Effect of genetic variation in *UGT1A* and *ABCB1* on moxifloxacin pharmacokinetics in South African patients with tuberculosis

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ABSTRACT:

AIM: We assessed the effect of genetic variability in *UGT1A* and *ABCB1* genes on moxifloxacin pharmacokinetics.

Methods: Genotypes for selected *UGT1A* and *ABCB1* SNPs were determined using a TaqMan® Genotyping OpenArray™ and high-resolution melt analysis for rs8175347. A nonlinear mixed-effects model was used to describe moxifloxacin pharmacokinetics.

Results: Genotypes of *UGT1A* SNPs, rs8175347 and rs3755319 (20.6% lower and 11.6% increased clearance, respectively) and ABCB1 SNP rs2032582 (40% reduced bioavailability in one individual) were significantly associated with changes in moxifloxacin pharmacokinetic parameters.

Conclusion: Genetic variation in *UGT1A* as represented by rs8175347 to a lesser extent rs3755319 and the *ABCB1* rs2032582 SNP is modestly associated with the interindividual variability reported in moxifloxacin pharmacokinetics and exposure. Clinical relevance of the effects of genetic variation on moxifloxacin pharmacokinetic requires further investigation.

Keywords: ABCB1; moxifloxacin; pharmacogenetics; pharmacokinetics; UGT1A

INTRODUCTION:

Moxifloxacin, an 8-methoxy fluoroquinolone, is active against both Gram-positive and Gram-negative bacteria, including anaerobes, and is used to treat a number of bacterial infections [1]. Moxifloxacin is also used in the treatment of *Mycobacterium tuberculosis.* It is recommended by the World Health Organisation for the treatment of multi-drug resistant tuberculosis (MDR-TB) and for drug-susceptible tuberculosis if toxicity develops to standard first line drugs or for isoniazid (INH) mono-resistance [2]. Moxifloxacin is emerging as a key drug in the development of novel shorter rifampicin-sparing drug regimens for the treatment of both drug susceptible and MDR-TB, being investigated in several phase III clinical trials which are currently underway [3, 4].

Although moxifloxacin demonstrates potent bactericidal activity in the treatment of tuberculosis, evidence suggests that the current standard 400mg dose may not be optimal in all patients, resulting in poor treatment outcomes and increased risk of acquired drug resistance. Moxifloxacin exhibits extensive inter-patient pharmacokinetic variability in both healthy individuals and patients with tuberculosis [5-9]. The observed variability in pharmacokinetics may be due to several factors including differences in patient age, weight, disease status, gender, poor treatment adherence, co-morbidities such as HIV and drug interactions. In addition, genetic variation associated with single nucleotide polymorphisms (SNPs), copy number variants and insertions or deletions in genes coding for drug metabolizing and transporter enzymes is emerging as an important contributing factor to tuberculosis drug exposure and variable pharmacokinetic parameters [10].

African populations have high levels of host genetic diversity resulting in differences in tuberculosis disease susceptibility [11]. Furthermore, genetic diversity in drug metabolising and drug transport enzymes has been shown to result in lower tuberculosis drug concentrations and variations in response to standard first line tuberculosis drugs [12-14].

Moxifloxacin is metabolized via glucuronide and sulphate conjugation by cytosolic enzymes glucuronosyltransferase and sulfotransferase [15].The uridine diphosphate (UDP) glucuronosyltransferases (UGT) form a family of enzymes, of which the UGT1A subfamily is encoded by a single gene locus through differential splicing of unique first exons (exon 1) to shared exons 2 - 5. The major human UGT's responsible for formation of the moxifloxacin M2 metabolite are UGT1A1 (main isoform), UGT1A3 and UGT1A9. Although there is no previous published data on polymorphisms in the genes encoding for UGT enzymes which might affect moxifloxacin metabolism or pharmacokinetics in TB treatment, these enzymes are known to be highly polymorphic leading to altered concentrations of other drugs and of moxifloxacin in healthy individuals [16-18]. Moxifloxacin is a substrate of the multidrug transporter P-glycoprotein, and this drug transporter protein found in the liver, kidneys and intestinal mucosa plays an important role in its absorption distribution and elimination [19, 20]. P-glycoprotein is coded by the ATP-binding cassette (*ABCB1*) gene, forms part of the ABCB1 family and is located on chromosome 7q21.12, spanning 209.6 kb and containing 29 exons [21]. Limited data from one study investigating polymorphisms in the *ABCB1* (MDR1 C3435T) gene coding for this transport protein found that the MDR1 3435 CC variant may affect the absorption of moxifloxacin in healthy individuals [7]. *ABCB1* exhibits high functional variance [22] shown to affect other drug exposures [21]. The effects of polymorphisms in *ABCB1* and genes coding for UGT enzymes on moxifloxacin pharmacokinetics need further investigation [16, 22].

The purpose of this study was to determine the prevalence of genetic variability in *UGT1A* and *ABCB1* in a cohort of South African patients with drug-susceptible recurrent tuberculosis, and to assess whether this variability might impact on moxifloxacin pharmacokinetics and drug exposure.

METHODS:

A prospective pharmacokinetic (PK) sub-study was conducted within the ongoing Improving Retreatment Success (IMPRESS) open-label randomised controlled trial (NCT02114684) from October 2013 in

KwaZulu-Natal, Durban, South Africa. Details on study objectives, design and inclusion or exclusion criteria are available at ClinicalTrials.gov (https://clinicaltrials.gov/ct2/show/NCT02114684)

Patients in the intervention arm of the study receiving moxifloxacin, who provided informed consent to be included in the pharmacokinetic sub-study, had blood samples collected for PK analysis at pre-defined time-points. Baseline whole blood samples were collected in all patients consenting to storage of samples for later genetic testing. All participants recruited to the study were; >18 years of age, had a confirmed history of tuberculosis within the last 3 years, and had been diagnosed with sputum smear positive, rifampicin sensitive, *mycobacterium tuberculosis* based on microscopy and GeneXpert technology. Only those with no predefined laboratory or clinical abnormalities were included, regardless of HIV status.

Drug regimens:

Patients randomized to the intervention arm of the study received daily moxifloxacin 400mg (Avelox®-Bayer Healthcare), weight based rifampicin 450mg or 600mg and isoniazid 225mg or 300mg, for patients 38-54kg and ≥ 55kg respectively, during the two-month intensive phase and four-month continuation phase of TB treatment. Pyrazinamide 1500mg or 2000mg, in patients between 38-54kg and ≥ 55kg respectively, was used during the intensive phase of treatment.

Follow up:

Patients were followed up for 24 months and clinical and safety monitoring was done bi-monthly for the first six months, or as clinically indicated. Adherence to tuberculosis treatment was measured using pill count based on the number of tablets dispensed, physically returned, reported remaining or lost as well as participant self-report of missed or incomplete doses in the four days prior to the day of study visit or PK sampling. HIV co-infected patients received standard first-line antiretroviral therapy (ART) containing efavirenz, emtricitabine and tenofovir. Treatment and prophylaxis for opportunistic infections and concomitant treatment used was recorded on case report forms.

PK Sample collection:

Plasma samples were collected prior to drug dose and at 2.5, 6 and 24 hours post dose at month one and/or two during the intensive phase of TB treatment, at month six during the continuation phase of TB

treatment and at approximately four weeks after completion of TB treatment following a single dose of moxifloxacin. Plasma, collected in EDTA tubes, was centrifuged at 3000rpm, placed on ice, sent within one hour of collection to the CAPRISA laboratory to be stored at –80 degrees Celsius. Moxifloxacin concentrations were quantified in clinical plasma samples at the KwaZulu-Natal Research Institute for Tuberculosis and HIV (KRITH) pharmacology laboratory using validated high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS). The bioanalytical method was developed and validated according to FDA guidelines (2011) [23]. Sample preparation included a protein precipitation with acetonitrile and subsequent dilution with water. Analytes were chromatographically separated using a Zorbax C18, 3.5µm, 50mm x 2.1mm column and detected using the ABI Sciex 5500 (AB Sciex LLC, Massachusetts, USA). QTrap mass spectrometer operated in positive mode. The following transitions were used; Precursor ion → Product ion (all in units of m/z): Moxifloxacin: 402.1 → 358.2 and 402.1 → 364.1. The internal standard used was Ciprofloxacin: 331.6 → 231.0 and 331.6 →288.1. Moxifloxacin was analysed isocratically with a 22% acetonitrile/water/0.1% formic acid mobile phase. The injection volume was 2 µL and the total analytical run time was 5 min. The method was validated over the concentration range of 50 - 5000 ng/mL. Overall precision, based on quality control samples evaluated at low, medium and high concentrations during the validation and analysis of samples ranged from 8.4 to 19.4% and accuracy ranged from 101.9 to 105%. Calculated carry over at the lower limit of quantification (LLOQ) was 5.4%. The LC-MS/MS system was interfaced with a DELL® Windows®7 computer running Analyst® software version 1.6.2, which was used for chromatographic data acquisition, peak integration and quantification of analytes.

Genotyping:

A total of 14 SNP's, six for *ABCB1* and eight for *UGT1A*, were selected for analysis in this study based on previous evidence of functional significance on drug response using the PharmGKB database and relevant literature [16, 22, 24]. The SNP's selected included rs10276036, rs1128503, rs2032582, rs1045642, rs2235033 and rs2235013 for *ABCB1*; and rs4148323, rs2003569, rs3755319, rs11692021, rs2070959, rs28900377, rs1983023 and rs8175347 for *UGT1A*.

Genotypes were determined for each of the SNP's using a TaqMan® Genotyping OpenArray™ (Thermo Fisher Scientific, Massachusetts, USA). For rs8175347, due to the limited ability of TaqMan® probes to

reliably distinguish all genotypes, a high resolution melt (HRM) analysis was used to determine genotype [25]. The TaqMan® Genotyping OpenArray™ was performed as follows: genomic DNA was extracted from whole blood for each sample using the Qiagen QIAamp MiniKit (Germany, Cat. No. 51306), according to manufacturer instructions. Approximately 50 µg/mL of DNA and 2 X TaqMan Genotyping OpenArray Master Mix was used for the assay. Plates were loaded using the Accufill liquid handling robot and run on the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR instrument according to Applied Biosystems™ user guide. Analysis was performed using the TaqMan® Genotyper Software version 3.1. The genotypes for *UGT1A1**28 (rs8175347) were determined using genomic DNA as previously described [25] with minor modifications. The real-time PCR conditions were changed as follows: 95 °C for 5 mins, followed by 45 cycles of 95 °C for 10s, 67 °C for 10s and 76 °C for 10s and finally 76 °C for 1min. Linkage disequilibrium (LD) was tested and haplotypes generated using LDlink 2.0. online software [26]. SNP's from *UGT1A* (rs11692021 and rs2070959) were found to be in perfect LD (D'=1, r^2=1). In addition, *ABCB1* SNP's (rs2235033 and rs2235013) were found to be in perfect LD (D'=1, r^2=1). The rs11692021 and rs2235033 SNP's were randomly selected in order to exclude them from the haplotypes generated.

Study oversight:

Ethical approval for the study was provided by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BFC029/13) and the Medicines Control Council of South Africa (MCC Ref:20130510).

Statistical Analysis:

The moxifloxacin concentration-time data were analysed using nonlinear mixed-effects (NLME) modelling, implemented with the software NONMEM (version 7.3) [27]. Perl-speaks-NONMEM (PSN), Xpose, and Pirana were used for model diagnostics and to track model development [28]. A previously reported model developed on the same pharmacokinetic data was used as a starting point.[29] The model had a semi-mechanistic structure describing the effect of the liver both on first-pass extraction and systemic clearance. The original analysis identified and characterized the effect of several factors on the pharmacokinetics of moxifloxacin, including body size (using fat-free mass (FFM) of the individual

patients, calculated based on weight, height and sex as suggested by Janmahasatian et al [30]), co-administration of efavirenz, and a difference between the exposure obtained at steady-state during rifampicin-based tuberculosis treatment and at single dose. After accounting for these effects, a moderate amount of variability still remained in the data, and this analysis aimed to further improve the model by exploring and including the effect of genetic polymorphisms. As a first exploratory step, random effects for the between-subject and -occasion variability (BSV and BOV) in intrinsic clearance (CL_{int}), pre-hepatic bioavailability (F_{preH}) and absorption parameters were extracted from the original model. These represent the portion of variability not yet explained by the factors previously included in the model (mentioned above). Linear mixed-effects models implemented in SAS, version 9.4 (SAS Institute, Cary, North Carolina, USA) were used to explore the relationship between this variability and genotypes for the *UGT1A* and *ABCB1* SNPs. A compound symmetry correlation structure was assumed to describe the correlation between individual variability estimates at different visits. After this screening step, all the effects for which a trend was identified ($p<0.05$) were further assessed within an updated version of the NLME model using Stepwise Covariate Model (SCM) building implemented in PSN [28]. Each SNP was included as a categorical variable, and grouped according to the corresponding genotype i.e. wild type, heterozygous, or homozygous mutant. In certain instances, the heterozygous group was combined with either the wild type or homozygous mutant whenever no statistical significant difference could be detected between two of the variants. A drop in the NONMEM objective function value (OFV) of 3.84 (corresponding 5% level of significance, 1 degree of freedom) was considered for inclusion of the effect of a SNP. A more stringent criterion of a change in OFV of 6.64 (corresponding to 1% level of significance) was applied for retaining the effect of a SNP in the final model. Further diagnostics were used, including inspection of goodness of fit plots and visual predictive checks, testing the robustness of the detected effect with a non-parametric bootstrap with replacement (n=200), and inspection of the individual contributions to the change of the OFV.

RESULTS:

Baseline data (Table 1)

We included 172 South African tuberculosis patients: 119 (69.2%) male, 170 (98.8%) of Black African ethnicity, and 127 (73.8%) HIV co-infected (**Table 1**). Moxifloxacin concentration-time data was available for 58 of 172 patients. Of these: median weight, fat-free mass, and age, were 56.9 kg (Interquartile range (IQR): 51.1-65.2), 46.8 kg (IQR: 42.5-50.3) and 37 years (IQR: 31-42), respectively. Forty-one (70.7%) patients were male, 42 (72.4%) HIV co-infected, with 40 (95%) on efavirenz-based ART (**Table 1**).

Genotype Frequencies: (Table 2)

The genotypic frequencies for *UGT1A* and *ABCB1* are presented in **Table 2** for all patients (n=172) included in the study and the subset of 58 patients with moxifloxacin drug level data. Allelic frequencies for all SNP's are presented in **Table 3**. Haplotypes were created for *UGT1A* based on SNPs rs2070959, rs1983023, rs28900377, rs3755319, rs2003569 and rs4148323 while haplotypes for *ABCB1* were created based on SNPs rs1128503, rs2032582 and rs1045642, in the order listed. Haplotype frequencies are presented in **Table 2**.

Effects of *UGT1A* and *ABCB1* SNP's on Moxifloxacin Pharmacokinetics:

The variability in mean moxifloxacin plasma concentrations (AUC) in each patient obtained from the base NLME model and stratified by genotypes for eight *UGT1A* and six *ABCB1* SNP's are shown in **Figure 1A/B**. These values are affected by other effects contained in the base model, and are only reported to show the distribution of variability in AUC among genotypes tested and for comparison of exposures with other studies. The exploration of pharmacogenetics was executed after adjusting for all the fixed effects already included in the model.

A linkage disequilibrium (LD) test was performed on *UGT1A* and *ABCB1* SNP's **(Figure 1C/D**). For *UGT1A* we tested seven SNPs. Of these, rs11692021 and rs2070959 were found to be in perfect LD (D'=1, r^2=1). For this reason, rs11692021 was randomly selected and excluded from the generation of haplotypes. The LD was not calculated for rs4148323 because only one genotype (GG) was observed in the population (**Figure 1C**). LD analysis was performed on five SNPs for *ABCB1*; rs2032582 was not included in the LD analysis as this SNP possessed multiple alleles that did not fit within the LD test. We

found rs2235033 and rs2235013 to be in perfect LD (D'=1, r^2=1) (**Figure 1D**); rs2235033 was randomly chosen to be excluded from the generation of haplotypes.

The SNPs found to be significant in the screening step employing linear models were then tested in NLME models that allow for re-estimation of other covariates that may affect moxifloxacin PK parameters. The effect of two *UGT* SNPs were confirmed to influence the pharmacokinetics of moxifloxacin in the final NLME PK model (**Table 4**). Having the TA 5/6 repeat in rs8175347 was associated with a 20.6% lower clearance and approximately 26% higher AUC, after adjusting for other covariates (such as efavirenz and/or rifampicin co-administration) compared to those with TA 6/6, 6/7, 7/7, and 7/8 (p=0.001). Patients with AC and AA genotypes for rs3755319 were found to have 11.6% increased clearance when compared to the CC genotype in the model (p=0.032). However, effect of the SNP was not strong enough to be retained in the final model using the cut-off value of p=0.01. Only one individual within the cohort examined had a CA genotype for rs2032582. When the effect of the rs2032582 SNP was included in the population PK model, a 40% reduced pre-hepatic bioavailability and similar decrease in AUC was observed for the patient with the CA genotype (p= 0.01). However, although this effect significantly improved the model fit in terms of OFV, it was not retained in the final model due to the fact that only one patient was affected. When testing the outcome of presence verses absence of haplotypes for both *ABCB1* and *UG*T within individual patients in the NLME models, no significant difference was observed. **Figure 2** depicts the visual predictive checks (VPC) for the final model stratified by genotypes for rs8175347. The VPC shows that the model describes the data adequately after inclusion of the effect of the SNP.

DISCUSSION:

We describe the prevalence of genetic variability in selected *UGT1A* and *ABCB1* SNPs in a cohort of South African tuberculosis patients, the majority being of Black African ethnicity. Our data adds to the current evidence of genetic variants having pharmacogenetic relevance among Africans. The impact of genetic variation on drug metabolising and transporter enzymes UGT and ABCB1, has not been described previously for moxifloxacin in the context of tuberculosis treatment. We found differences

between genotypes of *UGT1A* SNPs rs8175347 and rs3755319 which significantly associated with changes in moxifloxacin pharmacokinetic parameters. Genotypes of the *ABCB1* SNP rs2032582 were significantly associated with a decrease in pre-hepatic bioavailability in the NLME model.

Moxifloxacin exhibits extensive inter-individual variability in pharmacokinetic parameters [5-7, 31, 32]. We tested the effect of genetic variants in *UGT1A* and *ABCB1* as covariates in NLME models used to determine moxifloxacin concentration-time data and found that these are associated with some of the between subject variability observed in moxifloxacin PK parameters not accounted for by other relevant covariates in our study. We previously reported low overall moxifloxacin concentrations in this patient cohort [29] which are further decreased by drug interactions with efavirenz-based ART and rifampicin - a known inducer of UGT and p-glycoprotein [33]. After adjusting for these and other covariates, some of the unexplained between subject variability in moxifloxacin PK may be attributed to genetic variability.

More than 113 different *UGT1A1* variants have been described, which can confer reduced or increased activity as well as inactive or normal enzymatic phenotypes [16, 18]. The *UGT1A1* rs8175347 SNP in the gene promoter region covering the TATA box consists of variation in a short (TA)(n) sequence from five to eight TA repeats. This SNP has been extensively studied for association between the *UGT1A1**28 (TA7) genotype and toxicity related to the cancer drug irinotecan [16, 34]. The TA (6>7) variant is frequently seen in most populations studied, while the TA5 (*UGT1A1**36) and TA8 (*UGT1A1**37) are found almost exclusively in African populations, TA6/6 (*UGT1A1**1) being the wild-type [16, 35]. Frequencies of the TA repeats observed in our cohort were similar to those previously reported in South African patients however the frequency of the TA6 genotype was higher (0.63 vs 0.5) and TA7 lower (0.23 vs 0.37) in our cohort compared to a previous published black South African cohort [35]. Homozygotes for TA7 (*UGT1A1**28/28) and less commonly the TA8 genotype have been linked to decreased transcriptional activity and lower activity of the gene compared to the wild-type, resulting in decreased glucuronidation of the toxic SN-38 metabolite of irinotecan. The TA7 and TA8 genotypes are therefore expected to result in decreased metabolism, lower clearance, higher bioavailability and drug exposure as seen with thyroxine (T4) [36]. However, we demonstrate lower moxifloxacin concentrations with TA7 and TA8 genotypes compared to homozygous and heterozygous TA5 and TA6 genotypes. Similar findings were reported with

telmisartan [37] raloxifene [38] and moxifloxacin in healthy individuals [17], where higher concentrations of metabolites and resultant lower drug exposures were observed with TA7 (*UGT1A1**28). The reasons for this contrasting effect are not entirely clear, but may be linked to extensive sharing of exons in the *UGT1A* family and genetic linkage between many UGT variants (which may differ between ethnic groups) contributing to discordance between findings. Furthermore, uncertainty exists on the extent of functional activity of the UGT variants studied.

We report effects of the AC and AA variants in the rs3755319 *UGT1A* SNP resulting in higher CL and decreased moxifloxacin exposures, however these effects were not robust enough to be included in the final NLME model. Previous studies have reported non-significant effects of this SNP on exposure to other drugs such as ABT-751 used to treat patients with neoplasms [39]. No significant effects of *UGT* haplotypes were observed in our study.

We found very limited effects of genotypes in the *ABCB1* SNPs studied, with the exception of rs2032582 where we found 40% reduced pre-hepatic bioavailability associated with lower moxifloxacin concentrations in the presence of the CA genotype. Similar effects of this variant have been reported previously [21]. However, we had only one individual in this genotype group, likely as a result of our limited sample size. A previous study testing the effect of the rs2032582 SNP on efavirenz concentrations in South African patients found limited effects on drug exposure [40]. We found no significant effects of *ABCB1* haplotypes in our study. The *ABCB1* variants 1236C>T (rs1128503), 2677G>T/A (rs2032582) and 3435C>T (rs1045642) occur at high allele frequencies and create a common haplotype; therefore, they have been widely studied in relation to a number of drug classes [21]. A recent review investigating the effects of these SNPs on drug exposure and PK parameters in over 300 studies concluded that although these had significant effects on drug disposition and response, findings were in part conflicting and the clinical implications limited [21].

We acknowledge the limitations of our study. This includes a relatively small sample of patients with drug concentration data, and the lack of significant associations observed between SNPs studied and PK parameters for moxifloxacin may be due to inadequate numbers of patients in the groups for each of the

genotypes studied. The concentrations of moxifloxacin metabolites M1 and M2 were not measured and we are unable to draw any conclusions on the effects of the genetic variation in UGT on moxifloxacin glucuronidation to its inactive metabolites. We included a limited number of SNP's in our study based on previous literature that showed association between these SNPs and drug response, toxicity or disease susceptibility. Other relevant *UGT1A* and *ABCB1* SNPs that were not included in this study may significantly impact moxifloxacin PK parameters. Genotype frequencies in our population may differ from other populations and our findings may therefore be limited to similar African populations. The pharmacokinetic data was collected with sparse sampling. While NLME modelling has been shown to be suitable to analyse sparse data, our model had sometimes limited capability to separate effect on CL_{int} versus F_{preH}, parameters which both affect total exposure, as seen with TA 5/6 repeat in rs8175347. In such cases, the most statistically significant effect was included in the model.

In conclusion, we have observed increased prevalence of genetic variability in *UGT1A* and *ABCB1* in our cohort of South African patients. Genotypes of *UGT1A* SNPs (rs8175347) and to a lesser extent *UGT1A* (rs3755319) and *ABCB1* (rs2032582) were associated with significant differences in moxifloxacin drug concentrations or PK parameters. The clinical relevance of these changes in moxifloxacin drug exposure on tuberculosis treatment outcomes need further investigation, although given that moxifloxacin exhibits concentration-dependant bactericidal activity changes in drug concentrations are likely to impact efficacy.

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Author contributions:

Study concept and design: AN, NP, KN, HM, MSP, VR

Drafting of the manuscript: AN, VR, PD, MC

Statistical analysis: NLME modelling and data analysis: PD, MC; General statistical support: NY

Acquisition, analysis, or interpretation of data: All authors

Critical revision of the manuscript for important intellectual content: All authors.

Disclosure statement/ Declaration of Conflict of interest:

NP is the principal study investigator, on the Improving Retreatment Success Trial (IMPRESS) [NCT02114684]. Bayer Pharmaceuticals donated the study drug (moxifloxacin) used during the trial. All other authors have no potential conflicts of interest to declare.

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Figure and Table Legends:

Figure Legends

Figure 1. Distributions of (A) *UGT1A* and (B) *ABCB1* genotypes using variability in mean moxifloxacin concentration (AUC) μg·h/mL from the base NLME model, and linkage disequilibrium (LD) between (C) *UGT1A* (D) and *ABCB1* SNPs.

Figure 2. Visual predictive checks stratified by genotype of rs8175347. The dashed and solid lines are the 5th, median, and 95th percentiles of the observed concentrations, while the shaded regions represent the corresponding 95% confidence intervals for the same percentiles. The sub-plot in each stratum shows the same VPC with a logarithmic transformation applied to the y-axis.

Table Legends:

Table 1: Baseline data

*only for HIV+ patients, [a]Mixed Race, [b] 10/172, 4/58 missing data, [c]12/172, 5/58 missing data

Table 2: Genotype and haplotype frequency for *UGT1A* and *ABCB1* SNPs

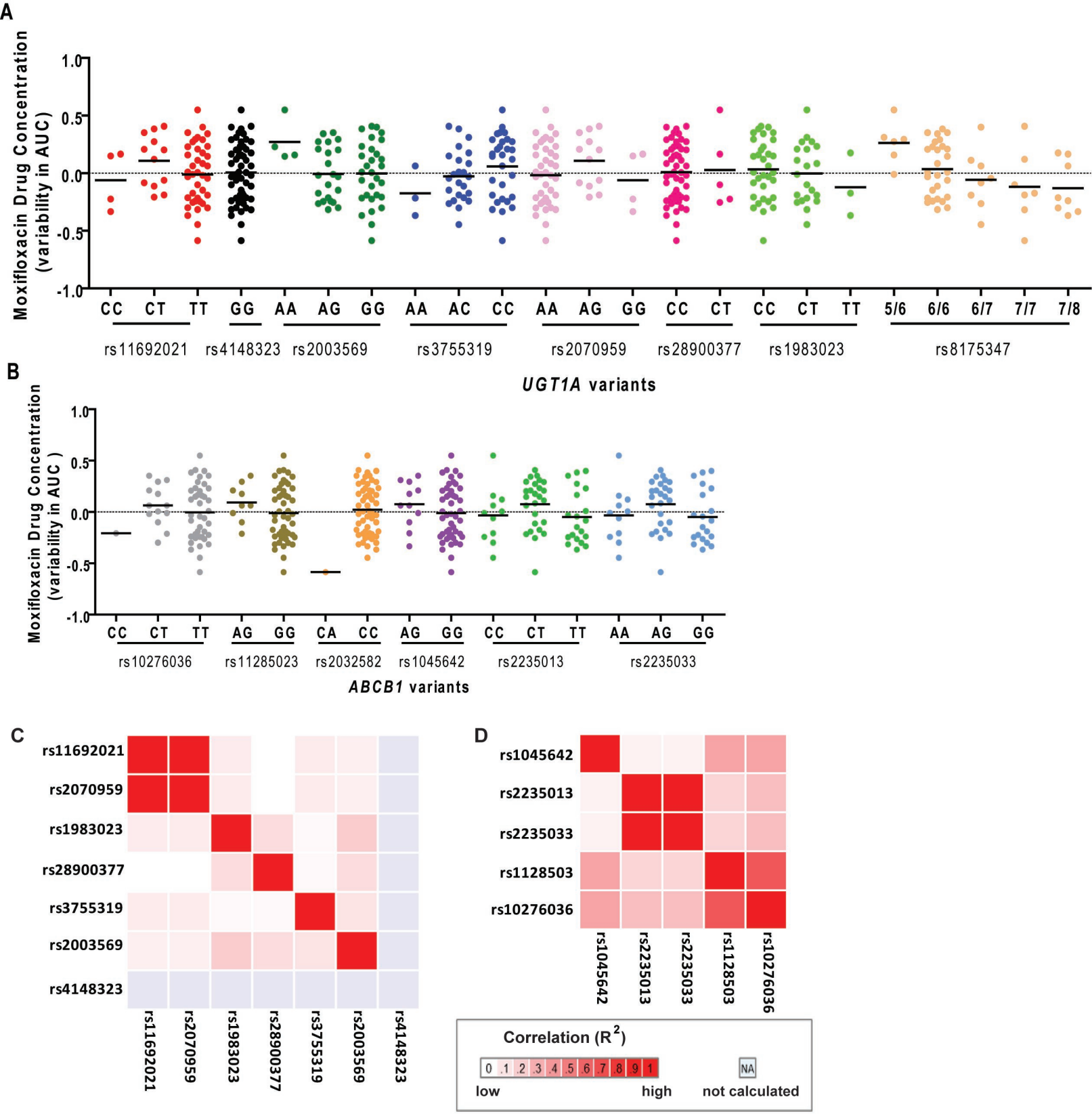
**UGT1A* haplotype consists of rs2070959, rs1983023, rs28900377, rs3755319, rs2003569 and rs4148323 respectively. ***ABCB1* haplotype consists of rs1128503, rs2032582 and rs1045642 respectively. The genotypes represented for *ABCB1* represent the forward orientation while the gene is transcribed in the reverse direction.

Table 3: Allele Frequencies for *UGT1A* and *ABCB1* SNPs (N=172)

*The genotypes represented for *ABCB1* represent the forward orientation while the gene is transcribed in the reverse direction.

Table 4: Population parameter estimates of moxifloxacin pharmacokinetics

[a] Intrinsic clearance of moxifloxacin when given at steady-state within rifampicin-based TB treatment and no efavirenz.
[b] All clearance and volume parameters have been allometrically scaled with fat-free mass, and the typical values reported here refer to the typical patient, with a FFM of 47 kg
[c]CI – Empirical 95% Confidence interval obtained with a nonparametric bootstrap (n=300)
[d] BSV- Between-subject variability; BOV- Between-occasion variability
[e] Pre-hepatic bioavailability is the fraction of the drug which is absorbed, crosses unchanged the gut wall, thus entering the portal vein and reaching the liver.
[f] These scaling factors modulate the size of the between-occasion variability in pre-hepatic bioavailability for the sections of data indicated (single dose and unobserved doses)
[prior] These parameters were estimated using a prior, as detailed in text of the original analysis[29].


A
Moxifloxacin Drug Concentration (variability in AUC)
1.0
0.5
0.0
-0.5
-1.0
CC CT TT GG AA AG GG AA AC CC AA AG GG CC CT CC CT TT 5/6 6/6 6/7 7/7 7/8
rs11692021 rs4148323 rs2003569 rs3755319 rs2070959 rs28900377 rs1983023 rs8175347
UGT1A variants
B
Moxifloxacin Drug Concentration (variability in AUC)
1.0
0.5
0.0
-0.5
-1.0
CC CT TT AG GG CA CC AG GG CC CT TT AA AG GG
rs10276036 rs11285023 rs2032582 rs1045642 rs2235013 rs2235033
ABCB1 variants
C
rs11692021
rs2070959
rs1983023
rs28900377
rs3755319
rs2003569
rs4148323
rs11692021 rs2070959 rs1983023 rs28900377 rs3755319 rs2003569 rs4148323
D
rs1045642
rs2235013
rs2235033
rs1128503
rs10276036
rs1045642 rs2235013 rs2235033 rs1128503 rs10276036
Correlation (R2)
0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1
NA
low
high
not calculated

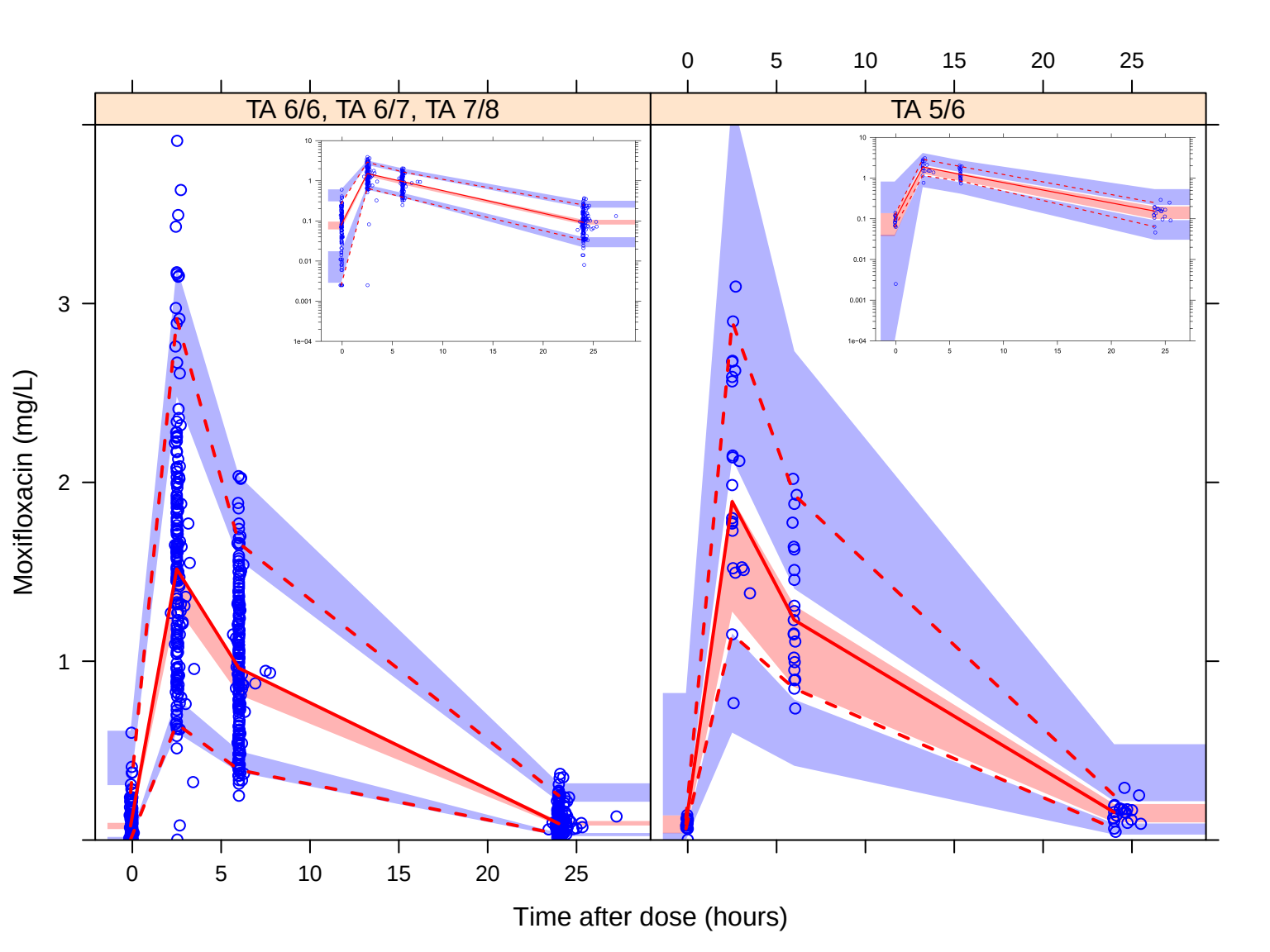

TA 6/6, TA 6/7, TA 7/8
TA 5/6
Moxifloxacin (mg/L)
Time after dose (hours)
0
5
10
15
20
25
1
2
3

Table 1: Baseline data

Variable	**N=172**	**N=58**
Age(years), median (IQR)	35 (30-41)	37 (31-42)
Male, n (%)	119 (69.2)	41 (70.7)
Race, n (%) – Black African Ethnicity/ Caucasian/ Coloured[a]	170 (98.8) / 1 (0.6) / 1 (0.6)	56 (96.6) / 1 (1.7) / 1 (1.7)
Weight (kg), median (IQR)	55.7 (50.3-62.1)	56.9 (51.1-65.2)
Fat-Free Mass (kg)	-	46.8 (42.5-50.3)
BMI(kg/m^2), median (IQR)	19.7 (18.3-22.5)	19.6 (18.0-23.3)
HIV status, n (%) Positive/ Negative	127 (73.8) / 45 (26.2)	42 (72.4) / 16 (27.6)
ART, n (%)*		
Efavirenz + Emtricitabine +Tenofovir	117 (95.1)	40 (95.2)
Lopinavir/Ritonavir + Lamivudine +Tenofovir	2 (1.6)	2 (4.8)
Lopinavir/Ritonavir + Lamivudine +Zidovudine	2 (1.6)	-
Lopinavir/Ritonavir + Emtricitabine +Tenofovir	1 (0.8)	-
Efavirenz/Lamivudine/Zidovudine	1 (0.8)	-
CD4+ count (cells/mm^3), median (IQR)*[b]	241.0 (129.0-407.0)	277.0 (139.0-384.0)
Viral load (log10 copies/mL)*[c]	3.7 (1.3-5.0)	3.3 (1.3-4.2)

*only for HIV+ patients, [a]Mixed Race, [b] 10/172, 4/58 missing data, [c]12/172, 5/58 missing data

Table 2: Genotype and haplotype frequency for *UGT1A* and *ABCB1* SNPs

Frequency *UGT1A*				Frequency *ABCB1*			
SNP	**Genotype**	**n=172**	**n=58**	**SNP**	**Genotype**	**n=172**	**n=58**
rs11692021 622T>C	CC	0.04	0.05	rs10276036 1000-44G>A	TT	0.74	0.74
	CT	0.18	0.21		CT	0.23	0.24
	TT	0.78	0.73		CC	0.03	0.02
rs4148323 211G>A	GG	1.00	1.00	rs1128503 1236C>T	AA	0.01	0.00
	GA	0.00	0.00		AG	0.14	0.16
	AA	0.00	0.00		GG	0.85	0.84
rs2003569 -997G>A	AA	0.18	0.09	rs2032582 2677G>T/A	AA	0.00	0.00
	AG	0.38	0.40		CA	0.02	0.03
	GG	0.45	0.51		CC	0.98	0.97
rs3755319 -1352A>C	AA	0.06	0.05	rs1045642 3435C>T	AA	0.02	0.00
	AC	0.32	0.41		AG	0.14	0.19
	CC	0.62	0.54		GG	0.84	0.81
rs2070959 855+20756A>G	AA	0.79	0.74	rs2235013 1725+38G>A	TT	0.29	0.38
	AG	0.18	0.21		CT	0.47	0.43
	GG	0.04	0.05		CC	0.24	0.20
rs28900377 856-23423C>T	TT	0.01	0.00	rs2235033 1554+24T>C	AA	0.24	0.20
	CT	0.06	0.09		AG	0.76	0.80
	CC	0.93	0.91		GG	0.00	0.00
rs1983023 −751T>C	TT	0.06	0.07				
	CT	0.39	0.36				
	CC	0.56	0.57				
rs8175347 TA[5][6][7][8]	TA(5) - UGT1A*36	0.07	0.05				
	TA(6) - UGT1A*1	0.63	0.62				
	TA(7) - UGT1A*28	0.23	0.25				
	TA(8) - UGT1A*37	0.08	0.07				
UGT1A	**Haplotype***	**n=172**	**n=58**	***ABCB1***	**Haplotype****	**n=172**	**n=58**
Hap1	ACCCGG	0.71	0.76		CGC	0.83	0.84
Hap2	GCCCGG	0.20	0.24		CGT	0.15	0.20
Hap3	ACCAGG	0.35	0.40		CTC	0.02	0.02
Hap4	ACCCAG	0.51	0.44		TGC	0.15	0.16
Hap5	ATCAGG	0.24	0.25		TGT	0.10	0.14
Hap6	ATCCAG	0.27	0.22				
Hap7	ATTCAG	0.05	0.05				
Hap8	GCTCGG	0.01	0.04				

**UGT1A* haplotype consists of rs2070959, rs1983023, rs28900377, rs3755319, rs2003569 and rs4148323 respectively. ***ABCB1* haplotype consists of rs1128503, rs2032582 and rs1045642 respectively. The genotypes represented for *ABCB1* represent the forward orientation while the gene is transcribed in the reverse direction.

Table 3: Allele Frequencies for *UGT1A* and *ABCB1* SNPs (N=172)

UGT1A							
211G>A	**-997G>A**	**-1352A>C**	**855+20756A>G**	**856-23423C>T**	**−751T>C**	**$TA_{(n)}$**	**622T>C**
rs4148323	**rs2003569**	**rs3755319**	**rs2070959**	**rs28900377**	**rs1983023**	**rs8175347**	**rs11692021**
G (1.00)	A (0.36)	A (0.22)	A (0.87)	T (0.04)	T (0.25)	5 (0.07)	C (0.13)
A (0.00)	G (0.64)	C (0.78)	G (0.13)	C (0.96)	C (0.75)	6 (0.63)	T (0.87)
						7 (0.23)	
						8 (0.08)	
ABCB1*							
1000-44G>A	**1236C>T**	**2677G>T/A**	**3435C>T**	**1725+38G>A**	**1554+24T>C**		
rs10276036	**rs1128503**	**rs2032582**	**rs1045642**	**rs2235013**	**rs2235033**		
T (0.85)	A (0.08)	A (0.01)	A (0.09)	T (0.53)	A (0.47)		
C (0.15)	G (0.92)	C (0.99)	G (0.91)	C (0.47)	G (0.53)		

*The genotypes represented for *ABCB1* represent the forward orientation while the gene is transcribed in the reverse direction.

Table 4: Population parameter estimates of moxifloxacin pharmacokinetics

Parameter description	Typical Value (CI) [c]	Random Variability (CI) [c]
Intrinsic clearance during rifampicin-based TB treatment (L/h) [a,b]	50.0 (45.9; 56.8)	BSV[d]: 12.1% (2.6; 16.7) BOV[d]: 12.2% (4.6; 18.9)
Volume of Central compartment (L) [b]	127 (109; 137)	BSV: 8.6% (0.5; 13.5)
Inter-compartmental clearance (L/h) [b]	2.12 (1.55; 4.51)	-
Volume of Peripheral compartment (L) [b]	31.0 (22.3; 50.8)	-
Pre-hepatic bioavailability (.) [e]	1 FIXED	BOV: 35.6% (28.2; 42.1)
Absorption lag Time (h)[prior]	0.55 (0.45; 0.73)	-
Ka - Absorption rate (1/h) [prior]	2.80 (1.18; 3.45)	BOV: 93.2% (0.9; 121.7)
Hepatic plasma flow (L/h)	50 FIXED	
Moxifloxacin fraction unbound (%)	50% FIXED	
Change in Intrinsic Clearance while on single dose of Moxifloxacin (%)	-28.9% (-36.5; -21.9)	
Change in pre-hepatic bioavailability while on single dose of Moxifloxacin (%)	-22.4% (-32.4; -11.6)	
Change in Intrinsic Clearance while on Efavirenz-based ART (%)	+42.9% (32.6; 56.1)	
Change in Intrinsic Clearance due to rs8175347 (5/6 genotype)	-20.6% (-29.3; -13.6)	
Scaling factor for variability in bioavailability while on single dose of Moxifloxacin (-fold) [f]	0.62-fold (0.41; 0.85)	
Scaling factor for variability in bioavailability for unobserved doses (-fold) [f]	2.48-fold (1.73; 3.75)	
Proportional Error (%)	17.4% (12.2; 21.2)	
Additive error (mg/L)	0.011 (0.004; 0.017)	

[a] Intrinsic clearance of moxifloxacin when given at steady-state within rifampicin-based TB treatment and no efavirenz.

[b] All clearance and volume parameters have been allometrically scaled with fat-free mass, and the typical values reported here refer to the typical patient, with a FFM of 47 kg

[c] CI – Empirical 95% Confidence interval obtained with a nonparametric bootstrap (n=300)

[d] BSV- Between-subject variability; BOV- Between-occasion variability

[e] Pre-hepatic bioavailability is the fraction of the drug which is absorbed, crosses unchanged the gut wall, thus entering the portal vein and reaching the liver.

[f] These scaling factors modulate the size of the between-occasion variability in pre-hepatic bioavailability for the sections of data indicated (single dose and unobserved doses)

[prior] These parameters were estimated using a prior, as detailed in text of the original analysis[29].