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Effect of *CYP3A4*, *CYP3A5*, and *ABCB1* Polymorphisms on Intravenous Tacrolimus Exposure and Adverse Events in Adult Allogeneic Stem Cell Transplant Patients

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Pharmacogenetics influences oral tacrolimus exposure; however, little data exist regarding i.v. tacrolimus. We investigated the impact of genetic polymorphisms in *CYP3A4, CYP3A5,* and *ABCB1* on i.v. tacrolimus exposure and toxicity in adult patients receiving an allogeneic hematopoietic stem cell transplant for hematologic malignancies. Germline DNA was extracted from buccal swabs and genotyped for *CYP3A4, CYP3A5,* and *ABCB1* polymorphisms. Continuous i.v. infusion of tacrolimus .03 mg/kg/day was initiated on day +5 post-transplant, and steady-state blood concentrations were measured 4 days later. We evaluated the association between phenotypes and prevalence of nontherapeutic target concentrations (below or above 5 to 15 ng/mL) as well as tacrolimus-related toxicities. Of 63 patients, 28.6% achieved the target concentration; 71.4% were >15 ng/mL, which was more common in CYP3A4 intermediate/normal metabolizers (compared with rapid) and those with at least 1 *ABCB1* C2677T loss-of-function allele ($P < .05$). *ABCB1* C2677T was significantly associated with concentrations >15 ng/mL (odds ratio, 6.2; 95% confidence interval, 1.8 to 23.6; $P = .004$) and tacrolimus-related toxicities (odds ratio, 7.5; 95% confidence interval, 1.6 to 55.2; $P = .02$). *ABCB1 C2677T* and *CYP3A4* are important determinants of i.v. tacrolimus exposure, whereas *ABCB1* C2677T also impacts tacrolimus-related toxicities in stem cell transplants.

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INTRODUCTION

Tacrolimus is a calcineurin inhibitor that constitutes the backbone of standard immunosuppression therapy for the prevention of graft-versus-host disease (GVHD) in patients receiving allogeneic hematopoietic stem cell transplants (SCT) [1,2]. Nonetheless, treatment with tacrolimus poses great challenges to clinicians because of its wide interpatient pharmacokinetic variability observed with the recommended initial i.v. dose of .03 mg/kg/day [3,4]. Current evidence suggests that tacrolimus blood concentrations less than 5 ng/mL portend a high risk for GVHD, whereas blood concentrations above 15 ng/mL are associated with tacrolimus-related toxicities such as nephrotoxicity and neurotoxicity [4-7]. Given the balance between drug efficacy and toxicity, therapeutic drug monitoring is routinely performed in clinical practice, particularly during the early period after SCT to maintain steady-state concentrations within the recommended therapeutic window of 5 to 15 ng/mL [8].

There has been great interest in identifying the genetic underpinnings of the considerable variability in tacrolimus exposure to provide a personalized rather than empiric dosing approach, with the goal to optimize drug exposure and clinical response. Findings from previous pharmacogenetic studies revealed that single nucleotide polymorphisms (SNPs) in the pharmacokinetic gene, *CYP3A5* (primarily the *3 allele, which is the most prevalent variant allele across most racial groups, especially whites), was the single most important determinant

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of the observed tacrolimus variability after oral administration [9–12]. Because the *CYP3A5*3* variant allele is associated with reduced expression of the enzyme responsible for metabolizing tacrolimus, patients homozygous for this polymorphism (ie, harbor 2 copies of *CYP3A5*3*, referred to as *CYP3A5* nonexpressers) require lower oral doses of the drug to achieve therapeutic concentrations compared with those carrying 1 copy of *CYP3A5*3* or homozygous for the wild-type allele (*CYP3A5*1/*1*) [13].

These findings prompted the Clinical Pharmacogenetics Implementation Consortium to develop guidelines to help guide selection of the optimal tacrolimus dose based on *CYP3A5* genotype [13]. Notably, these dosing recommendations emanated mainly from studies conducted in the solid organ transplant setting where the patient population, target therapeutic steady-state concentration, and route of administration (p.o. versus i.v.) are different, thereby limiting the generalizability and widespread adoption of these guidelines in the SCT setting where i.v. tacrolimus is often used.

In addition to *CYP3A5* polymorphisms, *CYP3A4* (also proposed to metabolize tacrolimus) and *ABCB1* (involved in drug transport/efflux out of cells) polymorphisms may also regulate tacrolimus disposition [14]. It has been demonstrated that *CYP3A4*1B* and *CYP3A4*22* confer enhanced and reduced enzymatic activity, respectively, compared with the wild-type allele [15,16], which may result in sub- or supratherapeutic tacrolimus concentrations. *ABCB1* SNPs *C1263T, C2677T,* and *C3435T* result in reduced transporter function [17] and may also result in supratherapeutic tacrolimus concentrations compared with the wild-type allele. However, prior studies have yielded inconclusive results [18-22], and thus guidelines have not addressed genotyping for these 2 genes.

Compared with studies performed in solid organ transplant and with orally administered tacrolimus, which has a poor bioavailability (approximately 25%) [23], there is a paucity of data about the genetic determinants contributing to the interindividual variability in i.v. tacrolimus exposure among SCT recipients [24,25]. Given the unmet need to optimize tacrolimus dosing in SCT patients, the main objective of this study was to discern the influence of polymorphisms in *CYP3A4, CYP3A5,* and *ABCB1* on i.v. tacrolimus exposure and adverse events in patients undergoing SCT for hematologic malignancies.

METHODS

Study Design, Patient Selection, and Treatment

This is a retrospective non-interventional study investigating the association between tacrolimus exposure and adverse events with polymorphisms in *CYP3A4, CYP3A5*, and *ABCB1*. Adult patients (≥18 years of age) with hematologic malignancies who underwent an allogeneic SCT (between April 2016 and October 2017) at the Levine Cancer Institute and received immunosuppression with i.v. tacrolimus for approximately 14 days before switching to oral formulation (exact duration of i.v. formulation depended on patient recovery) were included in this study. An Institutional Review Board–approved protocol allowed specimen collection (buccal swabs) on the day of SCT admission from patients who provided informed consent. All specimens were stored in a biorepository until requested for analysis.

Per standards of care for prevention of GVHD post-transplant at our institute, cyclophosphamide 50 mg/kg i.v. over 90 minutes (based on ideal body weight) was administered on days +3 and +4 each, whereas tacrolimus continuous i.v. infusion was initiated at the recommended dose of .03 mg/kg/day (based on ideal body weight, or actual body weight if lesser than the ideal body weight) on day +5 [26]. Because tacrolimus was administered as a continuous i.v. infusion and has a half-life of approximately 12 to 18 hours, steady-state blood concentrations were measured 4 days after initiation (at any time during continuous infusion given that steady-state was reached). Dose adjustments were made per provider discretion to target the recommended therapeutic range of 5 to 15 ng/mL. Thereafter, therapeutic drug monitoring was performed every 2 days.

Clinical data collected for the study while patients were treated with i.v. tacrolimus included patient demographics, initial steady-state tacrolimus concentrations, tacrolimus-related adverse events up to day +19 (2 weeks of dosing) or until discharge if sooner, and interacting concomitant medications. Of note, all patients received antifungal prophylaxis with i.v. micafungin starting on day +1 post-SCT, which was later switched to an azole antifungal (mainly voriconazole) when the oral route became feasible for drug administration.

Genotyping and Phenotype Assignment

Buccal swabs were collected from eligible patients on the day of admission after obtaining informed consent. Genomic DNA was extracted using the GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, St. Louis, MO), and DNA was stored at −80°C until requested for analysis. After identifying eligible patients for analysis, all samples were batch genotyped using the following procedure. A custom Ion AmpliSeq Pharmacogenetics Panel (Thermo Fisher Scientific, Waltham, MA) was used to genotype for *CYP3A4*1B* (rs2740574) and **22* (rs35599367); *CYP3A5*3* (rs776746), **6* (rs14690), and **7* (rs76293380); and *ABCB1 C1236T* (rs1128503), *C2677T* (rs2032582), and *C3435T* (rs1045642). Sequencing libraries were prepared using an Ion Ampliseq Library Kit (Thermo Fisher Scientific) per manufacturer's instructions. Briefly, amplicons are ligated to ion–compatible adapters, followed by nick repair to complete the linkage between adapters and DNA inserts. The libraries are clonally amplified by emulsion PCR on ion sphere particles using the Ion OneTouch 200 Template Kit (Thermo Fisher Scientific) as directed. After amplification the template-positive ion sphere particles were enriched to maximize the number of sequencing reads produced using the Ion PGM Sequencing 200 Kit (Thermo Fisher Scientific) on an Ion PGM Sequencer (Thermo Fisher Scientific) and Ion 318 Chips (Thermo Fisher Scientific). Raw data were transferred to the Ion PGM Torrent Server for base calling, preprocessing 3' trimming, quality control and assessment, and mapping. The Ion Pharmacogenomics Analysis Plugin was used to export data into AlleleTyper (Thermo Fisher Scientific) for mapping to star allele nomenclature together with translation tables developed from established guidelines.

CYP3A5 phenotypes were derived based on the guidelines set forth by Clinical Pharmacogenetics Implementation Consortium [13]. Accordingly, patients harboring 1 or 2 copies of the normal function (NF) wild-type allele (*CYP3A5*1*) were classified as CYP3A5 intermediate metabolizers (IMs) or normal metabolizers (NMs), respectively, whereas patients carrying 2 copies of any of the loss of function (LF) variant alleles (*CYP3A5*3, CYP3A5*6, CYP3A5*7*) were classified as CYP3A5 poor metabolizers (PMs). The CYP3A4 NM phenotype was assigned to patients with the **1/*1* genotype, and those with 1 or 2 copies of the decreased function *CYP3A4*22* allele were assigned the CYP3A4 IM or PM phenotypes, respectively [15,16]. Patients carrying at least a single copy of the enhanced function *CYP3A4*1B* variant allele were classified as CYP3A4 rapid metabolizers (RMs) [15,16]. For inferring ABCB1 phenotypes the presence of 1 or 2 copies of the LF T variant allele for *ABCB1 C1263T*, *C2677T*, or *C3435T* conferred ABCB1 intermediate function (IF) and LF phenotypes, respectively [17]. The presence of 2 copies of the wild-type C allele (for each of the *ABCB1* SNPs tested herein) defined the ABCB1 NF phenotype [17].

Measurement of Steady-State Tacrolimus Blood Concentration

Whole blood samples were stored at 2 to 8°C until assayed (within 7 days of collection) at the Carolinas Laboratory Network (a College of American Pathologists certified laboratory). The ARCHITECT tacrolimus assay (performed according to manufacturer's instructions; Abbott Laboratories, Chicago, IL) was used to measure or quantify steady-state tacrolimus concentration in whole blood. As described elsewhere, this assay is a delayed 1-step immunoassay based on Chemiluminescent Microparticle Immunoassay methodology [27]. The assay has a measurement range of 2 to 30 ng/mL and is designed to have a precision of ≤10% total coefficient of variation.

Statistical Analysis and Endpoints

The primary endpoint of this study was to evaluate the association between the prevalence of nontherapeutic tacrolimus blood concentrations at steady state and CYP3A4, CYP3A5, and ABCB1 phenotypes (individually and combined). Secondary endpoints included a comparison of median steady-state tacrolimus blood concentrations and the rates of tacrolimus-related toxicities by CYP3A4, CYP3A5, and ABCB1 phenotypes. Fisher's exact test was used to assess the association between prevalence of nontherapeutic tacrolimus concentrations and phenotypes. Univariate logistic regression analysis was performed to calculate the odds ratio (OR) and to identify potential risk factors associated with nontherapeutic tacrolimus concentration and tacrolimus-related adverse events.

All risk factors with $P < .10$ on univariate analysis were included in a multivariate logistic regression model and then followed by backward elimination (variable removed if $P > .10$). Steady-state tacrolimus concentrations stratified by phenotypes were compared using Student's *t*-test or one-way analysis of variance (followed by post-hoc Tukey's test for multiple comparison). Fisher's exact test with 1 degree of freedom was used to test for deviation from Hardy-Weinberg equilibrium between the observed and expected genotype frequencies for each of the genetic polymorphisms in *CYP3A4, CYP3A5,* and *ABCB1*

tested in this study. All analyses were performed in R statistical software (version 3.3.1; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 63 assessable patients were included in the final analysis. Baseline demographics are presented in Table 1. Median age at the time of SCT was 61 years (range, 25 to 78); 65% were men and 73% were white. The most commonly observed variants for *CYP3A4* and *CYP3A5* were *CYP3A4*1B* and *CYP3A5*3* with allele frequencies of 27% and 79.4%, respectively. The frequencies of the LF T allele for each of the *ABCB1* polymorphisms *C1236T*, *C2677T*, and *C3435T* were 42.1%, 39.7%, and 44.4%, respectively. The frequencies of the inferred phenotypes are outlined in Table 1. There was no deviation in the Hardy-Weinberg equilibrium in the observed genotype frequencies for *CYP3A4, CYP3A5*, and *ABCB1*.

Prevalence of Nontherapeutic Tacrolimus Concentrations by Phenotype

There was significant interindividual variability in tacrolimus exposure at the initial steady-state blood concentration (median, 16.9 ng/mL; interquartile range [IQR], 13.7 to 18.7). Forty-five patients (71.4%) had nontherapeutic concentrations, of which all were supratherapeutic (>15 ng/mL) and none was subtherapeutic (<5 ng/mL). Patients with the CYP3A4 IM and NM phenotypes had a significantly higher prevalence of supratherapeutic tacrolimus concentrations compared with patients with the RM phenotype (79.6% versus 42.9%, $P = .016$) (Figure 1A). Presence of at least 1 copy of the T allele for *ABCB1 C2677T* (IF or LF phenotype) also resulted in a statistically significant difference in the prevalence of supratherapeutic tacrolimus blood concentrations compared with patients with the NF phenotype (86.5% versus 50%, $P = .004$) (Figure 1B). Additionally, significant differences in prevalence of supratherapeutic tacrolimus concentrations were noted after combining the phenotypes for the 2 genes (CYP3A4 IM/NM and ABCB1 C2677T IF/LF: 85.7%; CYP3A4 IM/NM and ABCB1 C2677T NF: 64.3%; CYP3A4 RM and ABCB1 C2677T NF: 33.3%; $P = .002$) (Figure 1C). Only 2 patients harbored the combined CYP3A4 RM and ABCB1 C2677T IF/LF phenotype, both of which had supratherapeutic levels.

Alternatively, the prevalence of supratherapeutic tacrolimus concentrations was 77.1% in patients with the CYP3A5 PM phenotype and 53.3% in those with the CYP3A5 NM/IM phenotype ($P = .10$). There was no significant difference in the prevalence of supratherapeutic tacrolimus blood concentrations between patients who carried at least 1 copy of the T allele for either *ABCB1 C3435T* or *ABCB1 C1236T* (IF/LF phenotype) (76.2% for both) and those with the NF phenotype (61.9% for both) ($P = .25$).

In univariate logistic regression analysis, white race (OR, 5.5; 95% CI, 1.5 to 20.9; $P = .009$), CYP3A4 IM/NM phenotype (OR, 5.2; 95% CI, 1.5 to 19.4; $P = .01$), and ABCB1 C2677T IF/LF phenotype (OR, 6.4; 95% CI, 2.0 to 23.5; $P = .003$) were significantly associated with a higher prevalence of supratherapeutic tacrolimus concentrations at steady state (Table 2). Multivariate logistic regression with backward selection identified that only ABCB1 C2677T IF/LF phenotype (OR, 6.2; 95% CI, 1.9 to 23.6, $P = .004$) was independently associated with supratherapeutic tacrolimus steady-state concentrations (Table 2).

Median Tacrolimus Concentrations by Phenotype

Patients with CYP3A4 IM/NM phenotype had a median tacrolimus concentration of 17.1 ng/mL (IQR, 15.0 to 18.8) compared with 13.9 ng/mL (IQR, 11.2 to 17.6) in those with the CY3A4 RM phenotype ($P = .048$) (Figure 2A). The median tacrolimus concentration was 17.3 ng/mL (IQR, 15.6 to 19.0) for patients with the ABCB1 C2677T IF/LF phenotype compared with 14.9 ng/mL (IQR, 11.6 to 17.4; $P = .009$) in those with the ABCB1 C2677T NF phenotype (Figure 2B). Similarly, there was a significant difference in median tacrolimus steady-state concentration after combining the phenotypes for CYP3A4 and ABCB1 C2677T (CYP3A4 IM/NM and ABCB1 C2677T IF/LF: 17.3 ng/mL [IQR, 15.5 to 18.9]; CYP3A4 IM/NM and ABCB1 C2677T NF: 15.2 ng/mL [IQR, 12.7 to 17.5]; CYP3A4 RM and ABCB1 C2677T NF: 12.4 ng/mL [IQR, 10.9 to 16.4]; overall $P = .02$) (Figure 2C). Based on post-hoc analysis, the difference was statistically significant after comparing the CYP3A4 IM/NM and ABCB1 C2677T IF/LF combined phenotype with those with the CYP3A4 RM and ABCB1 C2677T NF combined phenotype ($P = .017$) (Figure 2C).

There were no significant differences in median tacrolimus steady-state concentrations between CYP3A5 PMs (median, 17.1 ng/mL; IQR: 14.9 to 18.7) versus CYP3A5 IM/NMs (median, 14.8 ng/mL; IQR, 11.5 to 17.9; $P = .34$), ABCB1 C3435T IF/LF (median, 17.0 ng/mL; IQR, 14.5 to 19.0) versus ABCB1

Table 1
Baseline Patient Characteristics (N = 63)

Characteristic	Value
Median age, yr (range)	61 (25-78)
Gender, male, %	65
Race	
Caucasian	46 (73)
African American	14 (22)
Others	3 (5)
Hematologic malignancies	
ALL	8 (13)
AML	27 (43)
CML	5 (8)
Lymphomas	10 (16)
MDS	10 (16)
Others	3 (4)
Conditioning regimen	
Flu/Cy/TBI	61 (97)
Bu/Cy	2 (3)
Transplant	
Haploidentical	46 (73)
Matched related	17 (27)
Median ideal body weight, kg (range)	68 (43-88)
Median tacrolimus dose, mg/day (range)	2.0 (1.3-2.6)
Median steady-state tacrolimus concentration, ng/mL (range)	16.9 (8.7-29.8)
CYP3A4 phenotypes	
IM (*1/*22)	4 (6)
NM (*1/*1)	45 (72)
RM (*1/*1B, *1/*1B)	14 (22)
CYP3A5 phenotypes	
PM (*3/*3, *3/*6, *3/*7, *6/*7)	48 (76)
IM (*1/*3, *1/*6, *1/*7)	12 (19)
NM (*1/*1)	3 (5)
ABCB1 C1236T phenotypes	
LF (T/T)	11 (18)
IF (C/T)	31 (49)
NF (C/C)	21 (33)
ABCB1 C2677T phenotypes	
LF (T/T)	13 (21)
IF (C/T)	24 (38)
NF (C/C)	26 (41)
ABCB1 C3435T phenotypes	
LF (T/T)	14 (22)
IF (C/T)	28 (45)
NF (C/C)	21 (33)

Values are n (%) unless otherwise defined. ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; Flu, fludarabine; Cy, cyclophosphamide; TBI, total body irradiation; Bu, busulfan.

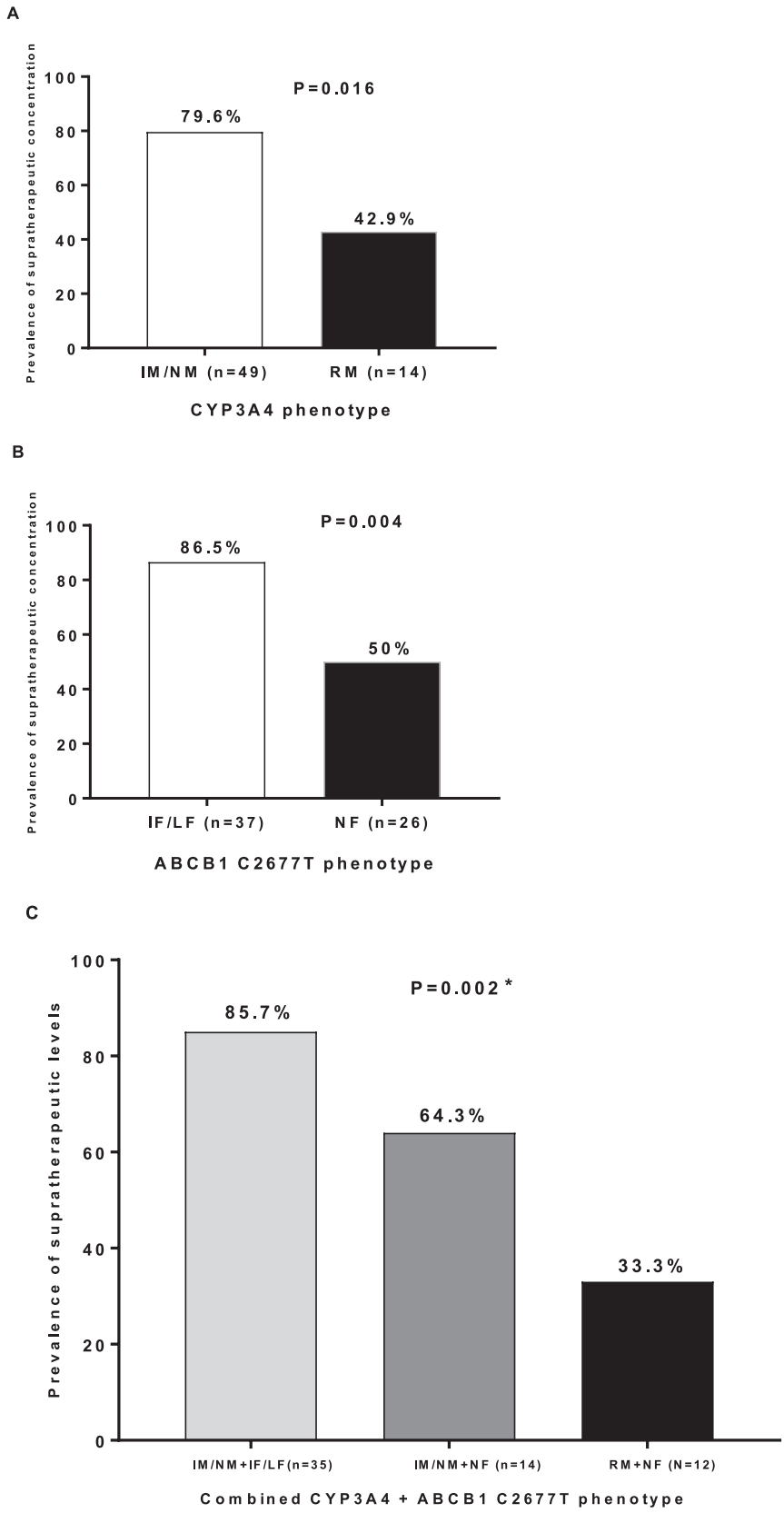

Figure 1. Prevalence of supratherapeutic tacrolimus blood concentration at steady state by CYP3A4 and ABCB1 C2677T phenotypes. (A) Comparison between CYP3A4 IMs + NMs and CYP3A4 RMs. (B) Comparison between ABCB1 C2677T IF + LF phenotype and ABCB1 C2677T NF phenotype. (C) Comparison between combined phenotypes for CYP3A4 and ABCB1 C2677T. *The combined RM + IF/LF phenotype (n = 2) was excluded from analysis because of small sample size.

Table 2
Logistic Regression Analysis for Odds of Supratherapeutic Tacrolimus Steady-State Concentrations

Variable	OR (95% CI)	*P*
Univariate logistic regression analysis		
Sex (male vs. female)	2.5 (.8-7.7)	.12
Race (African American as reference)		
White	5.5 (1.5-20.9)	.009
Other	2.7 (.2-65.8)	.46
Age	1.0 (1.0-1.1)	.05
Ideal body weight	1.0 (1.0-1.1)	.14
CYP3A4 IM/NM vs. RM	5.2 (1.5-19.4)	.01
CYP3A5 PM vs. IM/NM	2.9 (.9-10.1)	.08
ABCB1.1236 LF/IF vs. NF	2.0 (.6-6.2)	.24
ABCB1. 2677 LF/IF vs. NF	6.4 (2.0-23.5)	.003
ABCB1. 3435 LF/IF vs. NF	2.0 (.6-6.2)	.24
Multivariate logistic regression analysis*		
Age	1.0 (1.0-1.1)	.09
ABCB1. 2677 LF/IF vs. NF	6.2 (1.9-23.6)	.004

* Multivariate model uses backward selection with entry level of .1 and variable remains if it meets the .1 level.

C3435T NF (16.0 ng/mL; IQR, 12.1 to 17.6; $P = .09$), or ABCB1 C1236T IF/LF (median, 17.0 ng/mL; IQR, 14.5 to 18.8) versus ABCB1 C1236T NF (median, 15.2 ng/mL; IQR, 11.8 to 17.6; $P = .36$).

Tacrolimus-Related Adverse Events by Phenotype

Adverse events of any grade deemed possibly, probably, or definitely attributed to tacrolimus by the treating physician included hypertension (n = 16, 25.4%), severe headache (n = 10, 15.8%), acute kidney injury (n = 7, 11.1%), tremors (n = 6, 9.5%), tachycardia (n = 4, 6.3%), and hyperglycemia (n = 3, 4.7%). A combined total of 46 events across 32 unique patients were identified. In univariate logistic regression analysis the ABCB1 C2677T LF phenotype (homozygous for the T variant allele) was associated with higher odds of tacrolimus-related adverse events (84.6%) compared with NF (42.3%) phenotypes (OR, 7.5; 95% CI, 1.6 to 55.2; $P = .02$) (Table 3). The frequency of adverse events was similar between patients with the IF (42.0%) and NF phenotypes. Tacrolimus-related adverse events did not differ by CYP3A4, CYP3A5, ABCB1 C1236T, or ABCB1 C3435T phenotypes.

DISCUSSION

Significant interindividual variability in tacrolimus exposure was noted with the i.v. weight-based dose of .03 mg/kg/day used in the postallogeneic SCT setting. No patients had subtherapeutic concentrations at the time of the first steady-state blood draw. However, nearly three-fourths of all patients had steady-state concentrations above the recommended therapeutic window, which could predispose patients to developing tacrolimus-related toxicities as demonstrated in prior studies [4-7]. Based on this finding it may seem prudent to initiate i.v. tacrolimus at a reduced dose of .02 mg/kg/day instead of .03 mg/kg/day when genotype information is not available. Nonetheless, preemptive genotyping may be useful to help guide initial dose selection given that a subset of patients had therapeutic tacrolimus concentrations with the .03-mg/kg/day dose.

CYP3A4 and *ABCB1 C2677T* gene polymorphisms were found to be predictors of supratherapeutic concentrations in univariate analysis; however, only *ABCB1 C2677T* was retained in the final multivariable model, with a 6-fold increase in the risk of supratherapeutic concentration in patients with at least 1 T allele and a 7-fold increase in risk of toxicity in patients with the *TT* genotype (LF phenotype). Contrary to previous studies performed in the solid organ transplant [9-12,28,29] and SCT settings [24,25], there was no significant association between *CYP3A5* polymorphisms and i.v. tacrolimus exposure in our SCT cohort. It is worth noting that no patient received concomitant drugs that could potentially interact with i.v. tacrolimus during the early therapeutic drug monitoring period (such as azole antifungals or corticosteroids).

The data associating *ABCB1* polymorphisms with tacrolimus exposure are controversial. In the study by Anglicheau et al. [30], patients harboring at least 1 copy of the LF T allele for *ABCB1 C2677T* required significantly lower oral tacrolimus doses to achieve therapeutic tacrolimus blood concentrations after renal transplant. Furthermore, the normalized tacrolimus blood concentration (corrected by oral dose) was significantly higher among these patients. It is reasonable to assume that *ABCB1* has a role in modulating oral tacrolimus exposure; however, there are little data to suggest it impacts i.v. tacrolimus exposure, outside of these study findings. Nonetheless, ABCB1 is expressed not only in the intestines but also in the liver and kidneys. Tacrolimus undergoes extensive metabolism in the liver. This process is complex and involves an intricate interplay between the hepatic cytochromes (CYP3A4 and CYP3A5) and multidrug resistance protein (MDR1), the protein product of *ABCB1* gene [14]. As an efflux transporter, MDR1 pumps tacrolimus out of the hepatocytes into the biliary tract to not overwhelm the capacity of the hepatic cytochromes to metabolize and deactivate tacrolimus. Hence, in patients with LF variants such as the T allele for *ABCB1 C2677T,* this process is disrupted, which presumably results in elevated tacrolimus concentrations in the blood, as observed in this study. Therefore, to optimize drug exposure and response, these patients should likely be treated with initial i.v. tacrolimus doses lower than .03 mg/kg/day. Although other studies interrogated *ABCB1* haplotypes (comprising the 3 *ABCB1 C1236T*, *C2677T*, and *C3435T* SNPs) in relationship with tacrolimus disposition [31], we opted to analyze the effect of each SNP in *ABCB1* separately because they were not in linkage disequilibrium in our patient population.

An association between tacrolimus-related adverse events and the presence of genetic polymorphisms in *ABCB1 C2677T* was also noted in our cohort, where patients homozygous for the LF T allele experienced a higher incidence of adverse events. The higher prevalence of supratherapeutic tacrolimus blood concentrations translated into a significantly higher rate of adverse events throughout the study period. Because tacrolimus-related adverse events were identified retrospectively by chart review, it is imperative that these findings be validated prospectively with strict criteria to define related adverse events.

The current evidence for *CYP3A4* polymorphisms and tacrolimus exposure is not sufficiently robust or well established to support its clinical utility. However, a meta-analysis by Shi et al. [16], which included a total of 7 studies of mostly white patients (N = 1182), demonstrated that carriers of *CYP3A4*1B* allele (RMs) exhibited lower levels of tacrolimus and required significantly higher doses compared with noncarriers (ie, *CYP3A4*1/*1*) at various time points after renal transplant. Importantly, the effect of *CYP3A4* polymorphisms was independent of *CYP3A5*, which was also shown in another study by Tavira et al. [32]. Although our univariate results corroborate this hypothesis, this was not the case after accounting for other polymorphisms such as *ABCB1 C2677T* in the multivariable analysis. There was a very high correlation between CYP3A4 and ABCB1 C2677T phenotypes ($P < .01$ by Fisher's exact test), and the contribution of *CYP3A4* polymorphisms may be dependent on *ABCB1 C2677T*. Of note, the subset of patients with the CYP3A4 RM/ABCB1 C2677T NF combined phenotype had the

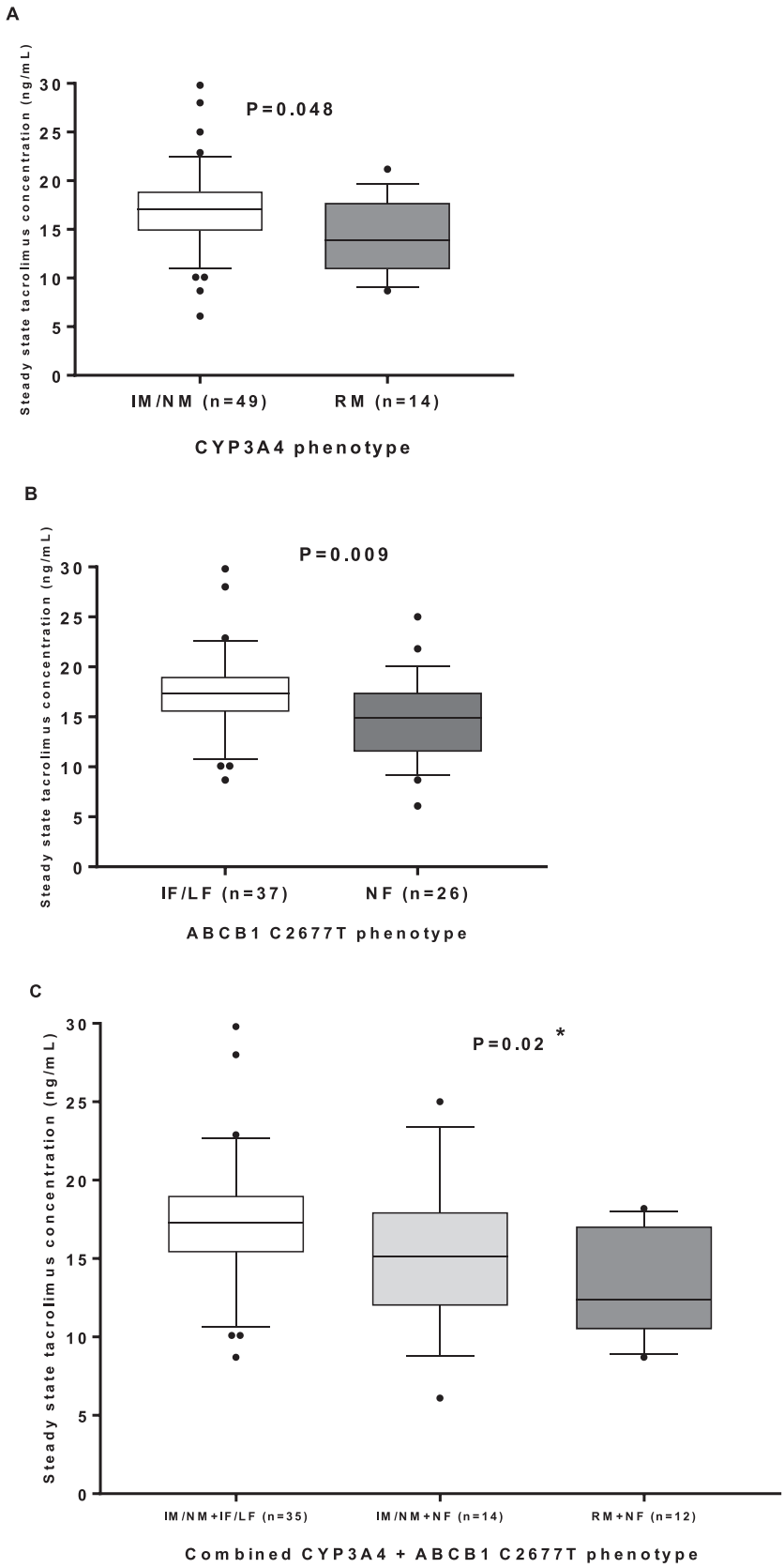

Figure 2. Box plot of steady-state tacrolimus blood concentrations by CYP3A4 and ABCB1 C2677T phenotypes. (A) Comparison between CYP3A4 IMs + NMs and CYP3A4 RMs. The bottom and top of the box represent the 25th and 75th percentiles and the middle band represents the 50th percentile (median). The lower and the upper whiskers represent the 10th and 90th percentiles. (B) Comparison between ABCB1 C2677T IF + LF phenotype and ABCB1 C2677T NF phenotype. (C) Comparison between combined phenotypes for *CYP3A4* and *ABCB1* C2677T. *Overall *P* value. The difference in median steady-state tacrolimus concentration was significantly different between the IM/NM + IF/LF and RM + NF combined phenotypes only ($P = .017$). The combined RM + IF/LF phenotype (n = 2) was excluded from analysis because of small sample size.

Table 3
Logistic Regression Analysis for Tacrolimus-Related Adverse Events

Variable	*P*	OR	95% CI
Sex (male vs. female)	.93	1.0	.4-3.0
Race (African American as reference)			
White	.23	2.1	.6-7.9
Other	.34	3.6	.3-89.8
Age	.90	1.0	1.0-1.0
Ideal body weight	.04	1.1	1.0-1.2
CYP3A4 NM (RM as reference)	.16	2.5	.7-9.1
CYP3A4IM (RM as reference)	.70	.6	.1-6.29
CYP3A5 IM (NM as reference)	.45	2.8	.2-70.8
CYP3A5 PM (NM as reference)	.58	2.0	.2-44.7
ABCB1 C1236T IF (NF as reference)	.69	.8	.3-2.4
ABCB1 C1236T LF (NF as reference)	.07	4.9	1.0-37.9
ABCB1 C2677T IF (NF as reference)	.96	1.0	.3-3.0
ABCB1 C2677T LF (NF as reference)	.02	7.5	1.6-55.2
ABCB1 C3435T IF (NF as reference)	.28	1.9	.6-6.1
ABCB1 C3435T LF (NF as reference)	.13	2.9	.7-12.7

lowest prevalence of supratherapeutic tacrolimus concentrations (33.3%) and median steady state tacrolimus concentration (12.4 ng/mL) compared with other phenotypic groups (including those with either the CYP3A4 RM phenotype or ABCB1 C2677T NF phenotype). These data suggest that the impact of both *CYP3A4* and *ABCB1 C2677T* polymorphisms should be accounted for when dosing i.v. tacrolimus.

A arge body of evidence in the literature links *CYP3A5* polymorphisms to interindividual variability in oral tacrolimus disposition. In the study by Zhang et al. [28], patients with the *CYP3A5 *1/*1* or *CYP3A5 *1/*3* genotypes (also referred to *CYP3A5* expressers due to the presence of *CYP3A5*1* wild-type allele) required significantly higher oral tacrolimus doses to achieve therapeutic blood concentrations compared with *CYP3A5 *3/*3* (nonexpressers). Clinical Pharmacogenetics Implementation Consortium guidelines recommend a 2-fold increase in the tacrolimus dose on therapy initiation for *CYP3A5* expressers (*CYP3A5*1/*1* and *CYP3A5*1/*3*) [13]. These recommendations were formulated based on studies conducted mainly in the solid organ transplant settings (particularly renal transplant) with oral tacrolimus dosing. Given the paucity of data reported in the SCT setting, further research is warranted before extrapolating these recommendations to SCT recipients where i.v. tacrolimus is commonly used.

Few studies have reported pharmacogenomics findings with i.v. tacrolimus [24,25]. Our study suggests there is no significant role for *CYP3A5* polymorphisms after i.v. administration of tacrolimus in SCT, contrary to the results reported in studies by Khaled et al. [24] and Onizuka et al. [25]. These conflicting results could be attributed to the following factors: differences in i.v. tacrolimus dosing (.02 mg/kg instead of .03 mg/kg), target therapeutic range (5 to 10 ng/mL versus 5 to 15 ng/mL), potential for drug interactions in prior studies, the use of ideal body weight to calculate daily i.v. tacrolimus dose, and, importantly, the more comprehensive genotyping methods used in this study for detecting *CYP3A5* and *CYP3A4* polymorphisms, which encompassed not only *CYP3A5*3* and *CYP3A4*22* (the common variant alleles tested in prior studies) but also other variants common in nonwhites (*CYP3A5*6, CYP3A5*7,* and *CYP3A4*1B*) given the diverse ethnic background of our patient population.

Previous studies have shown that both CYP3A4 and CYP3A5 are expressed in the liver and the intestines, with the latter being the predominant tacrolimus metabolizing enzyme in extrahepatic tissues such as the intestines [33-35]. The parenteral route for drug administration bypasses first-pass drug metabolism by CYP3A5 in the intestines, thereby providing a plausible explanation to our findings that liver mediated CYP3A4 metabolism may play a larger role after i.v. administration compared with CYP3A5 [35].

There are limitations of this study, including small sample size and the retrospective nature of the analysis. Additionally, there was a lack of data linking the genetic polymorphisms in these pharmacokinetic genes with variability of oral tacrolimus (in the same SCT setting). Although our patients were transferred to oral tacrolimus after approximately 1 week on i.v. dosing, patients were no longer receiving the same dose, and dose conversions from i.v. to oral were not consistent between all patients. The retrospective nature of this study also relied on accurate charting of tacrolimus-related adverse events; thus, only those toxicities possibly, probably, or definitely related to tacrolimus as noted in the physician progress note were included in the analysis. Although the primary role of tacrolimus in this setting is to prevent GVHD, we were unable to accurately depict the impact of pharmacogenetics or drug exposure on incidence of GVHD because patients received other immunosuppressants such as cyclophosphamide or mycophenolate mofetil post-transplant. Additionally, other genetic polymorphisms (eg, *PXR, POR,* as well as others) [18,36-38] are not accounted for in this study, which could possibly explain some of the observed variability in i.v. tacrolimus exposure among this patient population. Given the lack of data with i.v. tacrolimus in the SCT setting, we intended to confine our focus to the most studied pharmacogenes reported in the literature for tacrolimus.

In conclusion, *ABCB1 C2677T* and *CYP3A4* polymorphisms are important genetic determinants of tacrolimus exposure after i.v. administration in adult patients undergoing an allogeneic SCT. Furthermore, patients with *ABCB1 2677TT* genotype had more than a 7-fold risk of experiencing tacrolimus-related adverse events compared with those with NF. Given the relatively small number of patients included in this study and the retrospective design for data collection, larger prospective studies are warranted to verify these results and uncover other genetic markers potentially associated with tacrolimus exposure and response. Guidelines should discuss potential differences in pharmacogenetics findings between oral compared with i.v. tacrolimus and SCT compared with solid organ transplant. Ideally, using pharmacogenetics to identify patients at risk for i.v. tacrolimus overexposure (or underexposure) may pave the way for implementing a genotype-guided dosing approach, which when coupled with therapeutic drug monitoring may optimize drug exposure, minimize toxicity, and improve clinical outcomes in allogeneic SCT patients.

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REFERENCES

1. Nash RA, Pineiro LA, Storb R, et al. FK506 in combination with methotrexate for the prevention of graft-versus-host disease after marrow transplantation from matched unrelated donors. *Blood*. 1996;88:3634–3641.
2. Fay JW, Wingard JR, Antin JH, et al. FK506 (tacrolimus) monotherapy for prevention of graft-versus-host disease after histocompatible sibling allogenic bone marrow transplantation. *Blood*. 1996;87:3514–3519.

3. Venkataramanan R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet*. 1995;29:404–430.
4. Trifilio SM, Scheetz MH, Pi J, Mehta J. Tacrolimus use in adult allogeneic stem cell transplant recipients receiving voriconazole: preemptive dose modification and therapeutic drug monitoring. *Bone Marrow Transplant*. 2010;45:1352–1356.
5. Bottiger Y, Brattstrom C, Tyden G, Sawe J, Groth CG. Tacrolimus whole blood concentrations correlate closely to side-effects in renal transplant recipients. *Br J Clin Pharmacol*. 1999;48:445–448.
6. Staatz C, Taylor P, Tett S. Low tacrolimus concentrations and increased risk of early acute rejection in adult renal transplantation. *Nephrol Dial Transplant*. 2001;16:1905–1909.
7. Sikma MA, Hunault CC, van de Graaf EA, et al. High tacrolimus blood concentrations early after lung transplantation and the risk of kidney injury. *Eur J Clin Pharmacol*. 2017;73:573–580.
8. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14:641–650.
9. Quteineh L, Verstuyft C, Furlan V, et al. Influence of CYP3A5 genetic polymorphism on tacrolimus daily dose requirements and acute rejection in renal graft recipients. *Basic Clin Pharmacol Toxicol*. 2008;103:546–552.
10. Haufroid V, Wallemacq P, VanKerckhove V, et al. CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. *Am J Transplant*. 2006;6:2706–2713.
11. Garcia-Roca P, Medeiros M, Reyes H, et al. CYP3A5 polymorphism in Mexican renal transplant recipients and its association with tacrolimus dosing. *Arch Med Res*. 2012;43:283–287.
12. Ferraris JR, Argibay PF, Costa L, et al. Influence of CYP3A5 polymorphism on tacrolimus maintenance doses and serum levels after renal transplantation: age dependency and pharmacological interaction with steroids. *Pediatr Transplant*. 2011;15:525–532.
13. Birdwell KA, Decker B, Barbarino JM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. *Clin Pharmacol Ther*. 2015;98:19–24.
14. Barbarino JM, Staatz CE, Venkataramanan R, Klein TE, Altman RB. PharmGKB summary: cyclosporine and tacrolimus pathways. *Pharmacogenet Genomics*. 2013;23:563–585.
15. Elens L, van Schaik RH, Panin N, et al. Effect of a new functional CYP3A4 polymorphism on calcineurin inhibitors' dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenomics*. 2011;12:1383–1396.
16. Shi WL, Tang HL, Zhai SD. Effects of the CYP3A4*1B genetic polymorphism on the pharmacokinetics of tacrolimus in adult renal transplant recipients: a meta-analysis. *PLoS One*. 2015;10 e0127995.
17. Hodges LM, Markova SM, Chinn LW, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). *Pharmacogenet Genomics*. 2011;21:152–161.
18. Hesselink DA, Bouamar R, Elens L, van Schaik RH, van Gelder T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. *Clin Pharmacokinet*. 2014;53:123–139.
19. Haufroid V, Mourad M, Van Kerckhove V, et al. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics*. 2004;14:147–154.
20. Tsuchiya N, Satoh S, Tada H, et al. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplantation*. 2004;78:1182–1187.
21. Tada H, Tsuchiya N, Satoh S, et al. Impact of CYP3A5 and MDR1(ABCB1) C3435T polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplant Proc*. 2005;37:1730–1732.
22. Hesselink DA, van Schaik RH, van der Heiden IP, et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther*. 2003;74:245–254.
23. Undre NA. Pharmacokinetics of tacrolimus-based combination therapies. *Nephrol Dial Transplant*. 2003;18:1–5.
24. Khaled SK, Palmer JM, Herzog J, et al. Influence of absorption, distribution, metabolism and excretion genomic variants on tacrolimus/sirolimus blood levels and GVHD after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2016;22:268.. 267.
25. Onizuka M, Kunii N, Toyosaki M, et al. Cytochrome P450 genetic polymorphisms influence the serum concentration of calcineurin inhibitors in allogeneic hematopoietic SCT recipients. *Bone Marrow Transplant*. 2011;46:1113–1117.
26. Przepiorka D, Devine S, Fay J, Uberti J, Wingard J. Practical considerations in the use of tacrolimus for allogeneic marrow transplantation. *Bone Marrow Transplant*. 1999;24:1053–1056.
27. Wallemacq P, Goffinet JS, O'Morchoe S, et al. Multi-site analytical evaluation of the Abbott ARCHITECT tacrolimus assay. *Ther Drug Monitor*. 2009;31:198–204.
28. Zhang J, Zhang X, Liu L, Tong W. Value of CYP3A5 genotyping on determining initial dosages of tacrolimus for Chinese renal transplant recipients. *Transplant Proc*. 2010;42:3459–3464.
29. Zheng H, et al. Tacrolimus dosing in adult lung transplant patients is related to cytochrome P4503A5 gene polymorphism. *J Clin Pharmacol*. 2004;44:135–140.
30. Anglicheau D, Verstuyft C, Laurent-Puig P, et al. Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. *J Am Soc Nephrol*. 2003;14:1889–1896.
31. Wang J, Zeevi A, McCurry K, et al. Impact of ABCB1 (MDR1) haplotypes on tacrolimus dosing in adult lung transplant patients who are CYP3A5 *3/*3 non-expressors. *Transpl Immunol*. 2006;15:235–240.
32. Tavira B, Coto E, Diaz-Corte C, et al. Pharmacogenetics of tacrolimus after renal transplantation: analysis of polymorphisms in genes encoding 16 drug metabolizing enzymes. *Clin Chem Lab Med*. 2011;49:825–833.
33. Kamdem LK, Streit F, Zanger UM, et al. Contribution of CYP3A5 to the in vitro hepatic clearance of tacrolimus. *Clin Chem*. 2005;51:1374–1381.
34. Dai Y, Hebert MF, Isoherranen N, et al. Effect of CYP3A5 polymorphism on tacrolimus metabolic clearance in vitro. *Drug Metab Dispos*. 2006;34:836–847.
35. Pasternak AL, Zhang L, Hertz DL. CYP3A pharmacogenetic association with tacrolimus pharmacokinetics differs based on route of drug administration. *Pharmacogenomics*. 2018;19(6):563–576.
36. Liu MZ, He HY, Zhang YL, et al. IL-3 and CTLA4 gene polymorphisms may influence the tacrolimus dose requirement in Chinese kidney transplant recipients. *Acta Pharmacol Sin*. 2017;38:415–423.
37. Kotowski MM, Bogacz A, Bartkowiak-Wieczorek J, et al. The influence of the tumor necrosis factor-alpa-308G>A polymorphism on the efficacy of immunosuppressive therapy in patients after kidney transplantation. *J Physiol Pharmacol*. 2016;67:819–826.
38. Wang ZP, Zhao M, Qu QS, Miao SZ. Effect of pregnane X receptor polymorphisms on tacrolimus blood concentrations and the resulting adverse reactions in kidney transplantation recipients. *Genet Mol Res*. 2016;15(3). https://doi.org/10.4238/gmr.15038464.