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Tacrolimus Population Pharmacokinetics and Multiple CYP3A5 Genotypes in African American and White Renal Transplant Recipients

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Abstract

Tacrolimus exhibits inter-patient pharmacokinetic variability attributed to CYP3A5 isoenzymes and the efflux transporter, P-glycoprotein. Most African American renal transplant recipients require higher tacrolimus doses compared to whites to achieve similar troughs when race-adjusted recommendations are used. An established guideline provides tacrolimus genotype dosing recommendations based on *CYP3A5*^{*1}(*W/T*) and loss of protein function variants: *CYP3A5*^{*3} (rs776746), *CYP3A5*^{*6} (rs10264272), *CYP3A5*^{*7} (rs41303343) and may provide more comprehensive race-adjusted dosing recommendations. Our objective was to develop a tacrolimus population pharmacokinetic model evaluating demographic, clinical and genomic factors in stable African American and white renal transplant recipients. A secondary objective investigated race-based tacrolimus regimens and genotype-specific dosing. Sixty-seven recipients receiving oral tacrolimus and mycophenolic acid 6 months completed a 12-hour pharmacokinetic study. *CYP3A5*^{*3}, ^{*6}, ^{*7} and *ABCB1* 1236T>C, 2677T>GA, 3435T>C polymorphisms were characterized. Patients were classified as extensive, intermediate and poor metabolizers using a novel *CYP3A5*^{*3}^{*6}^{*7} metabolic composite. Modeling and simulation was performed with

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NONMEM 7.3. A two-compartment model with first-order elimination and absorption with lag time best described the data. The *CYP3A5**3*6*7 metabolic composite was significantly associated with tacrolimus clearance (P-value<0.05), which was faster in extensive (mean: 44.3 L·hr⁻¹) and intermediate (28.6 L·hr⁻¹) metabolizers than poor metabolizers (19.7 L·hr⁻¹). Simulations support *CYP3A5**3*6*7 genotype-based tacrolimus dosing to enhance general race-adjusted regimens, with dose increases of 1.5-fold and 2-fold, respectively, in intermediate and extensive metabolizers for comparable exposures to poor metabolizers. This model offers a novel approach to determine tacrolimus dosing adjustments that maintain comparable therapeutic exposure between African American and white recipients with different *CYP3A5* genotypes.

Keywords

Tacrolimus; Population pharmacokinetics; Race; *CYP3A5* Genotypes

INTRODUCTION

Tacrolimus is a calcineurin inhibitor immunosuppressant, which is widely prescribed with mycophenolic acid (MPA), corticosteroids, or alternate immunosuppressant agents (e.g., azathioprine) to prevent allograft rejection in solid organ transplant recipients.^{1, 2} Tacrolimus exhibits a narrow therapeutic index with troughs ranging from 4 to 15 ng/ml for renal transplant recipients based upon prescribing full or calcineurin inhibitor minimization protocols.³⁻⁸ Considerable inter- and intra-patient variability in tacrolimus pharmacokinetics exists that can be attributed to race, sex, clinical factors and drug-drug interactions that impact allograft survival.³⁻⁶ Compared to whites, most African American transplant recipients require larger tacrolimus doses to achieve therapeutic trough concentrations.⁹⁻¹¹ Therapeutic drug monitoring of trough concentrations is the standard of care to ensure patient treatment safety and allograft survival.⁸ However, there exists a poor correlation between tacrolimus dosage and troughs, requiring additional research into factors influencing patient drug exposure.^{3, 4, 6, 8, 12}

After oral administration, tacrolimus is rapidly absorbed and extensively bound to erythrocytes in the blood stream.¹³ Tacrolimus is highly metabolized in the liver and small intestine primarily by cytochrome P450 (CYP) 3A5 enzymes.^{6, 14, 15} It is also a substrate for the efflux transporter P-glycoprotein (P-gp).^{3, 6, 9} Fifteen metabolites of tacrolimus can be formed and excreted through the biliary route, with less than 1% of the parent drug remaining unchanged.¹⁶

Single nucleotide polymorphisms (SNPs) identified in the *CYP3A5* and *ABCB1* genes are associated with modulation of CYP3A5 enzyme and P-gp function respectively, which contribute to the inter-patient variability in tacrolimus pharmacokinetics.^{9, 17-19} Genetic variations in the *CYP3A5* gene have explained 40 to 50% of the variability in tacrolimus clearance.⁹ The variant *CYP3A5**3 (rs776746) has been commonly associated with reduction in the functional *CYP3A5* enzyme or loss of protein function and is detected primarily in whites.²⁰ In African Americans, *CYP3A5* variants including *CYP3A5**6 (rs10264272) and *CYP3A5**7 (rs41303343), are also associated with loss of protein

function, and may contribute to interracial variability in tacrolimus pharmacokinetics.^{20, 21} The Clinical Pharmacogenetics Implementation Consortium (CPIC) has assigned *CYP3A5* phenotypes based on *allele diplotypes.²² Patients are identified as extensive metabolizers (*1/*1), intermediate metabolizers (*1/*3, *1/*6, *1/*7), or poor metabolizers (*3/*3, *6/*6, *7/*7, *3/*6, *3/*7, *6/*7). Based on these phenotypes, CPIC has provided *CYP3A5* genotype based tacrolimus dosing guidelines to help achieve target trough concentrations for solid organ transplants in adults and children. For *CYP3A5* poor metabolizers, therapy should be initiated with the standard recommended dose, whereas for *CYP3A5**1 expressers which include intermediate and extensive metabolizers, a suggested regimen should be increased to 1.5-2 times the standard dose without exceeding 0.3 mg/kg/day.²²

The FDA initial dosing recommendations for oral tacrolimus are based on the type of organ transplant, race and time post-transplant, which is 0.1 mg/kg/day, administered as 0.05 mg/kg every 12 hours, for adult kidney transplant recipients in combination with mycophenolic acid.²³ However, higher tacrolimus dosing regimens are recommended for black patients compared to whites based on the time post-transplant. These recommendations are supported by several reports comparing clinical troughs and bioavailability between African American and white healthy subjects and renal transplant recipients.^{24, 25}

Over the last two decades, more than 50 population analyses have been conducted to investigate tacrolimus pharmacokinetics and identify significant covariates explaining inter-patient variability to better guide clinicians in defining an optimal dosing regimen.²⁶ Factors commonly reported to influence tacrolimus pharmacokinetics include: total body weight (TBW), hematocrit, time post-transplant, hepatic function, and *CYP3A5**3 polymorphisms.²⁶ In addition, approximately 50% of population-based pharmacokinetic analyses were performed using tacrolimus trough concentrations with limited inclusion of critical patient covariates.²⁶ To our knowledge, no population analysis has been conducted comparing whites and African Americans recipients.

The primary objectives of this study are to (i) develop a population pharmacokinetic model using intensively sampled tacrolimus profiles in stable African Americans and white renal transplant patients receiving tacrolimus minimization dosing with and MPA, and (ii) explore the effects of major demographic, clinical, and targeted genomic covariates on tacrolimus pharmacokinetic parameters. Using the final pharmacokinetic model, the secondary objective evaluates the CPIC and FDA tacrolimus dosing recommendations incorporating *CYP3A5**3*6*7 genotypes and race.

METHODS

Ethical approval and study design

The study protocol was approved by the University at Buffalo Health Sciences Institutional Review Board before enrollment (IRB# PHP0599703-4). The clinical trial was conducted in accordance with the Declaration of Helsinki, and is consistent with the Principles of the Declaration of Istanbul as outlined in the “*Declaration of Istanbul on Organ Trafficking and*

Transplant Tourism". Written informed consent was obtained from all individual patients included in the study.

Sixty-seven stable renal transplant recipients (35 African Americans and 32 whites) receiving maintenance oral tacrolimus (*Prograf*®) and MPA as enteric-coated mycophenolate sodium (ECMPS; *Myfortic*®) for 6 months were included in a cross-sectional, open-label, 12-hour pharmacokinetic study. The study was conducted at the University at Buffalo Research Center at the Erie County Medical Center from February 2010 until June 2011. Clinical stability was based on physical exam, comprehensive metabolic panel, fasting lipid panel and complete blood count at enrollment and on study morning. Tacrolimus doses were administered twice daily and adjusted to 4 to 9ng/mL trough concentrations using a minimization dosing protocol, time post-transplant and clinical response. ECMPS dose was adjusted based upon clinical response. Medication adherence was verified at enrollment and as well as one week and 48 hours prior to the study by research personnel with medication diaries provided by patients. Ethnicity for two previous generations was verified. The inclusion criteria were: 1) 6 months post-renal transplant; 2) age 25-70 years; 3) first or second time deceased-donor or living allograft recipient; 4) stabilized on same dose of immunosuppressive drugs for 7 days prior to study; 5) serum creatinine ≤ 3.25 mg/dL with no change in serum creatinine > 0.25 mg/dL during prior 2 visits; and 6) leukocyte count $\geq 3000/\text{mm}^3$ and hemoglobin ≥ 8.0 g/dL. Exclusion criteria were: 1) infection within 2 weeks; 2) acute rejection within 2 weeks; 3) concomitant drugs interfering with tacrolimus or MPA absorption; 4) concomitant cytochrome P450 3A4/3A5 or P-gp inhibitors or inducers within 4 weeks; and 5) significant cardiovascular, gastrointestinal, hematologic, psychiatric, neurological or oncological diseases that would limit participation.

Patients were studied at steady-state concentrations of tacrolimus and ECMPS, with the receipt on the same dose for 7 days prior to study. Patients took immunosuppressives between 5:30 to 6:30 PM followed by a 12-hour fast prior to study. Patients were admitted at 6:00 AM, vital signs were documented and an intravenous angiocatheter inserted. A 0 hour blood sample (~15 mL) was collected prior to immunosuppressive administration for drug troughs and fasting laboratory tests. Study medications were administered orally from a single lot of tacrolimus and ECMPS by 7:00 AM. Patients remained upright throughout the study. Standardized low fat meals were provided after 4 hours. Anti-hypertensive drugs were administered after 1.5 hours, with insulin, anti-lipidemic, and other medications were administered 4 hours after the immunosuppressives. Blood samples (~7 mL) were collected at times 0 (pre-dose), 1, 2, 3, 4, 6, 8, 10, and 12 hours post-dose. Whole blood samples were aliquoted immediately and stored at -70°C until analysis.

An additional 20 ml of blood was collected in *Cell Preparation Tubes (CPT®- BD Vacutainer)* pre-dose with separation of peripheral blood mononuclear cells (PBMCs), which were harvested; immediately frozen in liquid nitrogen and stored at -70°C until genotype analysis.

Bioanalytics

Blood tacrolimus concentrations were measured using the *ARCHITECT* chemiluminescent microparticle immunoassay system (Abbott, Abbott Park, IL). Assay validation was completed with a calibration standard curve ranging from 1 to 30 ng/mL with 0.5 ng/mL as the lower limit of detection. Three quality controls (QC) were prepared at 3.0, 12.0, and 25.0 ng/mL (Bio-Rad, Hercules, CA, USA) and were co-analyzed with all samples and standards. The inter-day coefficient of variation (CV) for each QC was < 4%, and intra-day CV was < 5%. Selected trough and peak concentrations (N=40 samples) were analyzed using a validated LC/MS/MS assay by an external analytical laboratory and compared to the results generated from the *ARCHITECT* tacrolimus assay with excellent agreement ($R^2=0.98$). For tacrolimus LC/MS/MS assay, the inter-day and intra-day CV were < 5% at the low and high concentration QC.

Genotyping assays

All patient samples were viable and analyzed in a genomics laboratory (University of New England Genomics, Analytics, and Proteomics Core). Laboratory personnel performing the genotyping analysis were blinded to patient treatment. Genomic DNA was isolated from PBMCs using the Wizard[®] *Genomic DNA Purification system* (Promega, Madison, WI) following manufacturer instructions. A total of 10 ng of genomic DNA was used in genotyping reactions for the following SNPs *ABCB1*: *1236C>T* (rs1128503), *2677G>T/A* (rs2032582), *3435C>T* (rs1045642) and *CYP3A5*3* (rs776746), *CYP3A5*6* (rs10264272), and *CYP3A5*7* (rs41303343) using TaqMan allelic discrimination assays (*Applied Biosystems*, Foster City, CA) with a *CFX96* Real-Time Polymerase Chain reaction Detection System (Bio-Rad). Each SNP was analyzed in duplicate experiments. Allele frequencies were confirmed in Hardy-Weinberg equilibrium when adjusted for race.

*CYP3A5*3*6*7* metabolic composite

CYP3A5 exhibits significant allelic heterogeneity. Three SNPs *CYP3A5*3*, *CYP3A5*6* and *CYP3A5*7* result in loss of protein gene expression. Loss of function by any of these SNPs can occur independent of allelic status at the other two loci. A *CYP3A5*3*6*7* metabolic composite based upon the combined allelic status for each loss of function SNP was used to characterize each patient as extensive (cEM_{CYP3A5}), intermediate (cIM_{CYP3A5}) or poor metabolizers (cPM_{CYP3A5}) as depicted in Figure 1. This composite followed the tacrolimus dosing guidelines provided by CPIC with the exception of the double heterozygous condition, which is not addressed by CPIC.²² Metabolic status using poor, intermediate and extensive metabolizer categories was also included for *CYP3A5*1* and **3* genotypes only and are summarized in Supplemental Data.

Pharmacokinetic modeling

Population pharmacokinetic modeling and simulation were performed using nonlinear mixed effects modeling with NONMEM 7.3.0 (ICON Development Solutions, Ellicott City, MD, USA). The first-order conditional estimation with interaction method was used to estimate the parameters. One- and two-compartment models with first-order absorption were compared, defined by apparent pharmacokinetic parameters in the absence of intravenous

data. The use of transit compartments and a lag-time for describing drug absorption were explored. Between-subject variability (BSV) was assumed to be log-normally distributed and tested by an exponential model on pharmacokinetics parameters. Additive, proportional, and mixed residual variability models were tested. Model selection was based upon the goodness-of-fit plots, precision of parameters estimates (relative standard error; RSE), and changes in the minimum objective function value (OFV). The R-based tool Xpose 4.5.3 and GraphPad Prism 6.04 (GraphPad Software Inc, La Jolla, CA) were used for graphical presentations and statistical analysis.

Covariate analysis

Covariate analysis was performed to explore the potential effects of patient characteristics on model parameters associated with BSV. All patient characteristics listed in Table 1 were investigated as potential covariates with the exception of study dose and patient number. No data was missing among patients. Individual SNPs for *ABCB1* and *CYP3A5*3* polymorphisms as well as the *CYP3A5*3*6*7* metabolic composite were examined. Significant covariates were selected using a classical stepwise approach.²⁷ The influence of continuous covariates on the pharmacokinetic parameters was modeled according to a power model scaled to the population median covariate value. The influence of categorical covariates was modeled using a fractional change due to the covariate value. Model refinements (addition of BSV and correlations between BSV) were tested before and after the covariate analysis.

Model evaluation

In addition to the standard goodness-of-fit plots, other methods were used to evaluate model performance. The precision of the final estimated pharmacokinetic parameters was evaluated using a nonparametric bootstrap resampling method.²⁸ 1000 replicates of the analysis dataset were generated by bootstrap and run with the final model to obtain the median and 95% percentile of all parameter estimates, which were compared to those of the final model. To evaluate the model predictive performance while accounting for different administered doses among patients and identified covariates, internal validation was performed using a prediction-corrected visual predictive check (PC-VPC).²⁹ 1000 replicates of the dataset were simulated conditional on the final pharmacokinetic parameters. The observed data and their 5th, 50th, and 95th percentiles were overlaid on the 90th confidence interval of the 5th, 50th, and 95th percentiles of the simulations to visually assess model performance. All data were corrected using the median of the population prediction within the associated time bin.

Dosing adjustment simulations based upon published guidelines

Using the final model, simulations (N = 1000) were conducted to explore the effect of significant covariates on tacrolimus exposure after oral administration of a standard dose of 0.075 mg/kg/day, given as 0.0375 mg/kg every 12 hours, which was selected as the most frequently administered standard tacrolimus dose based upon literature review.²⁶ Individual exposures were assessed by the area under the concentration curve between 0 and 12 hours at steady state ($AUC_{ss,0-12hr}$):

$$AUC_{ss,0-12hr} = \frac{Dose}{CL/F}$$

with Dose and CL/F as the individual study dose and model-estimated apparent clearance.

To evaluate the CPIC tacrolimus dosing guidelines, simulations (N=1000) were performed for cIM_{CYP3A5} and cEM_{CYP3A5} with new dose levels consisting of 1.5 and 2-fold change compared to cPM_{CYP3A5}. Tacrolimus exposure using simulated AUC_{ss,0-12hr} and 12-hour trough concentrations were compared across all *CYP3A5***3***6***7* metabolic composite and dose level groups. They were also compared to the suggested therapeutic ranges for tacrolimus AUC of 120 to 200 hr-ng/mL and trough concentrations of 4-12 ng/mL targeted for kidney transplant recipients greater than 6 months post-transplant using a tacrolimus minimization protocol. These ranges were selected based upon the tacrolimus minimization protocol used by the UB Transplant Center, literature review,^{4, 7, 30-32} This analysis was also completed for *CYP3A5***1* and **3* phenotypic groups.

Model simulations were also conducted based on the results reported in the FDA-approved monograph for African Americans and Caucasians for different time post-transplant.²³ Tacrolimus exposure (AUC_{ss,0-12hr}) and trough concentrations were generated using individual pharmacokinetic parameters estimated from the model after administration of 0.13 mg/kg/day for whites and 0.19 mg/kg/day for African Americans (i.e., 0.065 and 0.095 mg/kg every 12 hours). Both were compared to the targeted therapeutic ranges.⁽²⁰⁾ This analysis was also completed for *CYP3A5***1* and **3* phenotypic groups.

RESULTS

Data Summary and Patient Characteristics

A total of 594 tacrolimus steady-state concentrations from 67 patients were available for population pharmacokinetic analysis. The study cohort included 38 males and 29 females, 48% whites and 52% African Americans, with a median age of 46 years, and a median TBW of 85.9 kg. All concentrations were above the limit of quantification for the assay. Dose-normalized concentration-time profiles exhibited a large degree of variability within the *CYP3A5***3***6***7* metabolic composite groups (Figure 2). The distribution of the *CYP3A5***3***6***7* metabolic composite groups was highly correlated with race with 91% of whites identified as cPM_{CYP3A5} and 83% of African Americans identified as cIM_{CYP3A5} or cEM_{CYP3A5}. Patient characteristics were grouped according to *CYP3A5***3***6***7* metabolic composites and summarized in Table 1. The Supplemental Data (Section II) summarizes patient demographics and clinical characteristics in Table S2 stratified by *CYP3A5***1* and **3* genotype groups.

Population pharmacokinetic modeling

A two-compartment model with first-order absorption and elimination, and an absorption lag-time, best-described tacrolimus pharmacokinetics. BSV was included on the absorption rate constant (k_a), central clearance and volume of distribution (CL/F, V/F), and intercompartmental clearance (CL_p/F). The associated shrinkages were small (< 20%) and

supported an unbiased covariate inclusion. A proportional error model best described residual variability. Visual inspection of individual pharmacokinetic parameter estimates *versus* covariates showed potential trends for *CYP3A5**3*6*7 metabolic composite, race, African Americans females, and *ABCB1-2677* polymorphisms on CL/F, serum creatinine on CL_p/F, TBW and *ABCB1-3435* polymorphisms on V/F, and presence of diabetes on k_a. *CYP3A5**3*6*7 metabolic composite exhibited the strongest effect on CL/F (Figure 3). In the forward building step, inclusion of *CYP3A5**3*6*7 metabolic composite on CL/F, TBW on V/F, and diabetes on k_a produced a significant decrease in OFV (> 3.84, P-value < 0.05). After backward elimination, only *CYP3A5**3*6*7 metabolic composite and TBW were retained as significant covariates (P-value < 0.01), resulting in a total decrease in the OFV by 33.6 points (Supplemental Table S1). Individual CL/F_i and V/F_i values were predicted from the final model by the following equations:

$$CL/F_i = 19.7 \times 1.45^{H1} \times 2.25^{H2} \times e^{\eta_{CL,i}} \quad V/F_i = 234 \times \left(\frac{TBW}{85.9}\right)^1 \times e^{\eta_{V,i}}$$

in which H1 = 1 for cIM_{CYP3A5} and H2 = 1 for cEM_{CYP3A5} (0 otherwise), and η_i terms are the estimated BSV on CL/F and V/F.

Typical values of CL/F for cPM_{CYP3A5}, cIM_{CYP3A5} and cEM_{CYP3A5} were 19.7, 28.6, and 44.3 L.hr⁻¹. Inclusion of covariates on CL/F and V/F reduced the magnitude of BSV from 46.6 to 37% (CL/F) and from 83.2 to 76.7% (V/F). No correlation between random effects was significant. All final pharmacokinetic parameters were well estimated (RSE < 30%) and are summarized in Table 2. The supplemental data summarized in Tables S3 and S4 reflect the model using *CYP3A5**1 and *3 genotype groups only. Although there is a slight bias for the population-level fit (Fig. S1a), the goodness-of-fit plots (i.e., observed concentrations *versus* population and individual predictions, conditional weighted residuals *versus* population predictions and time) did not show significant misspecification or bias in the final model (Supplemental Figure S1). Results of the nonparametric bootstrap are listed in Table 2. Mean values for all parameters were within 5% of those obtained from the final model. The 95% confidence interval was relatively narrow and did not include the value zero suggesting reliable model-parameter estimates. The PC-VPCs for all patients and stratified by *CYP3A5**3*6*7 metabolic composite showed that the central tendency and variability of tacrolimus concentrations in the study population were well predicted by the final model (Figure 4).

Dosing adjustment simulations using published guidelines

After administration of a common clinical dose of 0.075 mg/kg/day of tacrolimus, administered as 0.0375 mg/kg every 12 hours, model simulations showed differences in steady-state concentration-time profiles across *CYP3A5**3*6*7 metabolic groups (Figure 5) suggesting a need for dosing adjustment. cPM_{CYP3A5} exhibited higher tacrolimus exposure (mean AUC_{ss,0-12hr} 170 hr.ng/mL) and trough concentrations (mean 10.9 ng/mL) than cIM_{CYP3A5} (AUC_{ss,0-12hr} 116 hr.ng/mL and trough 6.70 ng/mL) and EM_{CYP3A5} (AUC_{ss,0-12hr} 80.8 hr.ng/mL and trough 4.05 ng/mL).

Comparison with model-simulated tacrolimus exposures and troughs following 1.5- and 2-fold increase in doses for cIM_{CYP3A5} and cEM_{CYP3A5} are shown in Figures 6a and b. With the 0.075 mg/kg/day dosing regimen (0.0375 mg/kg every 12 hours), 47% of cPM_{CYP3A5} exhibited $AUC_{ss,0-12hr}$ within the therapeutic range, whereas the majority of cIM_{CYP3A5} and cEM_{CYP3A5} showed underexposure of 62% and 88%, respectively. In 40% of the cIM_{CYP3A5} recipients using a 1.5-fold increased dose, similar tacrolimus exposures to cPM_{CYP3A5} were predicted, but a higher risk of toxicity or overexposure was determined in 52% of this group with a 2-fold higher dose. In 44% of cEM_{CYP3A5} recipients, a 2-fold higher dose predicted similar exposure to cPM_{CYP3A5} .

Model-simulated exposures and trough concentrations for African Americans in this study receiving a tacrolimus dose of 0.19 mg/kg/day (0.095 mg/kg every 12 hours) as recommended in FDA monograph are shown in Figures 7a and b. Most of African Americans cEM_{CYP3A5} had tacrolimus exposures within the therapeutic range. 81% and 100% of African Americans classified as cIM_{CYP3A5} and cPM_{CYP3A5} exhibited overexposure. Simulations for whites receiving a tacrolimus dose of 0.13 mg/kg/day resulted in overexposure for most patients (data not shown). For all simulations, similar conclusions were observed considering troughs.

The Supplemental Data summarizes the dosing adjustment simulations performed for $CYP3A5^*1$ and $*3$ genotype groups in Figures. S6, S7, and S8.

DISCUSSION

This population-based pharmacokinetics model identifies a significant impact of multiple $CYP3A5$ genotypes on tacrolimus clearance using the novel $CYP3A5^*3^*6^*7$ metabolic composite. Notable differences were found in tacrolimus pharmacokinetics in patients identified as cPM_{CYP3A5} , cIM_{CYP3A5} and cEM_{CYP3A5} , and reinforces the need for well-defined dosing adjustments to achieve targeted troughs and $AUC_{ss,0-12hr}$. The pharmacokinetic analysis resulted in a two-compartment model with delayed absorption and first-order elimination to describe the full concentration-time profiles. This model is consistent to other pharmacokinetic models using intensive sampling.²⁶ Our final pharmacokinetic parameters and associated BSV are in agreement with previous estimates for tacrolimus.²⁶ Most tacrolimus population pharmacokinetic models using only trough concentrations resulted in one-compartment models and results in predictive limitations.³³⁻³⁶ The effect of TBW was included on V/F with an allometric coefficient of 1 as previously reported.^{35, 37-39} Time post-transplant and hematocrit had no impact in our tacrolimus pharmacokinetic model in spite of significance found in previous models.²⁶ This finding is likely due to the narrow inclusion criteria range for hemoglobin and hematocrit and the clinical stability of these patients.²⁶

The distribution of $CYP3A5$ genotypes differs by race, which may explain differences in tacrolimus pharmacokinetics between whites and African Americans who require higher tacrolimus doses to achieve comparable concentrations.^{9, 10, 40} In our covariate analysis, the impact of race on tacrolimus clearance was significant until the inclusion of the $CYP3A5^*3^*6^*7$ metabolic composite and a high correlation was found between these two

covariates. White patients were primarily poor metabolizers (cPM_{CYP3A5}) with none categorized as cEM_{CYP3A5} . African Americans were mainly intermediate (cIM_{CYP3A5}) and extensive (cEM_{CYP3A5}) metabolizers. These findings are consistent with the incidence for the loss of function $CYP3A5^*3$ variant of greater than 90% that is reported in whites and an approximate frequency of 30% found in African Americans.⁴¹ However, it is important to consider the other variant alleles $CYP3A5^*6$ and $CYP3A5^*7$ that also result in a loss of function of the respective enzymes. The $CYP3A5^*6$ and $CYP3A5^*7$ polymorphisms are rare in whites, but exhibit higher frequencies in African Americans of approximately 10%. Therefore, these variants contribute to the increased probability of non-functional enzymes resulting in poor metabolic phenotypes in African Americans and have important clinical implications.⁴¹ The different racial distributions of the $CYP3A5$ variants support the use of the $CYP3A5^*3^*6^*7$ metabolic composite for more comprehensive investigation of tacrolimus dosing adjustments.

The $CYP3A5^*3^*6^*7$ metabolic composite was the most significant covariate explaining tacrolimus pharmacokinetic variability in our model. Significant differences in CL/F were found relative to the three phenotypes with an approximate 1.5-fold and 2-fold increase for cIM_{CYP3A5} and cEM_{CYP3A5} compared to cPM_{CYP3A5} . The impact of the loss of function variants $CYP3A5^*3$, *6 and *7 on tacrolimus pharmacokinetics has been reported in two studies investigating only African American kidney transplant recipients using only trough concentrations.^{10, 21} Numerous tacrolimus population-based pharmacokinetic analyses were performed to explore the impact of the $CYP3A5^*3$ genotypic variant alone, without consideration of $CYP3A5^*6$ and *7 genotypes with limited inter-racial comparisons.²⁶ Therefore, to assess the superiority of the $CYP3A5^*3^*6^*7$ metabolic composite compared to $CYP3A5^*3$ variant only, as source of tacrolimus pharmacokinetic inter-individual variability, we performed the same pharmacokinetic analysis considering $CYP3A5^*1$ and *3 genotypes (See supplemental Data, section II). Significant differences on tacrolimus clearance were found between the three $CYP3A5^*3$ genotypic groups: poor metabolizers ($^*3/^*3$), intermediate metabolizers ($^*1/^*3$) and extensive metabolizers ($^*1/^*1$) (Supplemental Figure S3). Tacrolimus clearance estimates for the $CYP3A5^*3$ variant status were similar to those of the $CYP3A5^*3^*6^*7$ metabolic composite (Supplemental Table S4). However, the inclusion of the metabolic composite explained more inter-individual variability for tacrolimus clearance. In addition, using the metabolic composite provided a comprehensive identification of patients with the loss of function variants since the presence of $CYP3A5^*6$ and *7 are often missed in African Americans who may be categorized as intermediate or extensive metabolizers when the $CYP3A5^*3$ variant is only analyzed.⁴² Unlike our analysis, the majority of the studies exploring the $CYP3A5^*3$ variant, show differences in tacrolimus clearance between $CYP3A5$ non-expressers ($^*3/^*3$) or poor metabolizers in comparison to $CYP3A5^*1$ expressers, which combine both intermediate ($^*1/^*3$) and extensive ($^*1/^*1$) metabolizers. These findings may be explained by the limited incidence of $^*1/^*1$ variant in other study populations that primarily enrolled white patients.^{37, 43-46} In these analyses, $CYP3A5^*1$ expressers exhibited a 1.2- to 2.3-fold increase in tacrolimus clearance compared to $CYP3A5$ non-expressers.²⁶ Significant differences between the three $CYP3A5^*3$ genotype groups were found in two studies involving Korean adult³⁵ and Mexican pediatric renal transplant patients.⁴⁷ When combining these results with the African American

recipients¹⁰, these analyses reported a 1.5 to 2 fold faster tacrolimus clearance in intermediate and extensive metabolizers using tacrolimus trough concentrations only.^{10, 35, 47} Our results had a similar trend from the model developed using intensive sampling to generate a more accurate tacrolimus clearance.

Simulations were conducted with the final pharmacokinetic model to explore the impact of the *CYP3A5**3*6*7 metabolic composite on tacrolimus steady-state exposure and trough concentrations (Figures 5 and 6). A standard dose of 0.075 mg/kg/day (0.0375 mg/kg every 12 hours) was selected for our simulations based on the studies reviewed by Brook et al.²⁶ For comparison, the mean tacrolimus dose used in our study was 0.082 mg/kg. The primary goal of these simulations was to evaluate the dose adjustments required to achieve similar exposures between cPM_{CYP3A5}, cIM_{CYP3A5} and cEM_{CYP3A5}. Using the final model, simulations revealed that using an initial dose of 0.075 mg/kg/day (0.0375 mg/kg every 12 hours), about 50% of PM_{CYP3A5} would achieve steady-state exposures and troughs within the targeted therapeutic range (Figure 6).³⁰ However, based on this dosing regimen, 62% of cIM_{CYP3A5} and 88% of cEM_{CYP3A5} were at a risk for sub-therapeutic exposures which may increase the risk of allograft rejection. The CPIC guidelines recommend a 1.5- to 2-fold increase in the standard tacrolimus dose for intermediate and extensive metabolizers to achieve target troughs. To further individualize the CPIC guidelines, our simulations suggest that well-defined dosing increases should be considered to include 1.5-fold for cIM_{CYP3A5} and 2-fold for cEM_{CYP3A5} using the standard dose (Figure 6). For instance, a 2-fold increased dose for cIM_{CYP3A5} could result in a high proportion of patients exceeding the therapeutic range with an increased risk for toxicities or over immunosuppression as depicted in Figure 6. Similar findings were obtained when performing the same simulations with the tacrolimus pharmacokinetic model based on the *CYP3A5**1 and *3 variant (see supplemental Figures S6 and S7).

The updated FDA prescribing information suggests race adjusted tacrolimus dosing for black kidney recipients based on time post-transplant.²³ Using our final pharmacokinetic model, simulations in the African Americans patients demonstrated that the FDA recommended tacrolimus dose regimen of 0.19 mg/kg twice daily at 12 months post-transplant could result in overexposure for all cPM_{CYP3A5} and 81% of cIM_{CYP3A5}. See Figure 7. Similar results were also found with the same simulations performed with the pharmacokinetics model based on *CYP3A5**3 variant alone (Supplemental Figure S8). Therefore, consideration of the pertinent *CYP3A5* variants concurrent to race may optimize dosing strategy and subsequent regimen adjustments in the African American population. Other genotype factors may explain tacrolimus inter-patient variability, such as *CYP3A4* variants and P-gp, which modulate tacrolimus metabolism and cellular distribution.⁴⁸ The genotype, *CYP3A4**22 (rs35599367) associated with reduced enzymatic activity has been combined with the *CYP3A5**3 variant to define poor, intermediate and extensive metabolizers to explain tacrolimus pharmacokinetic variability.^{49, 50} These studies reported that well-defined doses for each metabolic group was required to achieve comparable tacrolimus exposure. In our model, P-gp may not have been adequately represented by using individual *ABCB1* polymorphisms in the covariate analysis. Use of *ABCB1* haplotypes may provide a more comprehensive investigation of this efflux transporter and the associated inter-patient pharmacokinetic variability of tacrolimus.⁵¹

Although this study included a small number of patients with no validation group included, the model developed has several advantages. This study was designed using intensive serial sampling strategy in clinically stable patients which provides more accurate estimates of tacrolimus pharmacokinetic parameters. All patients were enrolled based upon well-defined inclusion and exclusion criteria. In addition, medication adherence and steady state dosing conditions were carefully confirmed prior to study periods. All patients provided documentation of race for two previous generations.

CONCLUSION

The population pharmacokinetics model developed for tacrolimus identified the significant contribution of the *CYP3A5*3*6*7* metabolic composite to predict inter-patient variability between white and African American renal transplant recipients. Differences between these groups can be explained by the racial differences in the distribution of *CYP3A5*3*6*7* polymorphisms and metabolic enzyme activity. Well-defined dosing adjustments between the three metabolic phenotype groups comparing early to late post-transplant periods should be further investigated since extensive and intermediate metabolizers require higher doses than poor metabolizers. This model predicted tacrolimus exposures when applied to race specific FDA dosing guidelines and CPIC recommendations. This model predicted troughs and $AUC_{ss,0-12hr}$ using a genotype-based dosing approach between African American and white transplant recipients to incorporate precision medicine into immunosuppression. Development of a Bayesian estimator based on this population pharmacokinetics model with targeted *CYP3A5* genotypes could provide an efficient and feasible predictive tool for clinicians to facilitate personalized dosing of tacrolimus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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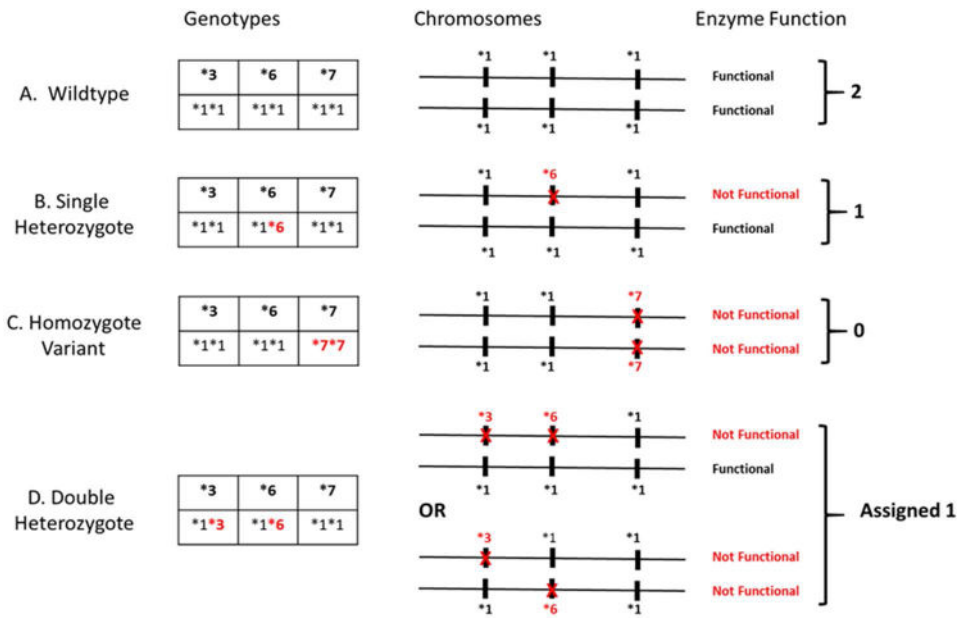


Figure 1. *CYP3A5*^{*3}^{*6}^{*7} Metabolic Composite scoring algorithm. Any of the three SNPs, *CYP3A5*^{*3}, *CYP3A5*^{*6}, and *CYP3A5*^{*7} independently result in loss of protein gene expression from the carrying chromosome. Metabolic composite status for each patient based upon the combined allelic status from each chromosome is summarized in fig. A–D above. **A** depicts an Extensive Metabolizer ($C_{EM_{CYP3A5}}$) with two completely functional genes. **C** depicts one possible example of a Poor Metabolizer ($C_{PM_{CYP3A5}}$): individuals who carry a loss of function allele on both chromosomes. Similarly **B** and **D** represents examples of possible Intermediate Metabolizers ($C_{IM_{CYP3A5}}$): individuals who are heterozygous at one or more loci. Note for the double heterozygote case (**D**) the genotyping assay used could not differentiate between cis and trans SNPs; therefore, the trans condition was conservatively assigned the intermediate metabolizer though such individuals would have no functional enzyme.

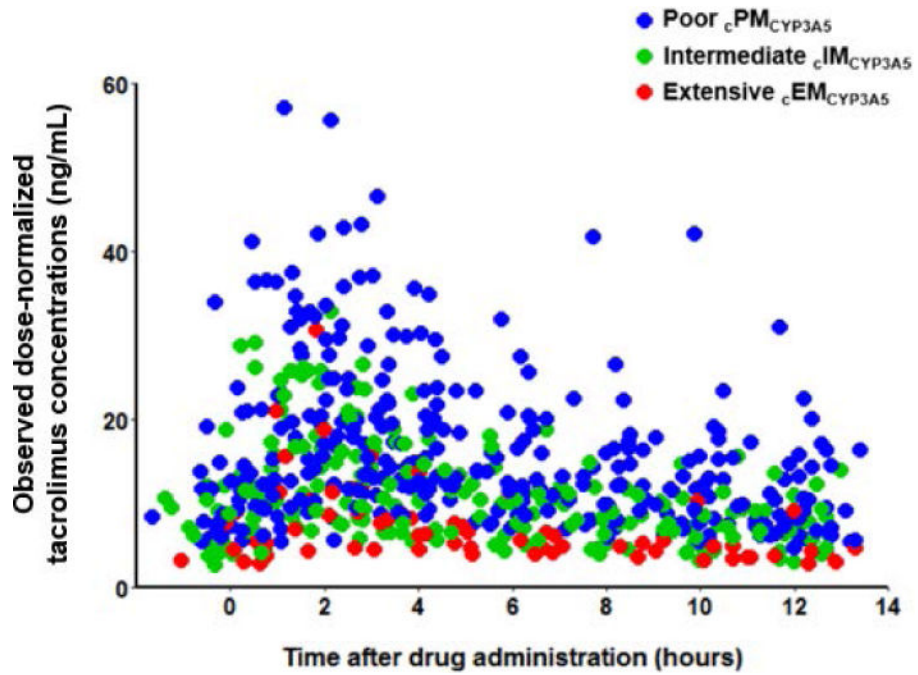


Figure 2. Observed tacrolimus dose-normalized concentrations (ng/mL) compared to time after drug administration stratified by *CYP3A5*^{*3*6*7} metabolic composite. For each patient, tacrolimus concentrations were divided by the recipient's dose and multiplied by the mean study dose for the population which was 3.4 mg. The red, green, and blue dots represent individual tacrolimus concentrations for *CYP3A5* extensive (cEM_{CYP3A5}), intermediate (cIM_{CYP3A5}) and poor metabolizers (cPM_{CYP3A5}).

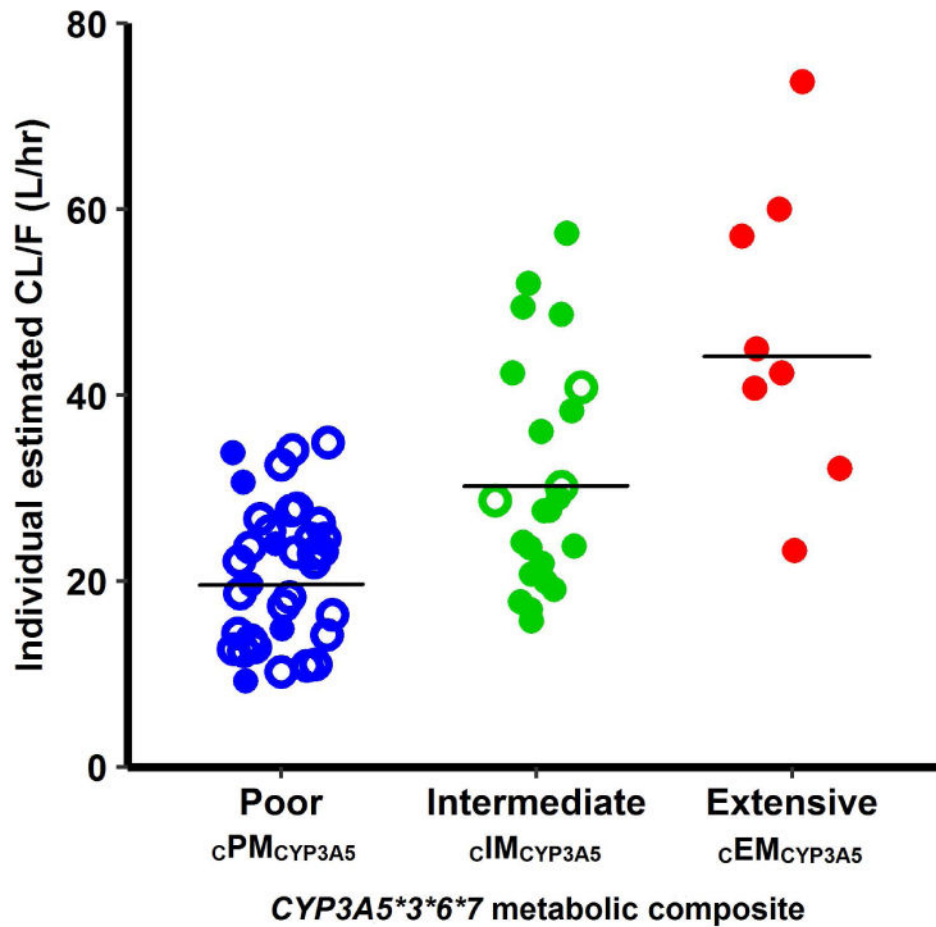


Figure 3. Individual base model estimated tacrolimus apparent clearance values (CL/F, symbols) and mean values (black lines) stratified by *CYP3A5*3*6*7* metabolic composite. Poor metabolizers (cPM_{CYP3A5}) exhibit lower CL/F (mean 19.8 L.hr⁻¹), compared to intermediate cIM_{CYP3A5} (mean 29.5 L.hr⁻¹) and extensive cEM_{CYP3A5} metabolizers (mean 45.0 L.hr⁻¹). Open-circles represent white patients and closed-circles represent African-American patients.

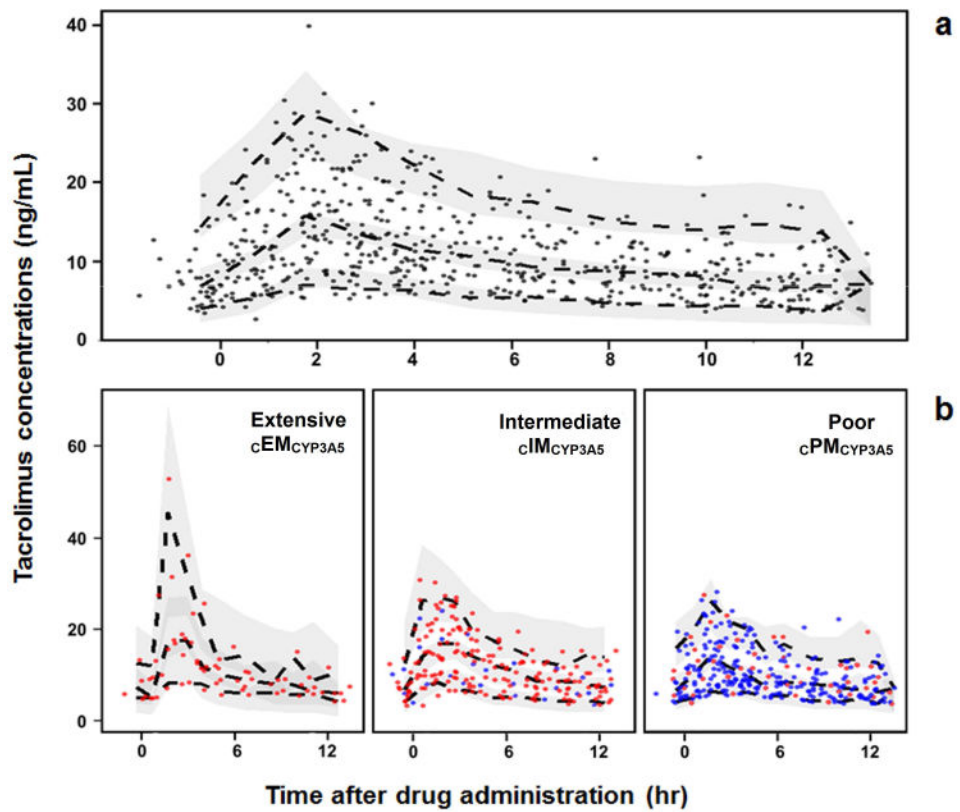


Figure 4. Prediction-corrected visual predictive check (PC-VPC) for the final tacrolimus pharmacokinetic model. PC-VPC for all patients **a**), and PC-VPC stratified by *CYP3A5**3*6*7 metabolic composite **b**). Solid circles represent the observed data. The dashed lines represent the 5th, 50th, and 95th percentiles of the observed data, and the shaded areas represent the 90–percent confidence intervals around the 5th, 50th, and 95th percentiles of the predicted data. Symbol colors in panel b represent white (blue) and African-American (red) patients.

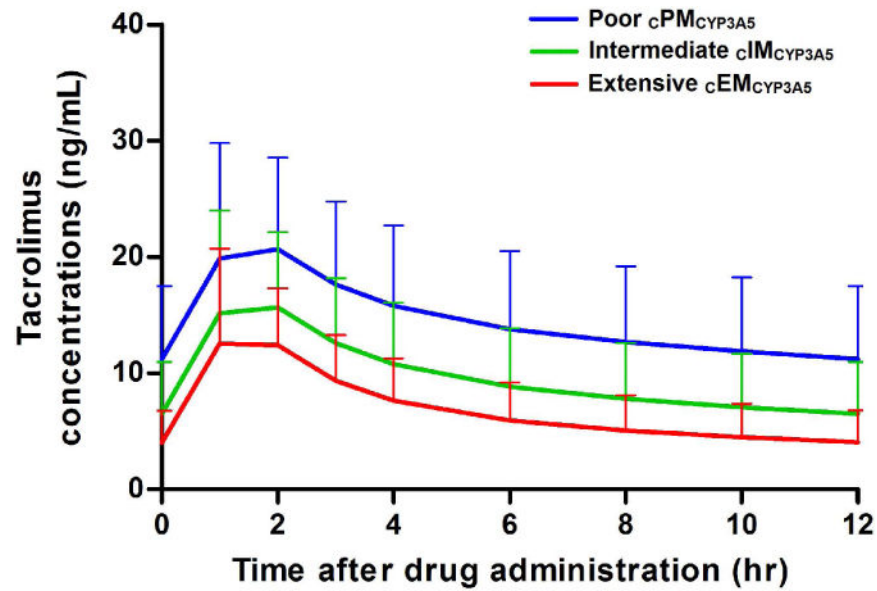


Figure 5. Simulations ($n = 1000$) of oral tacrolimus concentration-time profiles at steady-state after administration of 0.075 mg/kg/day (0.0375 mg/kg every 12 hours), represented by the mean and standard error bars according to $CYP3A5^{*3*6*7}$ metabolic composite. Poor metabolizers ($c_{PM_{CYP3A5}}$) exhibit higher exposures (mean $AUC_{ss,0-12hr}$ $170 \text{ hr}\cdot\text{ng/mL}$) and 12 hr trough concentrations (mean 10.9 ng/mL) compared to intermediate ($c_{IM_{CYP3A5}}$) ($AUC_{ss,0-12hr}$ $116 \text{ hr}\cdot\text{ng/mL}$ and trough 6.70 ng/mL) and extensive ($c_{EM_{CYP3A5}}$) metabolizers ($AUC_{ss,0-12hr}$ $80.8 \text{ hr}\cdot\text{ng/mL}$ and trough 4.05 ng/mL).

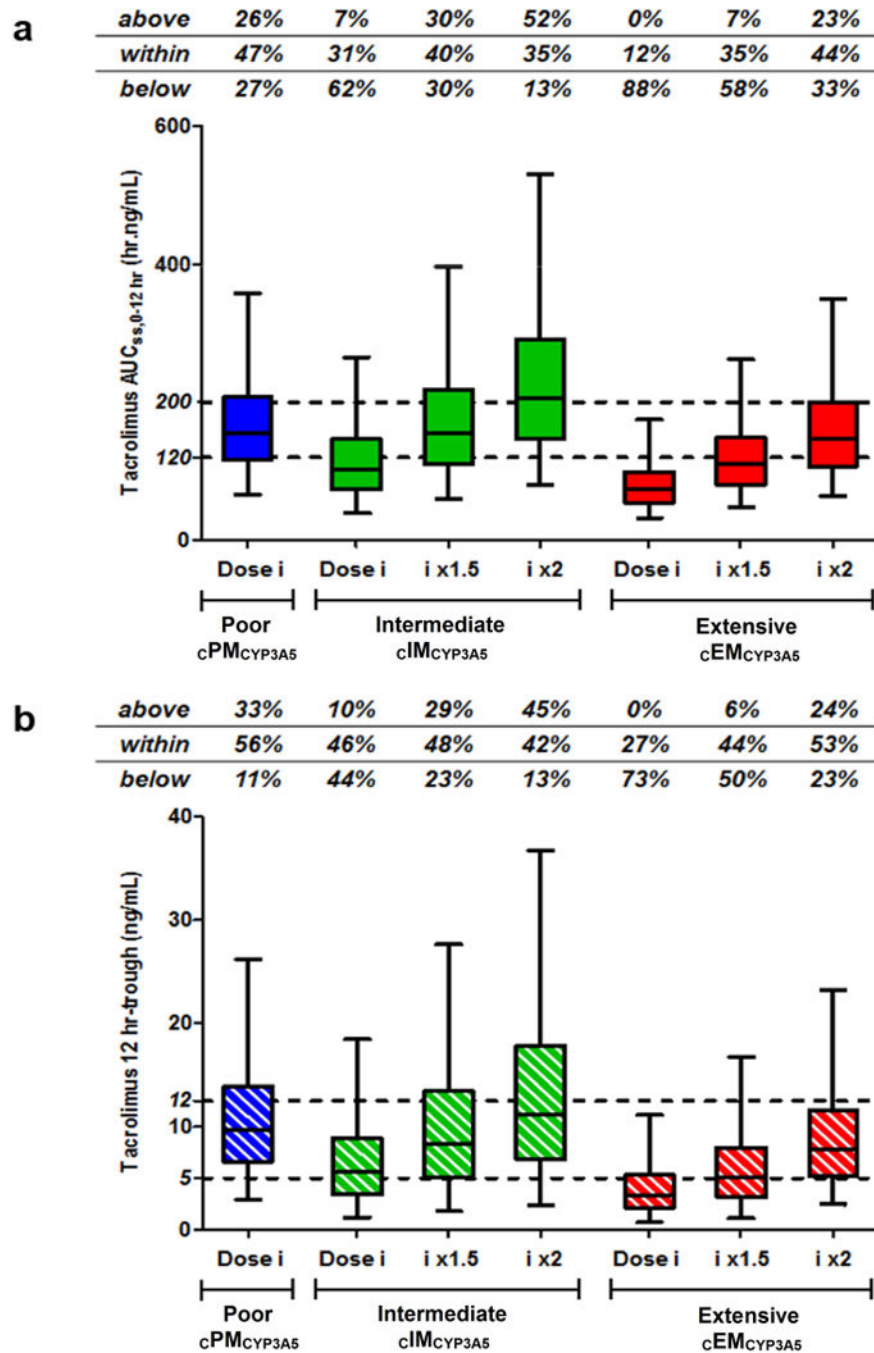


Figure 6. Boxplots of model-simulated tacrolimus $AUC_{ss,0-12hr}$ **a)** and 12-hour trough concentrations **b)** stratified by $CYP3A5^*3^*6^*7$ metabolic composite, obtained with different dosing regimens (i.e., Dose $i = 0.075$ mg/kg/day given as 0.0375 mg/kg every 12 hours, dose $i \times 1.5$, and $\times 2$).²⁰ The dashed lines represent the targeted therapeutic ranges: between 120 and 200 hr.ng/mL for $AUC_{ss,0-12hr}$, and between 5 and 12 ng/mL for 12-hour trough concentrations.²⁸ Percentages of patients exhibited $AUC_{ss,0-12hr}$ and trough concentrations above, within and below the therapeutic range are indicated in each graph.

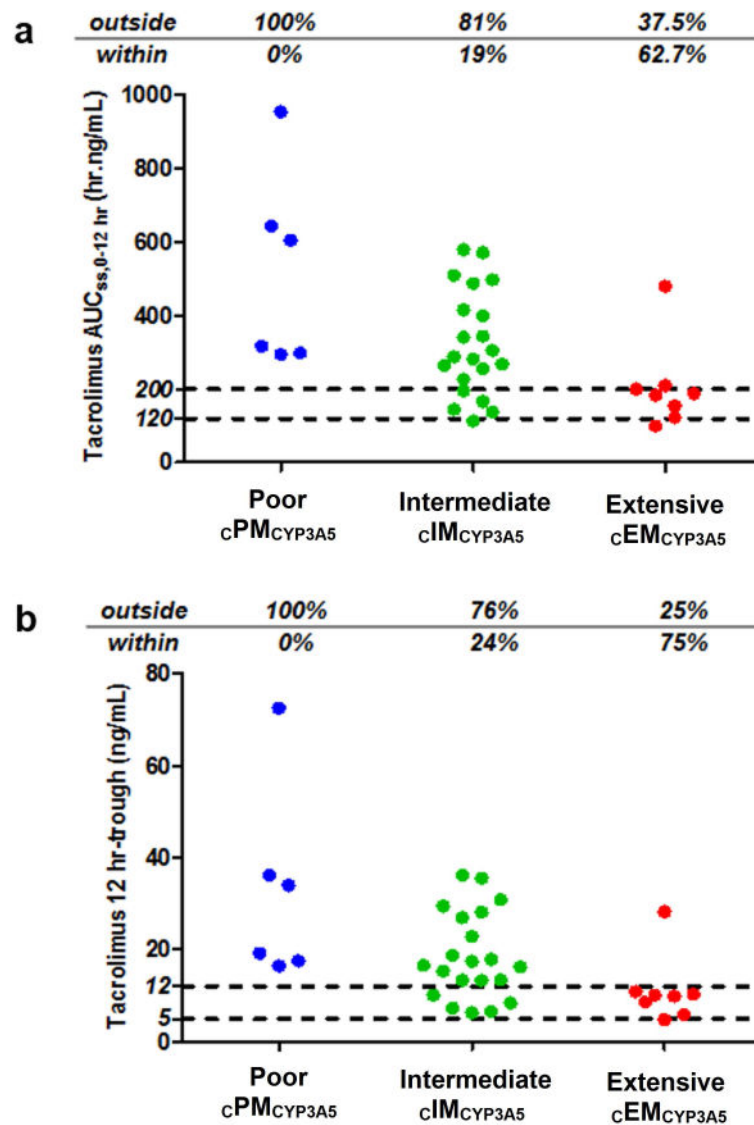


Figure 7. Individual model-simulated tacrolimus AUC_{ss,0-12hr} **a**) and 12-hour trough concentrations **b**) stratified by *CYP3A5**3*6*7 metabolic composite for the study population African American patients receiving a dosing regimen of 0.19 mg/kg/day (0.095 mg/kg every 12 hours).²¹ The dashed lines represent the targeted therapeutic ranges: between 120 and 200 hr.ng/mL for AUC_{ss,0-12hr}, and between 5 and 12 ng/mL for 12-hour trough concentrations.²⁸ Percentages of patients exhibited AUC_{ss,0-12hr} and trough concentrations above, within and below the therapeutic range are indicated in each graph.

Table 1

Patient demographics stratified by *CYP3A5***3***6***7* metabolic composite.

Parameters [#]	<i>CYP3A5</i> * <i>3</i> * <i>6</i> * <i>7</i> metabolic composite			P-values [†]
	Extensive	Intermediate	Poor	
No. of Patients, n (%)	8.0 (12)	24 (36)	35 (52)	–
Study dose (mg)	5.7 (1.5)	3.9 (1.7)	2.5 (1.1)	(Ext vs Poor) *** (Int vs Poor) **
Race	African-American, n (%)	21 (88)	6.0 (17)	***
	White, n (%)	0.0 (0.0)	3.0 (12)	
Sex	Male, n (%)	4.0 (50)	15 (63)	ns
	Female, n (%)	4.0 (50)	9.0 (37)	
Age (years)		46 (8.3)	49 (11)	ns
Total body weight (kg)		89 (22)	90 (21)	ns
Body mass index (kg/m ²)		31 (6.2)	30 (6.8)	ns
Albumin (g/dL)		4.1 (0.3)	4.1 (0.2)	ns
Glucose (mg/dL)		92 (19)	122 (77)	ns
Triglycerides (mg/dL)		134 (39)	125 (67)	ns
Total Cholesterol (mg/dL)		158 (24)	150 (26)	ns
HDL (mg/dL)		46 (14)	52 (15)	ns
LDL (mg/dL)		86 (21)	74 (22)	ns
eGFR (mL/min/1.73m ²)		46 (14)	54 (17)	ns
Serum creatinine (mg/dL)		1.9 (0.6)	1.6 (0.4)	(Ext vs Poor) ** (Int vs Poor) **
Hemoglobin (g/dL)		12 (1.2)	12 (1.4)	ns
Hematocrit (%)		37 (3.8)	38 (4.7)	ns
Platelet (cells/mm ³)		231 (67)	195 (59)	ns
Leukocytes (cells/mm ³)		5.7 (1.9)	4.9 (1.6)	ns
<i>ABCB1</i> 1236T>C	CC, n (%)	4.0 (50)	11 (46)	ns
	CT, n (%)	4.0 (50)	7.0 (29)	
	TT, n (%)	0.0 (0.0)	6.0 (25)	
<i>ABCB1</i> 2677T>G,A	GG, n (%)	7.0 (88)	17 (71)	*

Parameters [#]	CYP3A5*3/*6/7 metabolic composite			P-values [†]
	Extensive	Intermediate	Poor	
GT, n (%)	1.0 (12)	5.0 (21)	15 (43)	
TT, n (%)	0.0 (0.0)	2.0 (8.2)	7.0 (20)	
<i>ABCB1</i> 3435T>C, n (%)	4.0 (50)	13 (54)	13 (37)	ns
CT, n (%)	4.0 (50)	9.0 (38)	15 (43)	
TT, n (%)	0.0 (0.0)	2.0 (8.0)	7.0 (20)	
Time post-transplant (years)	2.3 (1.8)	3.4 (1.9)	2.9 (3.0)	ns
Diabete presence, n (%)	2.0 (25)	11 (46)	12 (34)	ns
ACEI use, n (%)	1.0 (13)	10 (42)	9.0 (26)	ns
ARB use, n (%)	1.0 (13)	3.0 (13)	4.0 (11)	ns
BB use, n (%)	7.0 (88)	21 (88)	22 (63)	ns
CCB use, n (%)	6.0 (75)	18 (79)	20 (49)	*
Prednisone use, n (%)	2.0 (25)	7.0 (29)	5.0 (14)	ns
Statin use, n (%)	1.0 (13)	10 (42)	12 (34)	ns

[#]Data represented as mean (standard deviation) or frequency (percentage)

HDL High-density lipoprotein, *LDL* Low-density lipoprotein, *eGFR* Glomerular filtration rate ⁴⁷, *ACEI* Angiotensin converting enzyme inhibitor, *ARB* Angiotensin receptor blocker, *BB* Beta-blocker, *CCB* Calcium-channel blocker

[†]P-value is based on χ^2 test for categorical variables, and on Kruskal-Wallis test (Dunn's multiple comparison test) for continuous variables. ns: non-significant.

* p-value < 0.05,

** p-value < 0.01,

*** p-value < 0.001.

Table 2
Estimated population pharmacokinetic parameters of tacrolimus and bootstrap validation

Parameter - Symbol	Population modeling			Bootstrap (n=1000)		
	Estimates	RSE (%)	Median	95% CI	Bias ^a (%)	
$CL/F (L \cdot hr^{-1}) - \theta_{CL/F}$	19.7	6.30	19.6	17.5 – 22.1	-0.10	
$CYP3A5^{*3*6*7}$ on CL/F						
$CL_{M_{CYP3A5}} - \theta_2$	1.45	9.90	1.46	1.20 – 1.78	0.01	
$CL_{EM_{CYP3A5}} - \theta_3$	2.25	13.6	2.28	1.64 – 3.01	0.03	
$V/F (L) - \theta_{V/F}$	234	10.2	231	190 – 276	-3.00	
$CL_p/F (L \cdot hr^{-1}) - \theta_{CL_p/F}$	52.6	9.50	53.2	44.1 – 63.6	0.60	
$V_p/F (L) - \theta_{V_p/F}$	403	20.1	404	275 – 572	1.00	
$k_a (hr^{-1}) - \theta_{k_a}$	4.21	20.3	4.14	2.77 – 5.74	-0.07	
Lag time (hr) – θ_{lag}	0.828	3.80	0.830	0.740 – 0.873	0.002	
Between-subject variability						
BSV CL/F (CV%) – η_1	37.0	6.20	36.1	30.9 – 40.7	-0.9	
BSV V/F (CV%) – η_2	76.7	12.1	76.0	56.0 – 93.5	-0.7	
BSV CL_p/F (CV%) – η_3	48.6	16.5	47.8	29.4 – 64.9	-0.8	
BSV k_a (CV%) – η_4	69.4	16.5	68.6	48.3 – 93.9	-0.8	
Residual variability						
Proportional error – ϵ_1	9.00	5.60	8.90	8.04 – 9.83	-0.1	

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RSE (%) Percent relative standard error of the estimate; $95\% CI_{95th}$ Confidence interval: 2.5 and 97.5th percentiles, BSV Between-subject variability expressed as percent coefficient of variation (CV%); CL/F =Apparent elimination clearance; CL_p/F Apparent distribution clearance; V/F Apparent central volume of distribution; V_p/F Apparent peripheral volume of distribution; k_d Absorption rate.

^aThe bias of each parameter was calculated by computing the difference between the median value derived from the bootstrap and the final parameter estimate.

99% of the bootstraps runs minimized successfully (990/1000).

Final pharmacokinetic model (typical individual values):

$$CL/F_i = \theta_{CL/F} \times \theta_2^{H1} \times \theta_3^{H2} \text{ and } V/F_i = \theta_{V1/F} \times \left(\frac{TBW}{85.9} \right)$$

where H1 = 1 for $CYP3A5$ intermediate metabolizers (cIM $CYP3A5$) and H2 = 1 for $CYP3A5$ extensive metabolizers (eM $CYP3A5$). TBW Total body weight (kg)