Title: Genetic determinants of the pharmacokinetic variability of rifampicin in Malawian adults with pulmonary tuberculosis

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Abstract

Variable exposure to anti-tuberculosis (TB) drugs, partially driven by genetic factors, may be associated with poor clinical outcomes. Previous studies have suggested an influence of the *SLCO1B1* locus on the plasma area under the concentration-time curve (AUC) of rifampicin. We evaluated the contribution of Single Nucleotide Polymorphisms (SNPs) in *SLCO1B1* and other candidate genes (*AADAC, CES-1*) to inter-individual pharmacokinetic variability in Malawi. 174 adults with pulmonary TB underwent sampling of plasma rifampicin concentrations at 2- and 6-hours post-dose. Data from a prior cohort of 47 intensively sampled, similar patients from the same setting were available to support population pharmacokinetic model development in NONMEM v7.2©, using a two-stage strategy to improve information during the absorption phase. Contrary to recent studies in South Africa and Uganda, SNPs in *SLCO1B1* did not explain variability in $AUC_{0-\infty}$ of rifampicin. No pharmacokinetic associations were identified with *AADAC* or *CES-1* SNPs, which were rare in the Malawian population. Pharmacogenetic determinants of rifampicin exposure may vary between African populations. *SLCO1B1* and other novel candidate genes, as well as non-genetic sources of inter-individual variability, should be further explored in geographically diverse, adequately powered cohorts.

Words: 186 (up to 250)

46 **Introduction**

47

48 Although effective chemotherapy for tuberculosis (TB) has been available for several decades, cure
49 rates are variable, remaining at 34-76% in many countries (1-6). The traditional view that treatment
50 failure, relapse and emergence of antimicrobial resistance are predominantly driven by poor
51 adherence has been challenged by reports that inter-individual variability in pharmacokinetic (PK)
52 exposure to rifampicin (RIF) accounts for some unfavourable outcomes, even amongst patients who
53 do not miss doses of medication (7).

54

55 Existing clinical data show up to 10-fold inter-individual variability in the plasma PK indices of key
56 drugs, especially RIF (8-11). Maximum plasma concentration (C_{max}) and area under the
57 concentration-time curve (AUC) measurements are often reported as "low" suggesting that some
58 patients are under-dosed according to currently clinical recommendations. Patient physiology, co-
59 morbidities, concomitant medications and dietary intake all influence drug exposure. However,
60 genetic polymorphisms in drug metabolising enzymes and transporters may explain up to 30% of PK
61 variability for all drugs (12-14). Although Africans have the highest degree of genetic diversity
62 worldwide (15) and sub-Saharan Africa accounts for a large proportion of global TB incidence and
63 mortality, data on the pharmacogenetic determinants of anti-tuberculosis drug exposure amongst
64 TB-endemic African populations are sparse.

65

66 RIF is believed to be the decisive drug that enables short-course chemotherapy. Metabolism by
67 hepatic esterases and biliary excretion of RIF occurs after first-pass metabolism and hepatocellular
68 uptake, which may be primarily mediated by organic anion-transporting polypeptide 1B1 (OATP1B1),
69 the product of the gene *SLCO1B1* (16-17). Reports from South Africa and Uganda suggested that two
70 *SLCO1B1* Single Nucleotide Polymorphisms (SNPs), rs11045819, rs4149032, are common in African
71 patients and associated with reduced RIF plasma exposure (18-20). However, a recent study from a

72 TB-endemic population in southern India reported lower variant allele frequency of these SNPs than
73 the prior African work and no genotypic effect on the pharmacokinetics of RIF (21). More data from
74 a broader range of global settings are required to assess the effect of SLCO1B1 genetics on
75 rifampicin exposure.
76
77 Within the hepatocyte, RIF is thought to be metabolised by microsomal hepatic esterases, which
78 have been incompletely characterised to date. *In vitro* data have suggested that the serine esterase
79 arylacetamide deacetylase (*AADAC*) may mediate 25-deacetylation of rifamycins (22) and that SNPs
80 in the genes encoding hepatic microsomal AADAC may alter RIF clearance (23). Alternative routes to
81 breaking ester linkages in RIF metabolism may involve the carboxyesterase (e.g. CES-1 and -2)
82 enzymes which are relatively abundant in the liver and gut. There are no clinical data to define the
83 effect of SNPs in *AADAC* or *CES* genes on plasma RIF exposure.
84
85 To assess previously described pharmacogenetic effects in a new population, and evaluate the
86 contribution of unexplored polymorphisms in other key metabolic processes, we assessed the
87 impact of critical SNPs in the candidate genes *SLCO1B1, AADAC* and *CES-1* on plasma exposure to RIF
88 amongst Malawian adults with smear-positive pulmonary TB. To maximise our study size with
89 limited resources in a low income setting, participants underwent sparse PK sampling at two time
90 points. Prior intensive PK data from the same population facilitated use of two-stage population
91 pharmacokinetic methods to improve information on RIF exposure, particularly during the
92 absorption phase.
93
94 **Results**
95
96 The sparsely sampled dataset comprised 174 participants. 121 (69.5%) were male, median age was
97 30 (range: 17-61) years and median weight was 52.0 (range: 34-74) kg. 98 (56.3%) were HIV-infected

with a median CD4 count of 174 (range: 6-783) cells/µl. 28/98 (28.6%) HIV-infected patients were on Antiretroviral Therapy (ART) at the time of recruitment. Full details are provided in Table 1.

The intensely sampled dataset used for the first stage of pharmacokinetic model-fitting comprised 47 participants. 24 (52%) were male, median age was 34 (range: 16-60) years and median weight was 52.5 (range: 35.8-74.3) kg. 30 (65%) were HIV-infected and 13/47 (27.7%) were on ART at the time of recruitment. All participants from both cohorts were black Africans newly diagnosed with tuberculosis at Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi.

In the first stage of the pharmacokinetic analysis, performed with the intensively sampled dataset alone, a one-compartment model appeared most appropriate with a transit compartment model best describing the absorption phase (Δ Objective Function Value (OFV) = 111). Inter-individual random effects (IIV) were supported for apparent clearance (CL/F) apparent volume of distribution (V/F) and absorption mean transit time (MMT).

The second stage of the analysis utilised only the sparsely-sampled dataset. Due to the relative lack of information on the absorption phase from this dataset, model parameters relating to absorption were fixed at the values estimated in the first stage of the analysis. Model refinement involved a comprehensive search of the covariates available. The inclusion of an allometric scaling weight model decreased the OFV significantly (ΔOFV =7). The only demographic covariate which significantly affected the model fit was sex, with a drop of OF of 10.5 ($P<0.01$). The final model showed an increase of clearance of 17% in male patients, resulting in a decreased of average AUC of 3% in males. Full PK parameters of the final two stage RIF model are included in Tables 2 and 3.

A Visual Predictive Check (VPC) of 1000 simulated datasets indicated that the final model performed adequately (Figure 1). The values of RIF $AUC_{0-\infty}$ and C_{Max} were obtained using empirical Bayesian

124 estimates of the individual parameters. A predicted RIF $AUC_{0-\infty}$ was generated for each subject using
125 their estimated CL/F from the final model (Dose/[CL/F] = AUC). The median predicted $AUC_{0-\infty}$ was
126 29.9 µg.hr/ml, with a range of 19.7 µg.hr/ml to 63.4 µg.hr/ml. The median CMax was 4.8 µg/ml with
127 a range of 1.4 µg/ml to 10.9 µg/ml.
128
129 Candidate SNPS in the *SLCO1B1*, *AADAC* and *CES-1* loci were assessed. The distribution of alleles at
130 these loci is presented in Table 1. The rs11045819 *SLCO1B1* SNP was rare, existing at an overall
131 frequency of 0.07 whilst the rs4149032 *SLCO1B1* SNP had a minor allele frequency of 0.32. No
132 subjects possessed the variant rs61733692 *AADAC* SNP whilst the rs1803155 *AADAC* SNP had a
133 minor allele frequency of 0.25. Only one patient was heterozygote for the variant rs121493368 *CES-1*
134 SNP. Since the metabolic action of these genes is believed to be exerted largely during first-pass
135 metabolism, their impact on relative bioavailability (F) was tested as well as CL/F. Additive,
136 dominant and recessive models of effect were tested for each SNP. Inclusion of *SLCO1B1* genotypes
137 did not significantly improve the model fit. The *AADAC* SNPs and haplotype, or the *CES-1* SNP did not
138 significantly alter the RIF model fit; as would be anticipated from the very low variant allele
139 frequency in our study population.
140
141 **Discussion**
142
143 This study is the first pharmacogenetic analysis of anti-tuberculosis therapy from Malawi, the largest
144 such study to date using a population modelling approach to relate pharmacogenetic
145 polymorphisms to plasma AUC and the first to evaluate the effect of SNPs in *AADAC* in this context.
146 The results of our analysis draw attention the importance of: fully considering SNP diversity within
147 Africa; the complexity of competing metabolic processes; and recognising the limitations of
148 observational pharmacogenetic designs.
149

150 A two-stage population PK modelling approach was used to quantify RIF exposure amongst adult
151 patients on treatment for pulmonary TB, benefiting from the availability of data from a pre-existing,
152 exchangeable population of intensively sampled patients (24). A sequential rather than simultaneous
153 approach was used to handle all the data because pharmacogenetic information was unavailable for
154 the intensive dataset, reducing the value of combined modelling with incomplete covariates.
155 The two-step approach enabled plausible and efficient estimation of plasma AUC from sparsely
156 sampled participants, which is a more reliable measure of total drug exposure than a single "peak"
157 concentration at a fixed time after dosing. Plasma AUC demonstrated up to 6-fold variability
158 between patients, like previous African cohorts. RIF is a crucial component of the current first-line
159 anti-tuberculosis regimen. In general, AUC/MIC (minimum inhibitory concentration) is the PK index
160 most closely related to the bactericidal activity of RIF (25), and a recent South African study reported
161 a threshold of AUC of RIF (≤ 13 μg.hr/ml) to be an independent predictor of poor outcome (26)
162 Therefore, identifying host pharmacogenetic factors which influence the AUC of this key drug may
163 help identify individuals at risk of treatment failure, and explain differences in treatment outcomes
164 between populations.
165
166 The *SLCO1B1* locus encodes an organic anion transporter (OATP1B1) implicated in the hepatic
167 uptake of several drugs and contains at least 17 non-synonymous SNPs. Much interest in Europe has
168 focused on the role of rs4149056 (521T>C, *5) in toxicity of statin therapy (27), whilst studies in
169 South Africa and Uganda have implicated two *SLCO1B1* SNPs (rs4149032 and rs11045819) in
170 reduced RIF exposure (18-20). However, in keeping with a recent report from southern India (21),
171 inclusion of these genotypes did not significantly improve the PK model fit for RIF in our study.
172
173 There are potential explanations for the discrepant results between *SLCO1B1* studies. The rs4149032
174 variant allele frequency was higher in South Africa (0.70-0.76), where an association with RIF was
175 reported, than our Malawian study (0.32) or southern India (0.46) where the results were negative.

Similarly, the rs11045819 variant allele frequency was higher in Uganda (0.15) that Malawi (0.07) or India (0.01). Therefore, it is possible that populations with lower allele frequencies require a larger sample size for adequate power to detect a pharmacokinetic effect. Additionally, whilst rs11045819 is believed to be functional (463C>A, *4), rs4149032 is an intronic SNP of unclear functional status which, in European populations, is in strong linkage disequilibrium with rs11045819 (28). However, these SNPs had a low level of linkage disequilibrium (D' 0.16) in Malawi, echoing findings from South Africa (18) and southern India (21). There are large differences in linkage disequilibrium worldwide and within Africa (15), so it is possible that previously recognised SNPs may tag functional genes in some populations but not others.

AADAC, a microsomal serine deacetylase expressed mainly in the liver and gastrointestinal tract, is responsible for 25-deacetylation of rifamycins in vitro (23). Previous pre-clinical studies identified that expression of the *AADAC*3* allele (rs1803155/rs61733692) significantly reduced RIF clearance (22). No subjects in our study possessed the *AADAC*3* allele, and inclusion of *AADAC* haplotype did not significantly improve the RIF model fit. Whilst the low incidence of these *AADAC* SNPs excluded their role in explaining inter-individual PK variability in this Malawian population, these genes are understudied. Similarly, the low incidence of one *CES-1* SNP in our population does not exclude the possibility that polymorphisms in this gene are relevant in other populations, or that other mutations are important. Future work may describe higher SNP incidence in other settings, or identify alternative SNPs of relevance to drug exposure.

The gene loci assessed here focussed on hepatic drug uptake and metabolism. Additional processes, including widely distributed mucosal P-glycoprotein transporters (29) and orphan nuclear receptor regulatory elements which influence gene induction (30) may also influence RIF metabolism. Investigation of these broader pharmacogenetic factors may be studied separately.

These data indicate that inter-individual variability in RIF PK amongst Malawian adults was not explained by polymorphisms in the candidate genes studied. They illustrate the importance of local ethnic background in the context of high SNP diversity within Africa and emphasise the need for caution in extrapolating findings across the continent. Growing recognition of the extent and clinical consequences of variable RIF PK (31-37) coupled with recent evidence that dose escalation is safe and tolerable (38) highlights the need for ongoing work to better define the relative importance of polymorphisms and non-genetic risk factors for low antibiotic exposure in a range of populations.

There were several limitations to our study. Standard Fixed Dose Combination (FDC) tablets were used in treatment of the participants under field conditions, but we have not been able to account for the effect of quality of the potentially varying drug formulation on PK variability between the two cohorts. This reflects the reality of the TB drug supply chain in most countries. The use of sparse PK sampling at three fixed time points over six hours limited the precision of the estimates in each model, particularly for the absorption parameters. The use of data from a similar, intensively sampled cohort, to develop a population PK model mitigated this to some extent, achieving reasonable precision for the key PK parameter, AUC. Finally, the size of the dataset was not predicated on this secondary pharmacogenetic analysis and the negative findings could be due to a relative lack of power. However, our study was larger than any reported African cohort to date and there are limitations on the number of PK profiles, even based on sparse sampling protocols, that can practically be obtained in the field situation. On the other hand, it is widely recognised that candidate gene studies can be prone to chance findings which may not be subsequently replicated.

In conclusion, high inter-individual variability in plasma exposure to RIF amongst Malawian adults with pulmonary TB cannot be explained by genetic heterogeneity in *SLCO1B1*, as suggested from other African populations. Similarly, the variability cannot be explained by the novel candidate gene SNPs in *AADAC*, *CES-1* that were evaluated but the low frequency of variant genes in our population

does not exclude an association elsewhere. The true significance of pharmacogenetic influences on disposition of anti-tuberculosis drugs by comparison with non-genetic factors may only be established through consistent observation across geographically diverse and adequately powered cohort studies.

Ethics

Ethical approval was obtained from the College of Medicine Research Ethics Committee, University of Malawi, and the Research Ethics Committee of Liverpool School of Tropical Medicine.

Materials and methods

Study participants

The sparse pharmacokinetic data sampling was conducted within a prospective cohort study conducted at QECH in Blantyre, Malawi from 2010-2012. Consenting adults aged 16-65 years with sputum smear-positive pulmonary TB were eligible. Exclusion criteria included haemoglobin<6g/dL, creatinine>177µmol/l, total bilirubin>51µmol/l, alanine transaminase >200IU/l, clinical status suggestive of imminent mortality (WHO Performance Score 4 (39)), pregnancy, TB treatment within five years, corticosteroid therapy or baseline resistance to rifampicin and isoniazid using the Genotype MTBDRplus 2.0 line probe assay (LPA, Hain Life Sciences). Patient characteristics have been reported previously (40). Participants received daily FDC tablets according to a WHO-approved weight-adjusted regimen (including rifampicin 8-12mg/kg) and standard National Tuberculosis Programme guidelines (41). Adherence was monitored by direct questioning and pill counts. All patients had point of care HIV serology. ART was provided per national protocols.

The intensively sampled pharmacokinetic data used for the first stage of model construction was obtained from a prior study of adult patients with sputum smear-positive pulmonary TB at the same

hospital, conducted from 2007-2008. Recruitment criteria, patient characteristics and treatment protocols have also been reported previously (24), and were similar to the sparse sampling dataset.

Genotyping

Genomic DNA extraction and genotyping were performed from whole blood samples collected at baseline from each patient, as previously described (18). TaqMan real-time PCR using fluorescent probes for allelic discrimination was used to detect SNPs of two RIF transporting or metabolising genes: *SLCO1B1* (rs11045819, rs4149032) and *AADAC* (rs1803155, rs61733693). These SNPs are previously reported to have functional significance for RIF PK (18,22,23). An exploratory SNP (rs12149368) in *CES-1* was also assessed.

Drug plasma concentration determination

For the sparse sampling cohort, blood collections to measure steady-state RIF concentrations were undertaken on day 14 or 21 after TB treatment initiation. Patients attended the study clinic after an overnight fast at 0730 hours. Samples were collected pre-dose, then 2 and 6 hours after medications. Plasma was separated by centrifugation and stored at -70^{o}C until analysis.

RIF concentrations were determined using a liquid chromatographic/tandem mass spectrometry method (24) using appropriate internal standards validated to internationally recognised acceptance criteria as previously described (42). The lower limit of quantification (LLQ) was 0.5 µg/ml for RIF. 5% of samples had RIF results below the LLQ. These data-points were handled by imputing a value which was 50% of the LLQ.

Population pharmacokinetic analysis

277 A population PK model for RIF was developed using NONMEM® (version 7.2.0, ICON Development
278 Solutions). Since the sparse sampling data contained little information on RIF absorption, a two-
279 stage model-building strategy was used.
280
281 In the first stage, a dataset previously obtained from 47 adult tuberculosis patients in Blantyre
282 employing intensive sampling and the same drug assay (24) was used to characterise the absorption
283 phase. No covariates were included. One- and two- compartment models with alternative models of
284 absorption were fitted to the data using the First Order Conditional method of estimation with
285 interaction. Among the models explored were simple first-order absorption or a sequence of zero-
286 and first-order absorption incorporating either lag times or transit compartment absorption.
287 Proportional, additive and combined proportional and additive error models were considered to
288 describe residual variability. The minimal OFV (equal to -2 log likelihood) was used as a goodness-of-
289 fit metric with a decrease of 3.84 corresponding to a statistically significant difference between
290 models (P=0.05, $\chi 2$ distribution, one degree of freedom). Residual plots were also examined.
291
292 Once the appropriate structural model was established, the values of the absorption parameters
293 were fixed and a second-stage analysis performed using data from the sparsely-sampled participants
294 of the current study. The following covariates were explored: body weight, age, gender, HIV status
295 and SNP genotypes.
296
297 Exponential errors following a log-normal distribution were assumed for the description of inter-
298 individual variability in pharmacokinetic parameters, as shown in the following equation:
299 (1) $\theta_{xi} = \theta_x * \exp(\eta_{xi})$
300 where θ_{xi} is pharmacokinetic parameter "x" of the *i*th individual; θ_x is the population parameter
301 estimate; and ηx_i is the log inter-individual variability for parameter "x" drawn from a normal
302 distribution with a mean of zero and variance ω^2. RIF was administered orally, CL and *V* represent

apparent values (CL/*F* and *V*/*F*, respectively, where *F* is the oral bioavailability, which was fixed to 1). An allometric weight model was applied to standardize the pharmacokinetic parameters using a standard weight (WT_{std}) of 70 kg. An allometric weight model for clearance parameters is given by $CL/F_{wt}=\left(\frac{WT}{WTstd}\right)^{3/4}$ and for volume parameters is given $V/F_{wt}=\left(\frac{WT}{WTstd}\right)^{1}$ where CL/F_{wt} and V_{wt} are the weight functions for clearance parameters and volume of distribution parameters, respectively, and *WT* is the individual weight value.

Dichotomous covariates were introduced as a power model and continuous variables were modelled using a power model with normalized covariate:

(2) $\theta_i = \theta_1 \times \theta_{cov}^{X}$

(3) $\theta_i = \theta_1 \times (COV_i/COV_{median})^{\theta cov}$

where θ_i is the pharmacokinetic parameter of the *i*th individual; θ_1 is the population parameter estimate; in equation 2 (dichotomous covariates) θ_{cov} is the ratio value of θ_i for the individuals X=0 or 1. In equation 3 (continuous covariates) COV_i is the value of the covariate for the *i*th individual, COV_{median} is the median value, θ_{cov} is the fraction of θ_i relative to the consider covariate effect.

Genotype information was coded as an index variable, shown below for CL/F:

$TVCL = \theta_0 + \theta_1 * X_1 + \theta_2 * X_2$

where θ_0 is the typical value of CL/F for individuals with homozygosity, θ_1 is the relative difference in CL/F for heterozygous for the mutant allele when X_1=1, and θ_2 is the relative difference in CL/F for patients homozygous for the mutant allele when X_2=1.

Graphical methods were used to explore the relationship of covariates versus individual predicted pharmacokinetic parameters. Each covariate was introduced separately into the model and only

retained if inclusion in the model produced a statistically significant decrease in OFV of 3.84 (P≤0.05). A backwards elimination step was then carried out once all relevant covariates were incorporated and covariates were retained if their removal from the model produced a significant increase in OFV (>6.63 points; P≤0.01, $\chi 2$ distribution, one degree of freedom).

To perform a VPC using Perl-speaks-NONMEM (PsN), 1000 datasets were simulated using the parameter estimates defined by the final model with the SIMULATION SUBPROBLEMS option of NONMEM®. From the simulated data, 90% prediction intervals (P5–P95) were constructed. Observed data from the original dataset were superimposed for both regimens. PsN was used to run a nonparametric bootstrap of 200 iterations to provide unbiased estimates of the standard errors and the 95% confidence intervals of the estimated parameters.

Estimates of $AUC_{0-\infty}$ for RIF were calculated from simulated values of *CL/F* using the equation:

$$AUC_{0-\infty} = \frac{Dose \in milligrams}{(CL/F)}$$

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353

354 **Author contributions:** D.J.S., A.D.M. and G.R.D wrote the article; D.J.S., H.C.M., S.H.K. and G.R.D.

355 designed the research; D.J.S., A.D.M., D.E., H.C.M., S.A.W., D.W., G.B., T.J.A., A.O., S.H.K. and G.R.D.

356 performed the research, D.J.S., A.D.M., A.S. and G.R.D. analysed the data.

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Figure legends

Figure 1: Visual predictive check for the final rifampicin model. The lower, middle and upper lines are the 5th percentile, median and 95th percentile of the observed data, respectively. The shaded areas are the 95% CIs for the 5th percentile, median and 95th percentile of the simulated data.

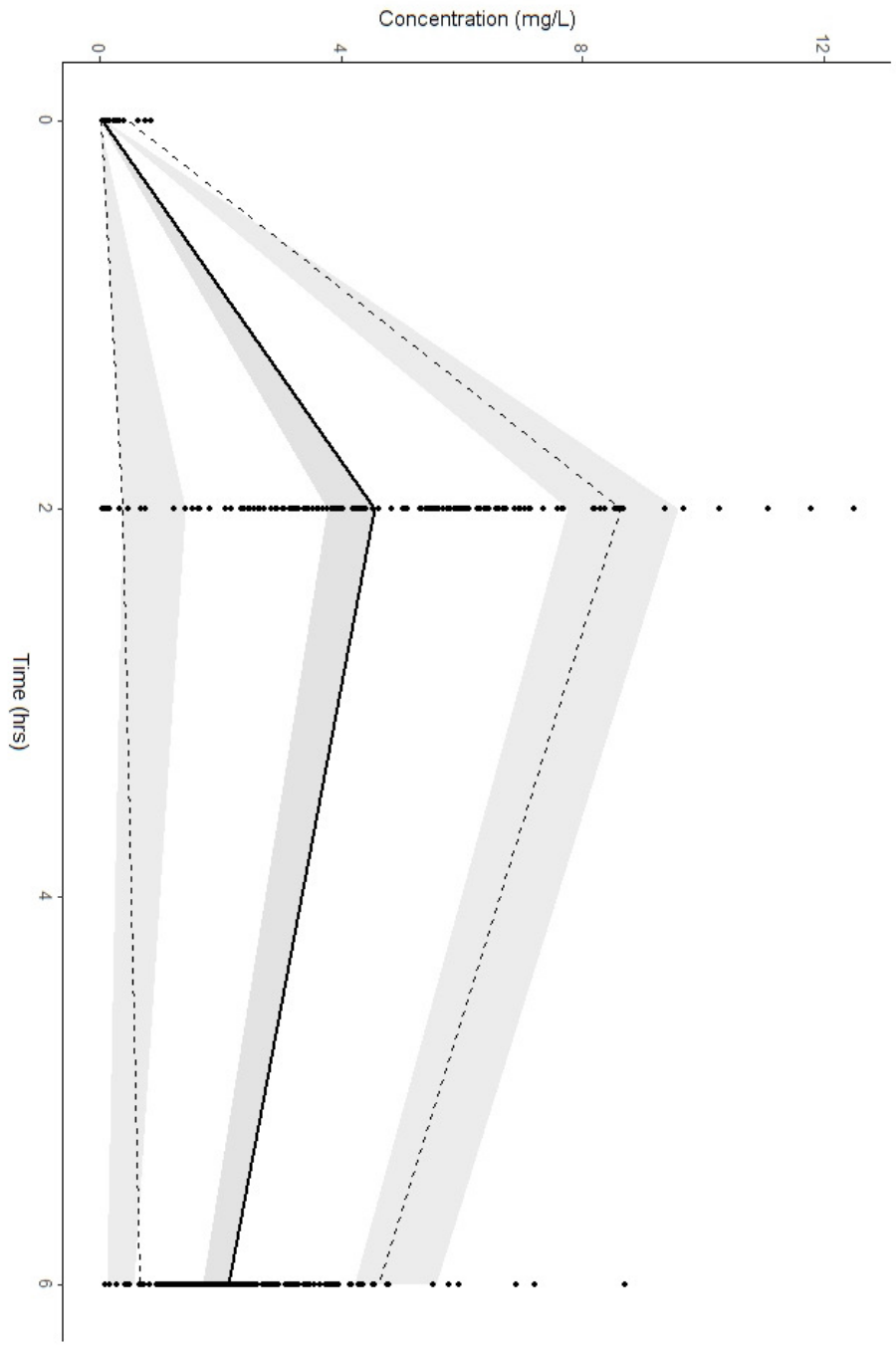

Table 1: Demographic and clinical description of cohort

Characteristic	Total (n=174)
Age in years, median (range)	30 (17-61)
Male sex, n (%)	121 (69.5)
HIV infected, n (%)	98 (56.3)
CD4 in HIV infected in cells/µl, median (range)	174 (6-783)
On ART at baseline if HIV infected, n (%)	28 (28.6)
Weight in kg, median (range)	52 (34-74)
Adherence, n (%)*	
Missed no doses	143 (82.2)
Missed <2 doses	10 (5.7)
Missed >2 doses	2 (1.1)
Rifampicin / isoniazid dose (mg), n (%)	
300 / 150	2 (1.1)
450 / 225	113 (64.9)
600 / 300	59 (33.9)
SLCO1B1 mutations	
rs11045819	
wild (CC)	150 (86.2)
heterozygote (AC)	24 (13.8)
variant (AA)	0 (0)
rs4149032	
wild (TT)	89 (51.1)
heterozygote (CT)	59 (33.9)
variant (CC)	26 (14.9)
AADAC mutations	
rs1803155	
wild (GG)	3 (1.7)
heterozygote (CG)	80 (46.0)
variant (CC)	91 (52.3)
rs61733692	
wild (TT)	174 (100)
heterozygote (CT)	0 (0)
variant (CC)	0 (0)
AADAC haplotype	
AADAC*1/*1	3 (1.7)
AADAC*1/*2	80 (46.0)
AADAC*2/*2	91 (52.3)
CES1 mutations	
rs12149368	
wild (GG)	173 (99.4)
heterozygote (CG)	1 (0.6)
variant (CC)	0 (0)

ART, anti-retroviral therapy; SNP, single nucleotide polymorphism; *NAT2*, *N*-acetyltransferase 2; *SLCO1B1*, solute carrier organic anion transporter family member 1B1; *AADAC*, arylacetamide deacetylase; *CES1*, carboxylesterase 1.
*Adherence data only available for 155 patients.

Table 2: parameter value estimates for the base model stage 1

Parameter	Typical value	%RSE [a]	95% CI [b]
CL/F (l/h)	19.5	3.9	18.2- 20.8
V/F (l)	27.1	13.9	20.9- 33.3
K_a (h^{-1})	0.277	9.8	0.23- 0.32
NN	1.5	41	0.09-3.9
MMT (h)	0.326	35	0.05- 1.0
Random effects			
IIVCL	0.165	15.3	0.12- 0.20
IIVV	0.425	27.1	0.25- 0.59
IIVMMT	0.0706	75	0.01- 1.79
Residual variability: Proportional error (%)	0.23	7.8	0.20- 0.26

CL/F, clearance *(F* is unknown bioavailability); V/F apparent volume of distribution; K_a, absorption rate constant NN, number of transit compartment; MMT, absorption mean transit time; IIVCL, inter individual variability on clearance; IIVV, inter individual variability on volume; IIVMMT, inter individual variability on absorption mean transit time. [a] RSE, relative standard error reported. [b] CI, confidence interval.

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Table 3: parameter value estimates for the final model stage 2

Parameter	Typical value (Shrinkage %)	%RSE [a]	95% CI [b]
CL/F (l/h)	19.6	12	16.7-22.5
V/F (l)	23.6	9	17.1-30.1
K_a (h^{-1})	0.277 (FIX)		
NN	1.5 (FIX)		
MMT (h)	0.326 (FIX)		
THETA_sex ^{male}	1.2	13	1.0-1.3
Random effects			
IIVCL	0.076 (22)	29	0.033-0.11
IIVV	0.397 (28)	29	0.17- 0.63
IIVMMT	0.0706(FIX)		
Residual variability: Proportional error (%)	0.22	12	0.19-0.26

CL/F, clearance *(F* is unknown bioavailability); V/F apparent volume of distribution; NN, number of transit compartment; MMT, absorption mean transit time; THETA_sex, fractional change in clearance for males; IIVCL, inter individual variability on clearance; IIVV, inter individual variability on volume; IIVMMT, inter individual variability on absorption mean transit time. [a] RSE, relative standard error reported. [b] CI, confidence interval.