeks of treatment). The pharmacokinetic parameter with the best fit was included in final models. Variables with p-values less than <0.1 in univariate models or factors known to be associated with culture conversion were tested in multivariate models (*e.g.* sex, ethnicity, cavity status, regimen). After exploring relationships with Cox modelling, we then proceeded to parametric survival analysis, a more sophisticated modelling technique that allows for evaluation of predictors' influence on both the shape and scale of the survival curve [16] (supplementary material, section A). Hazard function was defined by scale and shape parameters; covariates were tested on scale and shape parametersin analyses.

Pyrazinamide with standard versus higher-dose rifampicin

A population pharmacokinetic model for pyrazinamide was developed based on pharmacokinetic data from MAMS-TB using NONMEM software to generate primary pharmacokinetic parameters (supplementary material, section A, supplementary table S4 and figure S1). The relationship between pyrazinamide C_{max} or $AUC_{0-24\,h}$ and treatment outcomes was assessed in similar fashion to S27 and S28 in R program (version). A number of covariates were evaluated for inclusion in multivariate models (baseline mycobacterial load, weight, HIV status, age, sex, radiographic findings, rifampicin pharmacokinetics). Data were analysed using parametric survival analyses (supplementary material, section A). PK–PD assessments were restricted to 12 weeks.

Dosing and efficacy simulations

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

Pharmacokinetic-toxicity modelling

We evaluated the relationship between pyrazinamide pharmacokinetic parameters (*e.g.* C_{max} or $AUC_{0-24\,h}$) and change in LFTs from baseline on the basis of their relevance. Linear regression was conducted to measure the association between pyrazinamide C_{max} and individual maximal LFT values in R program (version version: 3.6.1). Multiple R-squared and p-value were calculated individually for ALT, AST and total bilirubin.

Results

Study population

Table 1 shows demographic and dose information for the 72 participants in the pharmacokinetic substudies of S27 and S28. In MAMS-TB, data for 363 participants were available and used in safety assessments.

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

	Study 27 participants	Study 28 participants	All study participants
Demographic factors			
Subjects n	9	63	72
Age years	50 (37–55)	33 (26–38)	33 (27–42)
Female sex	1 (11)	12 (19)	13 (18)
Enrolment from Africa[#]	0 (0)	37 (59)	37 (51)
Race			
Black	2 (22)	40 (63)	42 (58)
White	7 (78)	22 (35)	29 (40)
Asian	0 (0)	1 (1.6)	1 (1.4)
Hispanic ethnicity	7 (78)	18 (29)	25 (35)
Intensive phase treatment			
HRZE	7 (78)	15 (24)	22 (31)
HRZM[¶]	2 (22)		2 (3)
MRZE[+]		48 (76)	48 (67)
Thrice-weekly therapy[§]	4 (44)		4 (6)
Pyrazinamide dose mg	1000 (1000–1500)	1500 (1000–1500)	1500 (1000–1500)
Pyrazinamide dose mg·kg^{-1}	19.7 (18.6–23.9)	22.9 (20.2–25.4)	22.9 (19.9–25.3)
Clinical factors			
Cavity on baseline chest radiography	5 (56)	50 (79)	55 (76)
HIV positive	1 (11)	2 (3)	3 (4)
Weight kg	54.1 (53.3–74.0)	57.0 (51.1–63.0)	56.7 (51.3–63.3)

Data are presented as n, median (interquartile range) or n (%). HRZE: isoniazid-rifampicin-pyrazinamide-ethambutol intensive phase regimen; HRZM: isoniazid-rifampicin-pyrazinamide-moxifloxacin intensive phase regimen; MRZE: moxifloxacin-rifampicin-pyrazinamide-ethambutol intensive phase regimen. [#]: Uganda or South Africa; [¶]: HRZM not used in Study 28; [+]: MRZE not used in Study 27; [§]: in Study 27, pharmacokinetic sampling was performed during the first 2 weeks of therapy, when dosing was daily; after that, some patients received thrice-weekly dosing.

96 individuals participated in the pharmacokinetic substudy and had concentration data sufficient to produce pharmacokinetic estimates (characteristics in table 2); data for 86 subjects had dose and time of dose recorded adequately for population pharmacokinetic analysis.

Pharmacokinetic and MIC results

444 plasma samples were used in pharmacokinetic analyses from S27 and S28. *Post hoc* Bayesian estimates of pyrazinamide pharmacokinetic parameters and pharmacodynamic indices are in table 3. Predicted PZA C_{max} ranged from 15 to 55 μg·mL^{-1}; only 18 (25%) of participants had C_{max} above 35 μg·mL^{-1}. Pyrazinamide MICs were 25, 50 and 75 μg·mL^{-1} (27, 29 and four participants, respectively). 846 plasma pharmacokinetic samples from MAMS-TB were used in pharmacokinetic assessments (supplementary figure S1). Predicted pyrazinamide C_{max} ranged from 30 to 51 μg·mL^{-1}; 55 (64.0%) of participants had C_{max} above 35 μg·mL^{-1} (table 3). Time to maximum concentration (T_{max}) median was 4 h (range 3–6 h). Observed rifampin C_{max} varied depending on the dose level. Parameter estimates for the final population pharmacokinetic models are in supplementary tables S3 and S4 [14].

PK–PD of pyrazinamide, with standard-dose rifampicin (TBTC trials)

Time to culture conversion

In multivariate Cox regression analyses, the only significant predictors of time to culture conversion were pyrazinamide pharmacokinetic parameters (C_{max} p=0.046 or $AUC_{0–24\,h}$ p=0.015). In the more complex parametric survival analyses, pyrazinamide C_{max} and geographic site were the only covariates that improved the fit of the Weibull time-to-culture-conversion model significantly (pyrazinamide C_{max} influenced the shape parameter, and geographic site influenced both scale and shape parameters) (figure 1, supplementary figure S2a and table S5). Efficacy improved over the full range of clinically observed values of pyrazinamide C_{max}, without plateau (figure 2a).

TABLE 2 Demographic, treatment, and clinical characteristics among participants enrolled in the pharmacokinetic sub-study of Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA) multi-arm multi-stage tuberculosis (MAMS-TB)

	All study participants
Demographic factors	
Subjects	96
Age years	34.5 (28.9–39.2)
Male sex	67 (70)
Race	
Black	83 (86)
Mixed	13 (14)
Intensive phase treatment	
HRZE	19
$HR_{35}ZE$	20
HRZQ	19
$HR_{20}ZQ$	19
$HR_{20}ZM$	19
Pyrazinamide dose mg	1200 (1200–1600)
Pyrazinamide dose $mg \cdot kg^{-1}$	25.7 (24.0–28.3)
Clinical factors	
Weight kg	54.0 (48.9–56.5)
Body mass index $kg \cdot m^{-2}$	19.2 (17.6–20.5)[#]
Body mass index <18.0 $kg \cdot m^{-2}$	25 (26)[#]
HIV positive	2 (2)
Cavity on baseline chest radiography	66 (68.8)

Data are presented as n, median (interquartile range) or n (%). HRZE: isoniazid-rifampicin-pyrazinamide-ethambutol intensive phase regimen (subscript indicates $mg \cdot kg^{-1}$ dose); HRZQ: isoniazid-rifampicin-pyrazinamide-SQ109 (subscript indicates $mg \cdot kg^{-1}$ dose); HRZM: isoniazid-rifampicin-pyrazinamide-moxifloxacin (subscript indicates $mg \cdot kg^{-1}$ dose). [#]: one patient had height missing.

Probability of culture conversion

There was a positive relationship between pyrazinamide C_{max} and 2-month culture conversion in non-African but not African participants; however, there was a positive relationship between C_{max} and probability of culture conversion by 3 months across groups (supplementary table S6). Simulations show that to achieve culture conversion by 2 months in 90% of participants, pyrazinamide C_{max} of 43 and 93 $\mu g \cdot mL^{-1}$ would be needed for non-African and African participants, respectively. Average daily doses of 1800 mg and 4600 mg are required to achieve these targets (table 4).

PK–PD of pyrazinamide, with higher-dose rifampicin (PanACEA MAMS-TB trial)

In Cox regression models, pyrazinamide C_{max} or $AUC_{0-24\,h}$ were associated positively with time to culture conversion (p=0.0067 and 0.73, respectively). In parametric survival analysis, several factors were correlated with the scale parameter in the Weibull model (age, ethnicity, weight, baseline mycobacterial load, HIV status, pyrazinamide C_{max}, rifampicin $AUC_{0-24\,h}$), and several factors correlated with the shape parameter (age, ethnicity, baseline mycobacterial load, pyrazinamide C_{max}). The final model, which included rifampicin $AUC_{0-24\,h}$ and pyrazinamide C_{max} on scale and pyrazinamide C_{max} on shape, demonstrated a significant exposure–response relationship for pyrazinamide that depended on rifampicin exposures (supplementary tables S7 and S8, figure 3, supplementary figure S2b). Table 5 shows the doses of pyrazinamide and exposures of rifampicin needed to achieve 2-month or 3-month culture conversion proportions of 90 or 95% on liquid media. For context, in MAMS-TB, doses of 10, 20 and 35 $mg \cdot kg^{-1}$ of rifampicin achieved median $AUC_{0-24\,h}$ values of 20.6, 61.7 and 164.2 $\mu g \cdot mL^{-1}$ [13].

Pharmacokinetic-toxicity analysis

One of 72 participants in the TBTC trials and 12 of 363 participants in MAMS-TB had LFT values greater than 3-times the upper limit of normal during tuberculosis treatment. In TBTC trials, no association could be shown between pyrazinamide C_{max} and AST or total bilirubin (multiple R^2: 0.023 and 0.0007, p-value: 0.19 and 0.82, respectively) (figure 4a); Median C_{max} in those who had LFT>3-times normal was

TABLE 3 *Post hoc* Bayesian estimates of pyrazinamide pharmacokinetic parameters from Tuberculosis Trials Consortium (TBTC) Studies 27 and 28 and Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA) multi-arm multi-stage tuberculosis (MAMS-TB)

Parameter	Median (interquartile range)
TBTC trials (68 participants with MIC data)	
Pyrazinamide pharmacokinetic parameters	
Predicted C_{max} μg·mL^{-1}	29.2 (25.6–35.0)
Predicted $AUC_{0–24\ h}$ μg·h·mL^{-1}	306 (261–357)
Pharmacodynamic parameters	
Predicted $AUC_{0–24\ h}$/MIC	8.35 (5.36–12.7)
Predicted C_{max}/MIC	0.775 (0.549–1.18)
PanACEA MAMS-TB trial (86 participants)	
Pyrazinamide pharmacokinetic parameters	
Predicted C_{max} μg·mL^{-1}	37.2 (33.4–40.9)
Predicted $AUC_{0–24\ h}$ μg·h·mL^{-1}	331 (278–398)
Rifampicin pharmacokinetic parameters	
Observed C_{max} μg·mL^{-1}	
Control: $HR_{10}ZE$	5.56 (5.08–7.26)
Arm 1: $HR_{35}ZE$	26.7 (23.6–32.1)
Arm 2: $HR_{10}ZQ$	3.65 (2.49–5.04)
Arm 3: $HR_{20}ZQ$	12.1 (9.81–13.2)
Arm 4: $HR_{20}ZM$	12.1 (9.83–14.4)
Observed $AUC_{0–24\ h}$ μg·h·mL^{-1}	
Control: $HR_{10}ZE$	23.4 (17.4–29.3)
Arm 1: $HR_{35}ZE$	164 (131–199)
Arm 2: $HR_{10}ZQ$	18.3 (10.8–23.5)
Arm 3: $HR_{20}ZQ$	66.3 (56.7–82.9)
Arm 4: $HR_{20}ZM$	61.7 (50.5–78.7)

Data are presented as median (interquartile range). MIC: minimal inhibitory concentration; C_{max}: maximum concentration; AUC: area under the curve; HRZE: isoniazid-rifampicin-pyrazinamide-ethambutol (subscript indicates mg·kg^{-1} dose); HRZQ: isoniazid-rifampicin-pyrazinamide-SQ109 (subscript indicates mg·kg^{-1} dose); HRZM: isoniazid-rifampicin-pyrazinamide-moxifloxacin (subscript indicates mg·kg^{-1} dose).

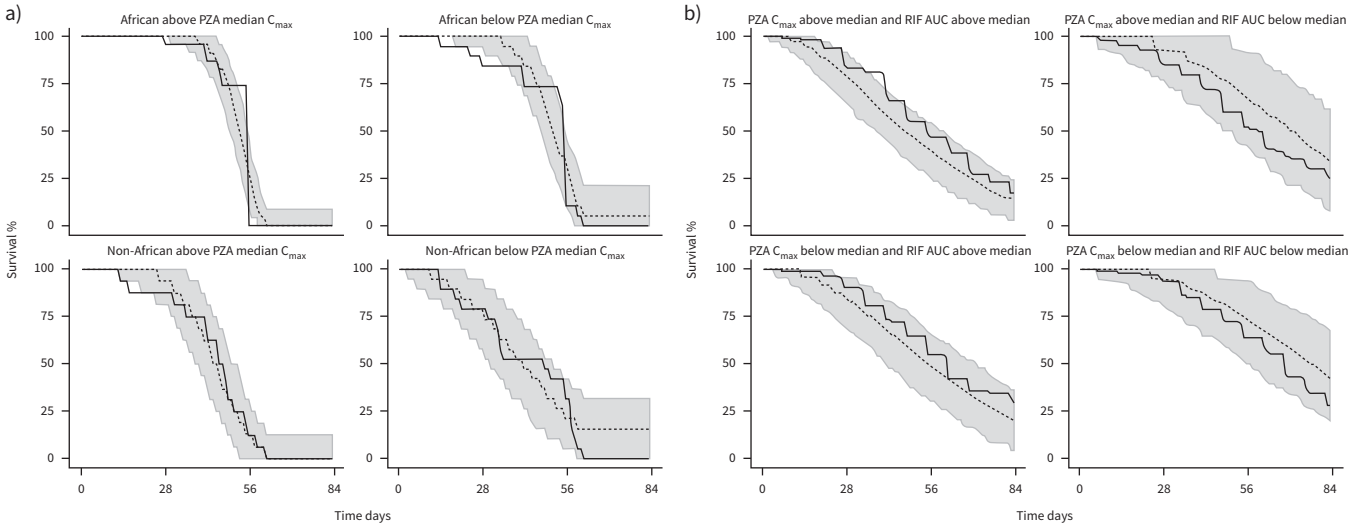

FIGURE 1 Visual predictive checks of the pharmacokinetic/outcome Weibull survival models for a) Tuberculosis Trials Consortium (TBTC) Studies 27 and 28, and b) Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA) multi-arm multi-stage tuberculosis (MAMS-TB) trials stratified by covariates identified in the survival model. The solid line is the observed data and the dashed line is the median of the simulated data. PZA: pyrazinamide; C_{max}: maximum concentration; RIF: rifampicin; AUC: area under the curve.

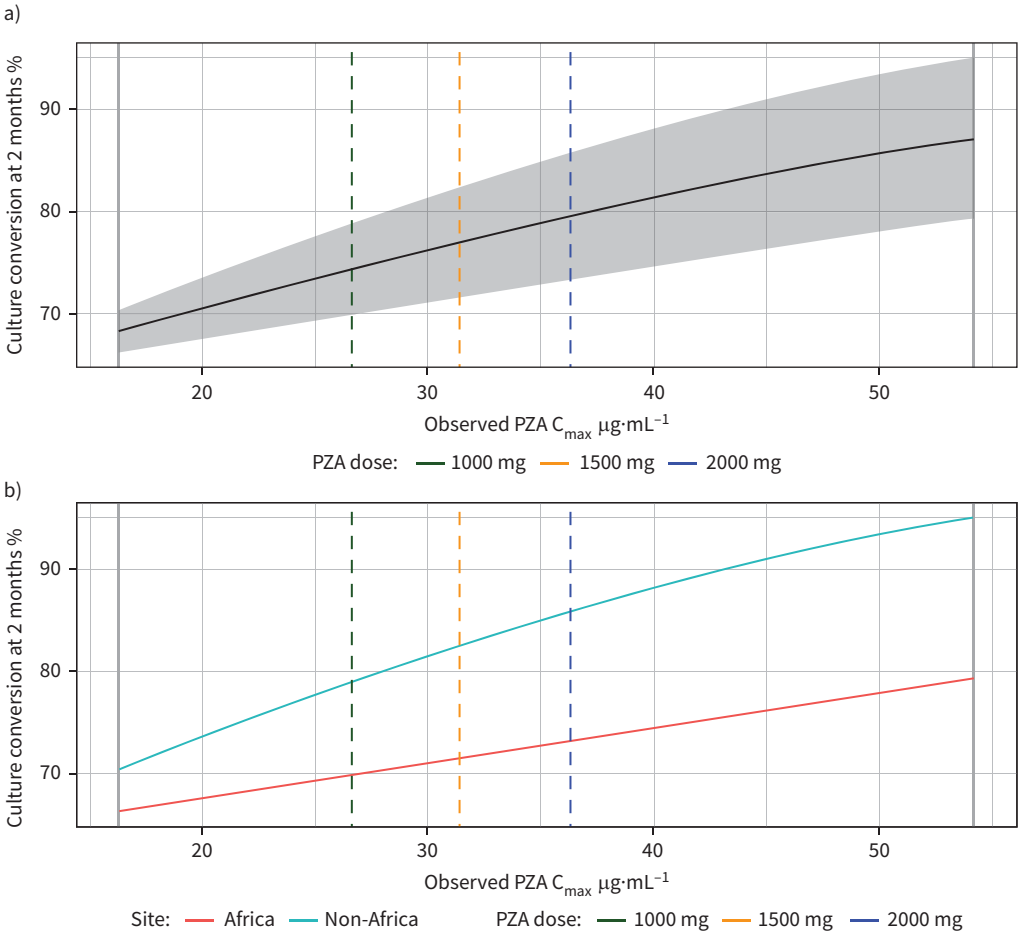

FIGURE 2 Among participants taking combination treatment including pyrazinamide (PZA) and standard-dose rifampicin in Tuberculosis Trials Consortium (TBTC) Studies 27 and 28, the relationship between maximum drug concentration (µg·mL^{-1}) and proportion with culture conversion to negative by 2 months of treatment. The median maximum concentration (C_{max}) with drug doses of 1000 mg, 1500 mg, and 2000 mg are shown in the vertical dashed lines, and the observed range of C_{max} values is contained within the vertical grey lines. a) The grey ribbon shows the 90% confidence interval of the proportion with culture conversion to negative with the black line as the median. b) The relationship between C_{max} and 2-month culture conversion is shown for African *versus* non-African participants.

28.9 µg·mL^{-1} *versus* 29.7 µg·mL^{-1} in those who did not. Similarly, there was not an association between pyrazinamide C_{max} and ALT, AST or total bilirubin, in MAMS-TB (multiple R^2=0.00063, 0.00026 and 0.019, p-value: 0.64, 0.76 and 0.16) (figure 4b). Median C_{max} was 35.3 µg·mL^{-1} in those who had LFT>3-times normal and 37.2 µg·mL^{-1} in those who did not.

TABLE 4 Clinically observed maximal concentration (C_{max}) and associated drug doses (90% CI) that would be required to achieve 90 and 95% culture conversion on solid media by 2 months of treatment in Tuberculosis Trials Consortium trials

Enrolment Site	90% Culture conversion	95% Culture conversion
Observed C_{max} µg·mL^{-1}		
Non-Africa	43	54
Africa	93	120
Expected dose level mg		
Non-Africa	1800 (2800–6000)	2200 (1400–4800)
Africa	4600 (3000–8400)	5800 (3800–8800)

TABLE 5 Doses (and clinically observed C_{max}) of pyrazinamide and rifampicin that would be needed to achieve 90% or 95% culture conversion on liquid media by 2 months or 3 months of treatment, using PanACEA MAMS-TB data

Pyrazinamide dose mg[#]	Pyrazinamide C_{max} µg·mL^{-1}	Rifampicin AUC (for conversion by 2 months) µg·h·mL^{-1}	Rifampicin AUC (for conversion by 3 months) µg·h·mL^{-1}	Culture conversion %
1500	42	>688	>450	95
3000	83	>352	⩾113	95
1500	42	>588	>349	90
3000	83	>251	⩾13	90

[#]: For reference, median weight in MAMS-TB was 54 kg. C_{max}: maximum concentration; AUC: area under the curve.

Discussion

Pyrazinamide is a standard component of first-line tuberculosis treatment, yet the "right dose" is not established. In this study using data from three international phase 2 clinical trials, higher concentrations of pyrazinamide were associated with higher culture conversion rates at 2 and 3 months of treatment. These analyses suggest that current dosing may be insufficient to maximise efficacy [17]. In the trials that enrolled from geographically diverse settings, parametric survival modelling revealed that PK–PD relationships differed for participants from African *versus* non-African sites. To achieve targets associated with treatment-shortening, drug doses that are beyond the range of tolerability would likely be needed in African patients in the absence of other new drugs. Modelling and simulation showed that increasing doses of both rifampicin and pyrazinamide appears to be a more promising strategy. The range of pyrazinamide concentrations was broad, yet elevations in liver enzymes were rare, and there was not an association between pyrazinamide levels and hepatotoxicity.

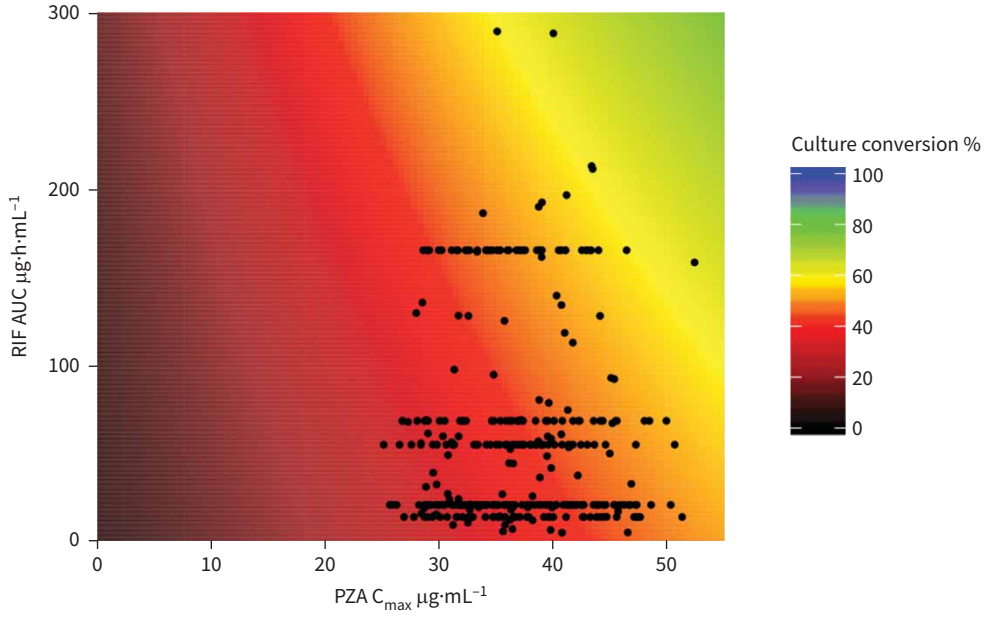

FIGURE 3 Simulated relationship between culture conversion on liquid medium by 2 months of treatment with maximum concentration (C_{max}) of pyrazinamide (PZA) and area under the curve ($AUC_{0–24\,h}$) rifampicin (RIF) from Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA) multi-arm multi-stage tuberculosis (MAMS-TB) trial. Black dots are C_{max} values of pyrazinamide and $AUC_{0–24\,h}$ of rifampicin obtained by population pharmacokinetic models. Notes: for patients whose pharmacokinetic concentrations were not measured, pyrazinamide C_{max} values were imputed using the population pharmacokinetic model and rifampcin $AUC_{0–24\,h}$ values were inputed using geometric mean values of the regimen taken.

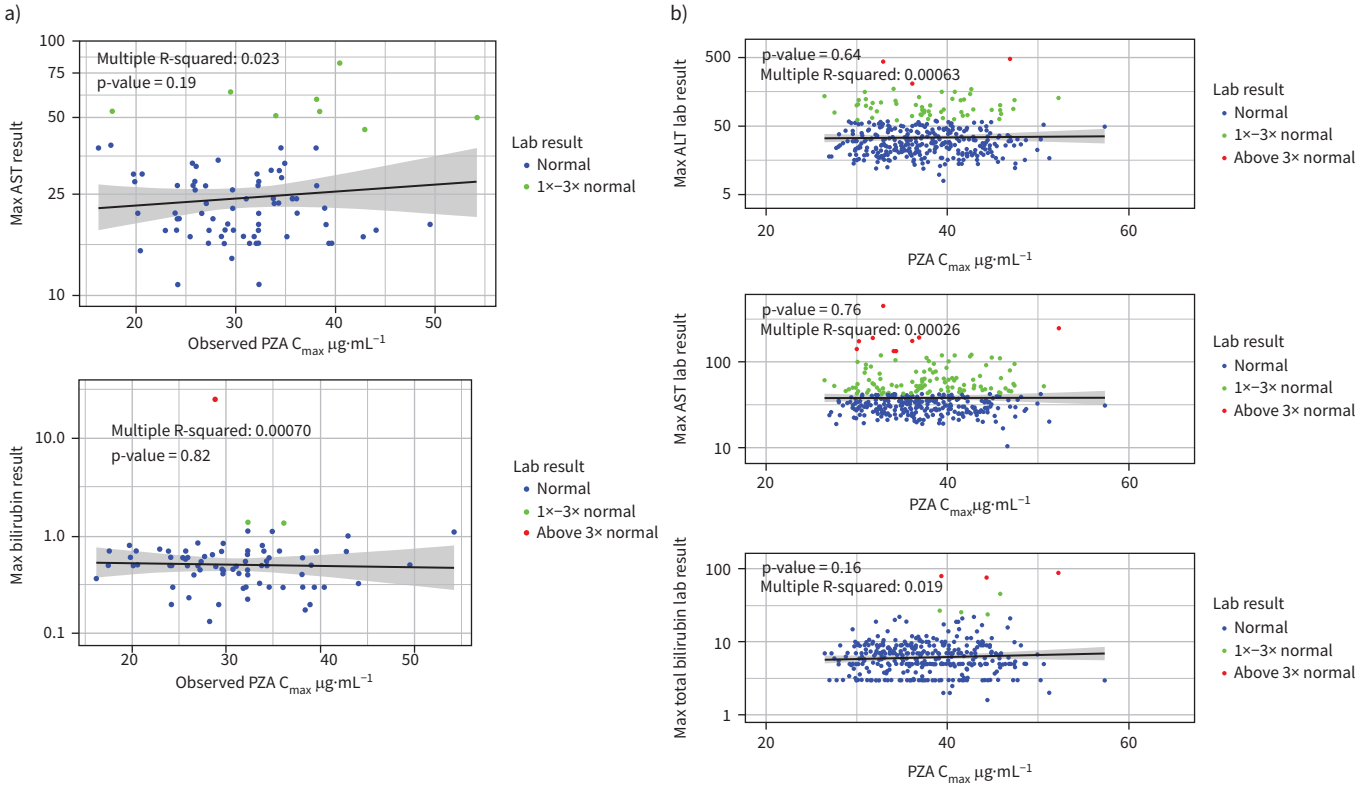

FIGURE 4 a) Distribution and regression of individual maximal aspartate aminotransferase (AST) (top) and maximal total bilirubin (bottom) *versus* observed maximum concentration (C_{max}) of pyrazinamide (PZA) in Tuberculosis Trials Consortium (TBTC) 27 and 28 trials. b) Distribution and regression of individual maximal alanine aminotransferase (ALT) (top), individual maximal AST (middle), and individual total bilirubin (bottom) *versus* pyrazinamide C_{max} in Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA) multi-arm multi-stage tuberculosis (MAMS-TB) trial.

Pyrazinamide is an important sterilising agent. Early on, it was highly effective in two-drug combinations with isoniazid, provided it was given at a high enough dose and for sufficient duration [8, 18–22]. Following the discovery of rifampicin, adding pyrazinamide to rifampicin-containing regimens reduced relapses [2, 23–25], and pyrazinamide became an essential part of current "short-course" treatment. Giving it during the first 8 weeks allows for the shortening of treatment from nine to 6 months. In the trials demonstrating its treatment shortening activity, though, the doses given were 30–40 mg·kg^{-1} daily, not the currently recommended 20–25 mg·kg^{-1} for adults [26–28].

In our study, culture conversion rates increased with increasing pyrazinamide exposure both when pyrazinamide was combined with standard-dose rifampicin or higher-dose rifampicin, and the best activity was seen when exposures of both drugs were high, demonstrating that there was an observable exposure–response relationship even when the companion drug was a potent sterilising agent given at a high dose. Interestingly, in the TBTC studies, PK–PD relationships were different for non-African and African participants. African participants tended to have higher baseline extent of disease (higher likelihood of three or more sputum smear grade or large lung cavities) than non-Africans, but these factors were not significant in our multivariate models, and there are likely other unobserved factors contributing to lower treatment response. This same phenomenon of lower treatment response, even after adjusting for known risk factors, was seen in the larger S28 study population and in TBTC Studies 29 and 29X and remains unexplained [29, 30]. While optimising pyrazinamide in the context of first-line therapy is important, pyrazinamide also has a role in multidrug-resistant tuberculosis treatment; treatment outcomes are significantly worse if the multi-drug resistant tuberculosis strain is pyrazinamide-resistant [28]. Pyrazinamide also enhances the activity of new and investigational drugs, namely bedaquiline, delamanid and pretomanid, so optimisation of pyrazinamide may be valuable in multiple contexts [31–33].

Our study was not the first modern study to find that pyrazinamide pharmacokinetic influenced treatment outcomes. In our study, C_{max} was the covariate identified in the final PK–PD model as the most informative. However, we note that C_{max} and $AUC_{0-24\,h}$ are highly correlated and each has a strong association with outcomes; depending on the sampling strategy of a given study, which influences how well that parameter is estimated, one might have a modestly stronger correlation or better precision. For example, single samples can sometimes fail to capture C_{max} well. In the TBTC studies, coefficient of variation % for C_{max} and $AUC_{0-24\,h}$ were similar at 23.1% and 26.1%, respectively, so variability in these estimates was similar. In a study in Botswana, after adjusting for HIV infection and CD4 cell count, patients with pyrazinamide C_{max} less than 35 $\mu g \cdot mL^{-1}$ (a putative pyrazinamide PK target) had a 3.4-fold higher risk of poor treatment outcome [6]. In South Africa, pyrazinamide $AUC_{0-24\,h}$ was a top predictor of poor long-term outcomes [7]. In children with and without HIV in India, low pyrazinamide and rifampicin C_{max} were associated with unfavourable outcomes [34]. In a recent meta-analysis, low pyrazinamide concentrations were shown to increase the risk of poor outcomes with relative risk of 1.73 [35]. At currently recommended doses, a high proportion of patients do not have drug concentrations that reach 35 $\mu g \cdot mL^{-1}$, let alone a proposed alternative target of 58 $\mu g \cdot mL^{-1}$ [10], and the evidence base for selection of a 20–25 $mg \cdot kg^{-1}$ dose is limited. Likely, the optimal dose for this drug lies somewhere between 35 and 45 $mg \cdot kg^{-1}$. Higher doses would produce exposures exceeding 5000 $\mu g \cdot h \cdot mL^{-1}$ (see below) in some patients [29, 36]. At the current dose, we are undertreating many.

Pyrazinamide commonly causes arthralgias, but its most dreaded toxicity is liver injury. In early studies, doses of at least 40–50 $mg \cdot kg^{-1}$ given for 24 weeks or longer caused unacceptable rates of liver toxicity (5–10%), while rates were substantially lower (2–5%) if the duration or dose was reduced [37]. Currently, doses of 30–40 $mg \cdot kg^{-1}$ daily are well-tolerated as part of MDR-TB treatment [38]. In a meta-analysis involving 4490 individuals, risk of liver toxicity did not appear to increase as a function of drug exposure until exposures were quite high (weekly AUC of >5000 $\mu g \cdot h \cdot mL^{-1}$) [39]. Pyrazinamide toxicity appears to be idiosyncratic up until a point, after which dose-related increases in liver toxicity are seen [10]. The mechanism for and contributing factors to pyrazinamide-associated liver injury are not clearly understood and may differ for different companion drugs [40]. Consistent with previous reports, in our study, median weekly AUC were 2100 (TBTC) and 2310 (PanACEA MAMS-TB) $\mu g \cdot h \cdot mL^{-1}$, and liver injury was rare and not related to exposure in the ranges seen.

Our study has limitations. Because of differences in culture methodology and covariates collected between TBTC and PanACEA trials, we could not combine pharmacokinetic-efficacy data into a single model. Model-predicted doses assumed proportional dose effects at doses higher than those observed, which may not be the case. In our parametric survival analyses, we did not consider interval censoring. The concentration that increases risk of liver toxicity could not be determined, as liver toxicity was rare and the dose range limited. Adjusting pyrazinamide pharmacokinetic parameters for isolates' MIC values did not improve model fit; likely this is because the MIC range was narrow, MICs were not measured precisely, and many participants did not have MIC data. In one TBTC trial, some patients had intermittent dosing-sensitivity analyses suggested removing those patients did not change model parameters or fit. Lastly, predicting an effective treatment-shortening regimen using microbiologic data from phase 2 trials is an uncertain science; while 90–95% culture conversion on solid media by 8 weeks of treatment has been proposed, there are no well-validated prediction models using liquid culture [41].

It is important that the dose of each drug in a tuberculosis treatment regimen be optimised. Higher-dose rifampicin and pyrazinamide have the potential to shorten tuberculosis treatment. Simply prolonging the duration over which pyrazinamide is given was not sufficient to reduce treatment duration from 6 to 4 months in historical trials [4]. Using parametric survival modelling and trial simulations, we discovered that increasing just the pyrazinamide dose does not seem as though it will improve outcomes in the hardest-to-treat patients [29]. Indeed, the predicted doses of pyrazinamide that would be required as part of the standard regimen to produce rapid and sustained culture conversion (90% conversion by 2 months, for example) for all patients were high and would not be safe. Whether or not an enhanced multidrug regimen containing high-dose rifampicin (*e.g.* $\geqslant$35 $mg \cdot kg^{-1}$) and higher-dose pyrazinamide (*e.g.* 30–40 $mg \cdot kg^{-1}$) will be sufficient to meaningfully reduce tuberculosis treatment duration must be explored prospectively, with attention to safety and tolerability.

Acknowledgements: We thank the participants who volunteered to participate in these studies. The authors are grateful to Philip LoBue for his support of the Tuberculosis Trials Consortium within the US Centers for Disease Control and Prevention, and Andrew Vernon, Beverly Metchock and Angela Starks for their support of the clinical and laboratory work of the Tuberculosis Trials Consortium.

This study is registered at clinicialtrials.gov (NCT00140309, NCT00144417, NCT01785186). Following de-identification, data may be shared with investigators following an approved use request to the study funders.

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Conflict of interest: N. Zhang has nothing to disclose. R.M. Savic has nothing to disclose. M.J. Boeree has nothing to disclose. C.A. Peloquin has nothing to disclose. M. Weiner has nothing to disclose. N. Heinrich reports grants from EDCTP (EU), during the conduct of the study; personal fees for lectures from AstraZeneca, outside the submitted work. E. Bliven-Sizemore has nothing to disclose. P.P.J. Phillips reports grants from EDCTP, during the conduct of the study. M. Hoelscher has nothing to disclose. W. Whitworth has nothing to disclose. G. Morlock has nothing to disclose. J. Posey has nothing to disclose. J.E. Stout has nothing to disclose. W. Mac Kenzie has nothing to disclose. R. Aarnoutse has nothing to disclose. K.E. Dooley has nothing to disclose.

Support statement: TBTC Studies S27 and S28 were supported by the Centers for Disease Control and Prevention, Division of Tuberculosis Elimination, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, and the Global Alliance for Tuberculosis Drug Development. Bayer donated drugs for S27 and S28. PanACEA MAMS-TB was funded by the European and Developing Countries Clinical Trials partnership (EDCTP Project-ID: IP.2007.32011.011), the German Ministry for Education and Research (BMBF, grant 01KA0901), and the Medical Research Council UK (MRC). The EDCTP, BMBF and MRC were not involved in the study conduct, data analysis and writing of the report. Funding information for this article has been deposited with the Crossref Funder Registry.

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