

ARTICLE

Evaluation of the performance of a prior tacrolimus population pharmacokinetic kidney transplant model among adult allogeneic hematopoietic stem cell transplant patients

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Abstract

Tacrolimus is a calcineurin inhibitor used to prevent acute graft versus host disease in adult patients receiving allogeneic hematopoietic stem cell transplantation (HCT). Previous population pharmacokinetic (PK) models have been developed in solid organ transplant, yet none exists for patients receiving HCT. The primary objectives of this study were to (1) use a previously published population PK model in adult patients who underwent kidney transplant and apply it to allogeneic HCT; (2) evaluate model-predicted tacrolimus steady-state trough concentrations and simulations in patients receiving HCT; and (3) evaluate covariates that affect tacrolimus PK in allogeneic HCT. A total of 252 adult patients receiving allogeneic HCT were included in the study. They received oral tacrolimus twice daily (0.03 mg/kg) starting 3 days prior to transplant. Data for these analyses included baseline clinical and demographic data, genotype data for single nucleotide polymorphisms in *CYP3A4/5* and *ABCB1*, and the first tacrolimus steady-state trough concentration. A dosing simulation strategy based on observed trough concentrations (rather than model-based predictions) resulted in 12% more patients successfully achieving tacrolimus trough concentrations within the institutional target range (5–10 ng/ml). Stepwise covariate analyses identified HLA match and conditioning regimen (myeloablative vs. reduced intensity) as

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significant covariates. Ultimately, a previously published tacrolimus population PK model in kidney transplant provided a platform to help establish a model-based dose adjustment strategy in patients receiving allogeneic HCT, and identified HCT-specific covariates to be considered for future prospective studies.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Tacrolimus is a cornerstone immunosuppressant used in patients who undergo organ transplantations. However, because of its narrow therapeutic index and wide inter-patient pharmacokinetic (PK) variability, optimizing its dose is crucial to maximize efficacy and minimize tacrolimus-induced toxicities. Prior to this study, no tacrolimus population PK models have been developed for adult patients receiving allogeneic hematopoietic stem cell transplantation (HCT). Therefore, research effort was warranted to develop a population PK model that begins to propose more precision tacrolimus dosing and begins to address both a clinical and scientific gap in this patient population.

WHAT QUESTION DID THIS STUDY ADDRESS?

The study addressed whether there is value in utilizing the observed tacrolimus steady-state trough concentrations from patients receiving allogeneic HCT within the context of a pre-existing population PK model developed for kidney transplant. The study also addressed whether there are clinically relevant covariates specific to adult patients receiving allogeneic HCT.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Inclusion of a single steady-state tacrolimus trough concentration is beneficial to model predictions. The dosing simulation strategy based on observed tacrolimus concentration, rather than the model-predicted concentration, resulted in more patients achieving the target range at first steady-state collection. Future studies should evaluate HLA matching and myeloablative conditioning versus reduced intensity conditioning regimens as covariates. These data and model-informed dose adjustments should be included in future prospective studies. This research could also serve as a template as to how to assess the utility of prior information for other disease settings.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The M2 model fitting method and D2 dosing simulation method can be applied to other clinical pharmacology studies where only a single steady-state trough concentration is available per patient in the presence of a previously published population PK model.

INTRODUCTION

Tacrolimus, a calcineurin inhibitor, has been used as a cornerstone immunosuppressive therapy for patients receiving allogeneic hematopoietic stem cell transplantation (HCT) for almost 3 decades.¹ By inhibiting calcineurin phosphatase activity, tacrolimus binds to FK506 binding protein, which then inhibits calcineurin phosphatase to prevent subsequent calcium-dependent events (e.g., *IL2* gene transcription, cytokine release, etc.).² The net effect is inhibition of T-lymphocyte activation, which results in immunosuppression.³

Although tacrolimus has been used in patients receiving allogeneic HCT for almost 3 decades, dosing information is not included in the tacrolimus package insert.⁴ Clinicians have primarily derived tacrolimus dosing in allogeneic HCT from

a 1999 consensus report, which recommended a weight-based dosing strategy of 0.03 mg/kg/day continually infused to prevent acute graft-versus-host disease (aGVHD). Then, the continuous infusion is converted to twice daily oral tacrolimus administration once the patient tolerates oral intake.^{1,5} However, tacrolimus exhibits a narrow therapeutic index that necessitates therapeutic drug monitoring, and its wide interindividual and intraindividual PK variability complicates therapeutic drug monitoring.^{6,7} Interindividual oral tacrolimus pharmacokinetic/pharmacodynamic (PK/PD) variability observed clinically has been partially attributed to differences in patients' clinical and demographic characteristics, such as age, race, hepatic function, concomitant medications, and genetic variation.⁸

Germline genetic variations in genes that encode for proteins central to tacrolimus metabolism and transport are

associated with interindividual tacrolimus in PK/PD variability in both the solid organ and the HCT patient populations.^{9–11} *CYP3A5* has been identified as a gene with functional variants known to alter tacrolimus PK in seminal guidelines from the Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group.^{12,13} *CYP3A5* encodes CYP3A5, which contributes to tacrolimus hepatic metabolism and is responsible for an estimated 40–50% variability in tacrolimus clearance.^{14,15} *CYP3A5*3* is a single nucleotide polymorphism (SNP) that results in an alternatively spliced mRNA, which leads to a premature termination codon and a nonfunctional protein.^{15–17} Patients with at least one *CYP3A5*1* allele have been shown to have lower tacrolimus concentrations than patients with a *CYP3A5*3/*3* genotype, which predisposes them to suboptimal immunosuppression.^{15–18} *CYP3A5*6* causes a splicing defect, whereas *CYP3A5*7* is a single base insertion that causes a frameshift. Both *CYP3A5*6* and **7* result in premature termination of *CYP3A5*, leading to decreased tacrolimus metabolism and increased tacrolimus plasma concentrations.¹²

Several tacrolimus population PK/PD models that evaluated *CYP3A5* genotype as a significant covariate have been developed in solid organ transplantation.^{19–22} Specifically, Campagne et al. published a two-compartmental model, with first-order elimination and absorption with a lag time, to describe tacrolimus PK in patients who underwent kidney transplantation. They concluded that tacrolimus clearance was significantly associated with *CYP3A5* metabolizer phenotype, with more rapid clearance among extensive metabolizers (EMs) compared with poor metabolizers (PMs; 45 vs. 19.8 L/h).²² *CYP3A5* metabolizer phenotype was based on the *CYP3A5*3*, *CYP3A5*6*, and *CYP3A7*7* haplotype.²² Their model concluded that intermediate metabolizers (IMs) and EMs require a 1.5- and 2-fold increased dose, respectively, to achieve plasma concentrations within the desired therapeutic range.

To date, there has been a plethora of literature focused on tacrolimus dose optimization in solid organ transplant (e.g., kidney, liver, and heart).^{22–26} However, solid organ transplant aims to prevent organ rejection, whereas HCT aims to mitigate aGVHD and develop complete tolerance so that graft versus leukemia can be maintained.^{27,28} Additionally, baseline organ function differences between patients receiving solid organ transplant and allogeneic HCT also exist. These underlying differences may mean that solid organ transplant models are not entirely applicable to patients receiving allogeneic HCT. Few studies have been conducted to derive dosing recommendations for patients receiving allogeneic HCT and, to date, there are no population PK models that have been developed for tacrolimus in this population. Previously published data showed that of 252 patients, only 37.2% (95) patients were at therapeutic trough goal (5–10 ng/ml) as they reached steady-state on the day of receiving allogeneic HCT.⁹ Ultimately, this is a significant and unresolved clinical

concern for patients receiving HCT because suboptimal tacrolimus concentrations can result in either increased risk of aGVHD or tacrolimus-induced toxicities. It has been shown that getting to target tacrolimus steady-state trough concentrations quicker have been associated with a lower incidence of aGVHD and tacrolimus-induced toxicities.^{29,30} Therefore, to explore optimized tacrolimus dosing for patients receiving allogeneic HCT, the primary objectives of this study were three-fold: (1) to use a previously published population PK model in adult patients undergoing kidney transplant and apply it to allogeneic HCT, (2) to evaluate model-predicted tacrolimus steady-state trough concentrations and simulations in patients receiving HCT, and (3) to evaluate covariates that affect tacrolimus PK in allogeneic HCT.

METHODS

Patients

All study patients provided informed consent prior to study enrollment, and the study was approved by the University of North Carolina Institutional Review Board (UNC IRB 16–1480). Eligible patients were consented if they were greater than or equal to 18 years old and treated at the University of North Carolina Medical Center (UNCMC) from January 11, 2011 to May 31, 2016, had received their first allogeneic HCT, were prescribed oral tacrolimus as part of their aGVHD prophylaxis regimen (often with methotrexate), and received active follow-up at UNCMC post-transplant. Detailed information regarding patient enrollment was previously described.⁹ Baseline clinical and demographic data were collected beginning on the first date of the patient's inpatient admission. Clinical data that were collected included diagnosis that led to allogeneic HCT, date of HCT, baseline total body weight (TBW), baseline liver function tests (aspartate aminotransferase, alanine transaminase, and total bilirubin), baseline serum creatinine (SCr), HLA match/mismatch, transplant type (matched related or matched unrelated donor), hemoglobin levels, source of transplanted cells (peripheral blood stem cell, bone marrow, or cord blood), conditioning regimen intensity (myeloablative conditioning [MAC] versus reduced intensity conditioning [RIC]), and Karnofsky performance status score (0–100). Non-MAC regimens were grouped with RIC. Following the UNCMC institutional Bone Marrow Transplant protocol, oral tacrolimus was started 3 days prior to transplant, using a weight-based dosing strategy of 0.03 mg/kg twice daily.

Genotyping

Germline DNA was obtained from blood samples at UNCMC McLendon Laboratories, or a buccal sample was

collected from the patients. DNA collection and extraction methods have been described previously.⁹ Eight SNPs were included in this study: rs776746 (A>G, *CYP3A5**3), rs10264272 (G>A, *CYP3A5**6), rs41303343 (insT, *CYP3A5**7), rs274057 (A>G, *CYP3A4**1b), rs35599367 (C>T, *CYP3A4**22), rs1128503 (*ABCB1*, C1236T), rs2032582 (*ABCB1*, C2677T), and rs1045642 (*ABCB1*, C3435T). For the *CYP3A4* SNPs, genotyping was performed using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA), and was carried out according to the manufacturer's instructions using a QuantStudio 6 Real-Time PCR System (Applied Biosystems) as previously described.⁹ Genotyping for the three *ABCB1* SNPs, *CYP3A5**3, *6, and *7 were performed using molecular inversion probes and next generation sequencing in the UNC Center for Pharmacogenomics and Individualized Therapy as previously described.³¹ Sanger-based DNA sequencing (Eurofins Genomics, Louisville, KY) was performed on a randomly selected subset of patient DNA samples (10%, $n = 25$) to validate genotype calls and to confirm thresholds for allelic discrimination.

Patient pharmacokinetic sample analysis

Tacrolimus concentrations from whole blood samples were quantified using liquid chromatography-tandem mass spectrometry and treated with a protein precipitant reagent containing internal standard. Samples were centrifuged and chromatographed using a Waters Alliance 2795 Separations Module and Waters Xbridge C18 2.5 μ M, 4.6 \times 50 mm column. Tandem mass spectrometry detection was performed in multiple reaction monitoring mode using ion transitions. The analytic measurement range was 1–40 ng/ml, and the maximum dilution factor for sample measurement was 10-fold. Plasma concentrations of tacrolimus were collected twice weekly starting the day of allogeneic HCT. Steady-state trough concentrations were collected after at least five doses of oral tacrolimus were administered.

Pharmacokinetic data analysis

First, the population PK model published by Campagne et al. was recapitulated in Phoenix 64 version 8.2 (Certara, Princeton, NJ) using the textual model function.²² For model fitting, two independent model fitting methods were used. The first method (M1) examined how well the prior kidney transplant model predicted the observed tacrolimus trough concentrations among UNCMC patients receiving HCT, based on UNCMC patient characteristics. The second method (M2) also examined the prior model predictions, but was based on Bayesian predictions with utilization of

observed tacrolimus trough concentrations and patient demographic information. For M2, the model was not refitted to the data because it was too sparse. Rather, additional samples were used to generate model-predicted estimates of steady-state trough concentrations. For both models, the TBW-based doses were used to predict tacrolimus exposure. For M1 and M2, model simulated steady-state tacrolimus trough concentrations were generated and plotted against observed trough concentrations. These values were then compared for M1 and M2 via computation of the mean absolute prediction error as well as by their goodness of fit plots using the DV versus individual predicted values. The method with better predictive performance was used for dosing simulations.

Model-based dosing simulations

Dosing simulations were also performed in Phoenix 64 version 8.2. Based on the assumption that there was a linear relationship between dose and concentration, the dose adjustment was performed using the schematic shown in Figure 1. Briefly, a dose adjustment was only deemed necessary if the patient's first steady-state tacrolimus trough concentration was outside of the institutional target concentration range of 5–10 ng/ml. The dose adjustment ratio was calculated by

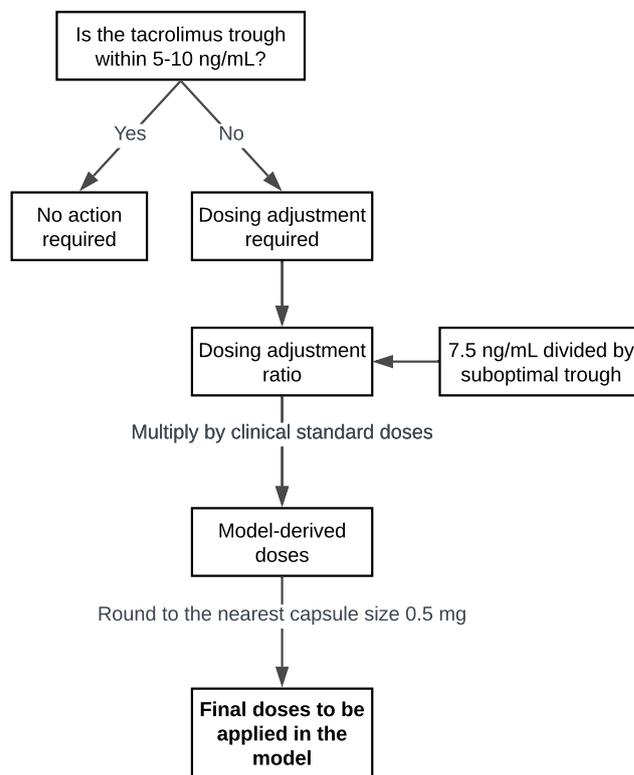


FIGURE 1 Dose adjustment schematic. Model-based dose adjustment was performed based on this schematic to derive the final doses to be applied in the M2 model.

dividing the target range median (7.5 ng/ml) by the suboptimal tacrolimus trough concentration. Then, for each patient who achieved suboptimal steady-state tacrolimus trough concentrations, their standard weight-based dose was adjusted by multiplying the dose adjustment ratio to derive new model-based doses. New doses were then rounded to the nearest 0.5 mg tacrolimus capsule size.

The M2 method was then used to evaluate the performance of the proposed dose adjustment. Two independent methods were used to determine the patients who required dose adjustment. The first dosing simulation method (D1) utilized the model predictions from M2. If the model-predicted values were suboptimal, then patients received the dose adjustment based on the Figure 1 schematic. The second dosing simulation method (D2) based the decision of whether a dose adjustment was needed on the observed tacrolimus trough concentrations. For both dosing simulation methods, the newly derived doses were then applied in the model to generate simulated initial tacrolimus steady-state trough concentrations. These two sets of predicted concentrations, from the two dosing simulation methods, were then compared to determine which dose adjustment method would likely help more patients to achieve tacrolimus plasma concentrations within the institutional target range. Last, the two dosing methods were evaluated against the relevant covariates for their impact on oral tacrolimus PK.

Stepwise covariate analyses

In addition to the covariates included in the Campagne et al. model (CYP3A5 metabolizer phenotype and TBW), additional clinical factors specific to adult patients receiving allogeneic HCT were evaluated. Specifically, a stepwise regression analysis was used where each covariate was analyzed in the order of significance, and in the presence of other significant covariates. The covariate selection was conducted using a forward selection ($p < 0.05$) and backward elimination ($p < 0.01$) approach. Observed first steady-state tacrolimus trough concentrations were \log_{10} transformed, and used as the dependent variable. Covariates that remained after forward selection followed by backward elimination were deemed to be statistically significant. All covariate effects were estimated by the model and p values were generated for each effect. Covariate effects were evaluated using JMP Pro 15 (SAS Institute, Cary, NC).

Statistical analyses

Concentrations were expressed as medians (range). An analysis of variance test was utilized to compare tacrolimus

concentrations among the three CYP3A5 metabolizer phenotype groups, and if the analysis of variance showed significance, then pairwise comparisons were conducted. All statistical testing was 2-sided, with an *a priori* significance (α) level of 0.05 ($p < 0.05$). Hardy-Weinberg equilibrium (HWE) was evaluated using Fisher's exact test with 1 degree of freedom, and SNP genotype calls were considered inconsistent with HWE if $p < 1 \times 10^{-3}$. Statistical analyses were performed using GraphPad Prism version 8.2 (GraphPad Software, La Jolla, CA) and SAS JMP Pro 15.

RESULTS

Patients

A total of 252 patients were included in the final data analysis, and baseline characteristics have been previously described.⁹ Briefly, 57.5% of the study patients were male, 83.7% were White, 11.9% were Black, and 4.4% self-identified as Native American or Asian. The median age was 52 years (range 19–76 years) and median TBW of 85.1 kg (range 42.8–123.8 kg). Table 1 compares baseline clinical, demographic, and genotypic characteristics between the UNCMC and Campagne et al. cohorts.²² Differences among characteristics were not observed between the two cohorts, with the exception of baseline SCr values (1.5 mg/dl in Campagne et al. vs. 0.7 mg/dl in the UNCMC cohort). Allele calls for all *ABCB1* and *CYP3A4/5* SNPs are included in Table S1. When stratified by race, the allele frequencies for *CYP3A5*3* and *CYP3A4*1b* did not significantly deviate from HWE, whereas *CYP3A4*22* could not be properly evaluated for HWE because the predicted minor allele frequency for *CYP3A4*22* is less than 5% among all races. None of the *ABCB1* SNPs deviated from HWE.⁹

Patient sample analyses

First steady-state trough concentrations were available for all patients, and used for PK analyses. The median steady-state trough concentration was 5.1 ng/ml (0.6–27.1 ng/ml). Based on *CYP3A5* genotyping results, 182 patients (72.2%) were classified as CYP3A5 PMs, 63 patients (25.0%) were IMs, and 7 patients (2.8%) were EMs. Median steady-state tacrolimus trough concentrations were significantly higher in PM patients than IM patients (6.3 ng/ml [1.3–27.1 ng/ml] vs. 2.8 ng/ml [1.1–20.6 ng/ml]; $p < 0.0001$). Similarly, median steady-state trough concentrations were significantly higher in PM patients than EM patients (6.3 ng/ml [1.3–27.1 ng/ml] vs. 1.3 ng/ml [0.6–5 ng/ml]; $p < 0.0001$). Last, median steady-state trough concentrations were significantly higher in IM patients than EM patients ($p = 0.04$; Figure 2).

TABLE 1 Baseline clinical and demographic characteristics

Characteristic	Zhu et al.			Campagne et al.		
	CYP3A5 Metabolizer Phenotype			CYP3A5 Metabolizer Phenotype		
	EM	IM	PM	EM	IM	PM
No. of patients, <i>n</i> (%)	7 (3)	63 (25)	182 (72)	8 (12)	24 (36)	35 (52)
Race Black, <i>n</i> (%)	4 (57)	20 (32)	6 (3.3)	8 (100)	21 (88)	6 (17)
White, <i>n</i> (%)	2 (29)	40 (63)	173 (95)	0 (0)	3 (12)	29 (83)
Female, <i>n</i> (%)	2 (29)	29 (46)	76 (42)	4 (50)	9 (37)	16 (46)
Age, year	59	51	54	46	49	50
TBW, kg	83	84	85	89	90	85
SCr, mg/dL	0.92	0.82	0.7	1.9	1.6	1.3
<i>ABCB1 1236C>T</i>						
CC, <i>n</i> (%)	4 (80)	28 (47)	41 (26)	4 (50)	11 (46)	14 (40)
CT, <i>n</i> (%)	1 (20)	24 (40)	91 (57)	4 (50)	7 (29)	14 (40)
TT, <i>n</i> (%)	0 (0)	8 (13)	27 (17)	0 (0)	6 (25)	7 (20)
<i>ABCB1 2677G>T/A</i>						
CC, <i>n</i> (%)	4 (100)	33 (66)	45 (34)	7 (88)	17 (71)	13 (37)
CT, <i>n</i> (%)	0 (0)	10 (20)	61 (46)	1 (12)	5 (21)	15 (43)
TT, <i>n</i> (%)	0 (0)	7 (14)	27 (20)	0 (0)	2 (8.2)	7 (20)
<i>ABCB1 3435C>T</i>						
CC, <i>n</i> (%)	3 (60)	22 (37)	31 (20)	4 (50)	13 (54)	13 (37)
CT, <i>n</i> (%)	2 (40)	28 (47)	86 (54)	4 (50)	59 (38)	15 (43)
TT, <i>n</i> (%)	0 (0)	10 (16)	41 (26)	0 (0)	2 (8)	7 (20)

Abbreviations: ABCB1, ATP-binding cassette B1; CYP3A5, cytochrome P450 isoform 5; EM, extensive metabolizers; IM, intermediate metabolizers; PM, poor metabolizers; SCr, serum creatinine; TBW, total body weight.

Baseline characteristics are compared between patients in the current study and the previously published study by Campagne et al.²²

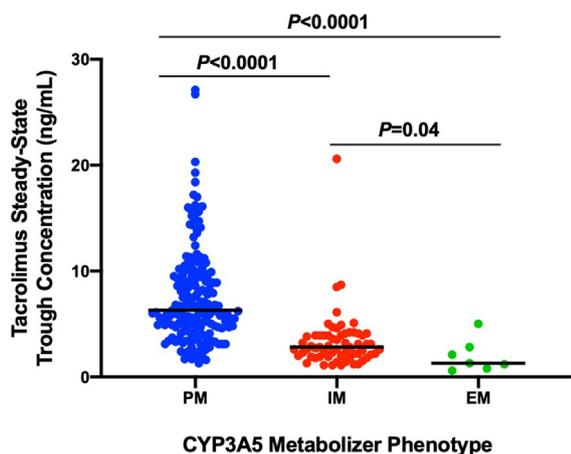


FIGURE 2 Steady-state tacrolimus trough by CYP3A5 metabolizer phenotype. Tacrolimus trough concentration at steady-state for CYP3A5 metabolizer phenotype. Associations between steady-state tacrolimus trough concentrations measured on the day of allogeneic HCT (day 0) were evaluated. The black lines denote the median tacrolimus concentration. Abbreviations: CYP3A5, cytochrome P450 isoform 5; EM, extensive metabolizers; HCT, hematopoietic stem cell transplantation; IM, intermediate metabolizers; PM, poor metabolizers.

Pharmacokinetic data analyses

The model-predicted tacrolimus trough concentrations from the M1 and M2 methods were evaluated against the observed steady-state tacrolimus trough concentrations (Figure 3). In comparison to the observed tacrolimus concentrations, in the setting of method M1, the model prediction improved in patients who had subtherapeutic concentrations, as evidenced by a reduction in the percentage of patients with subtherapeutic concentrations from 46.4% to 41.3%, whereas the percentage of suprathreshold patients remained unchanged (16.3%). Overall, M1 increased the percentage of patients who reached the tacrolimus target range from 37.3% to 42.5% by the first steady-state collection. In the setting of method M2, the model prediction improved in patients who had suprathreshold concentrations, as evidenced by a reduction in the percentage of patients with suprathreshold concentrations from 16.3% to 4.8%, whereas the percentage of subtherapeutic patients decreased only slightly from 46.8% to 46.4%. Overall, M2 increased the percentage of patients who reached the tacrolimus target range from 37.3% to 48.8% at first steady-state

collection. The mean absolute prediction error for M1 and M2 were 80.3% and 10.3%, respectively. The M1 and M2 goodness of fit plots showed that M2 predicted the observed values better than M1, as evidenced by the closer proximity of observed concentrations to the line of unity for M2 (Figure S1). Additionally, both M1 and M2 revealed slight under predictions at higher concentrations, which was similar to the Campagne et al. model results.

Model-based dosing simulations

M2 was deemed superior to M1 based on its predictability of the observed tacrolimus data. Therefore, M2 was used to perform dosing simulations. The model-predicted tacrolimus steady-state concentrations after dose adjustments (D1 and

D2) were evaluated against the observed steady-state tacrolimus trough concentrations (Figure 4). When compared with the observed tacrolimus concentrations, model-predicted results from D1 and D2 primarily benefited patients with supratherapeutic steady-state tacrolimus trough concentrations. D1 and D2 reduced the percentage of patients with supratherapeutic concentrations from 16.3% to 8.7% and 9.9%, respectively. Additionally, D2 also benefited patients with subtherapeutic trough concentrations by reducing the percentage of subtherapeutic patients from 46.4% to 44.0%. D1 did not benefit subtherapeutic patients. Overall, D2 demonstrated an improved percentage of patients reaching the tacrolimus target range (37.3% to 46.0%) when compared with D1 (37.3% to 43.3%).

Subsequently, the model-predicted tacrolimus steady-state concentrations were evaluated against the two covariates included in the model: CYP3A5 metabolizer phenotype and TBW.

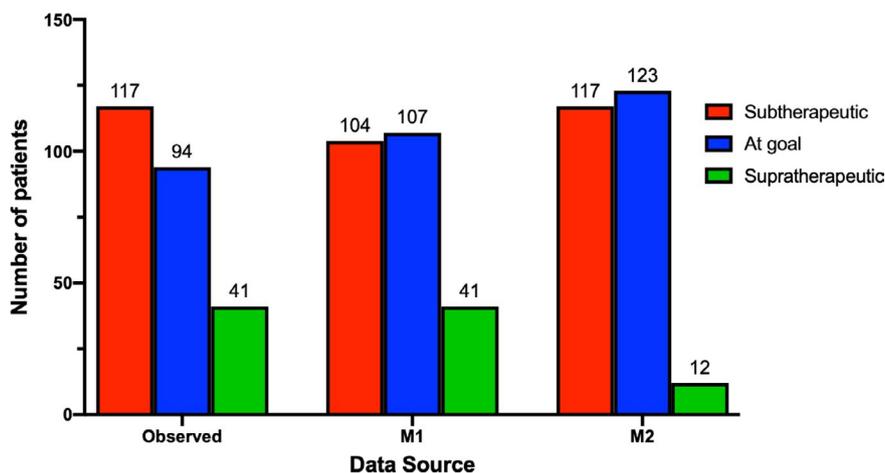


FIGURE 3 Modeling methods comparisons. Model-predicted tacrolimus steady-state trough concentration post-dose adjustments were compared across the observed data, the M1 modeling method, and M2 modeling method. Vertical bars depict the number of patients who were subtherapeutic (< 5 ng/ml), at target range (5–10 ng/ml), and supratherapeutic (> 10 ng/ml), respectively.

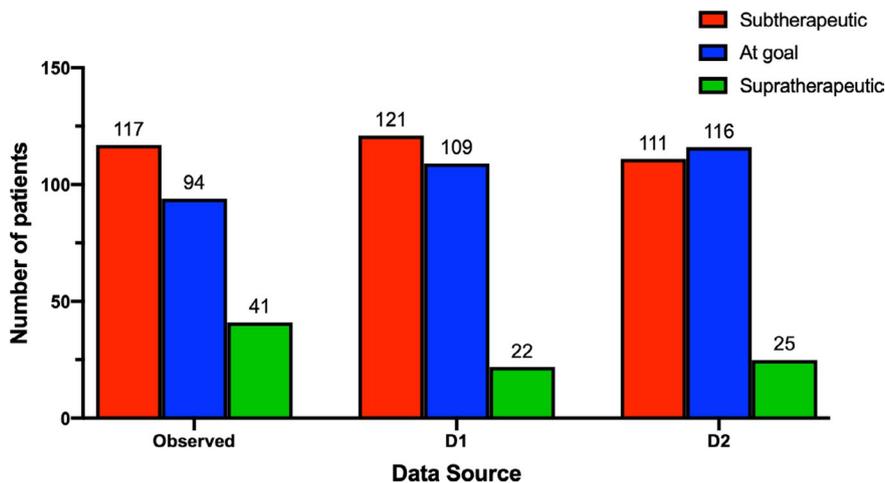


FIGURE 4 Dose adjustment method comparisons. Model-predicted tacrolimus steady-state trough concentration post-dose adjustments were compared across the observed data, D1 dose adjustment method, and D2 dose adjustment method. Vertical bars depict the number of patients who were subtherapeutic (< 5 ng/ml), at target range (5–10 ng/ml), and supratherapeutic (> 10 ng/ml), respectively.

Both D1 and D2 adjustment strategies benefited CYP3A5 PMs to a greater extent than EMs and IMs based on the increased percentage of PM patients whose trough concentrations were at goal post-D1 and D2 (3.4% and 13.2% respectively; Figure S2). Both D1 and D2 slightly benefited patients with TBW of less than 90 kg (2.0% and 3.2% more patients at target concentrations, respectively; Figure S3). Although neither D1 nor D2 relied on weight-based dosing, both were less effective in patients with higher TBW, particularly with TBW greater than 90 kg.

Stepwise covariate analyses

Estimations of covariate effects are included in Table 2. After using the stepwise analysis approach, covariates that remained included CYP3A5 metabolizer phenotype, TBW, diagnosis that led to HCT, HLA matching, and MAC versus RIC conditioning regimen. Based on the effect summary of each covariate, the most impactful were the CYP3A5 metabolizer phenotype, MAC versus RIC conditioning regimen and HLA matching (Table S2). Based on the leverage plots, the effects of TBW and diagnosis were minimal (Figure S4). Least square mean values for the most significant covariate (CYP3A5 metabolizer phenotype) showed that, after adjusting for other covariates in the model, only CYP3A5 PM patients were at the target range, whereas both EM (1.55 ng/ml) and IM (2.77 ng/ml) remained subtherapeutic (Table S3). For other covariates, predicted tacrolimus trough concentrations, based on each level of covariates, can be found in Table S3. For the final model, a plot of the predicted versus residual concentrations illustrated a lack of any bias (Figure S5).

DISCUSSION

This is the first study that has used population PK modeling to help inform model-based predictions and precision dosing

strategies in adult patients receiving allogeneic HCT. The study explored how to apply a published tacrolimus population PK model from kidney transplant to an allogeneic HCT patient cohort.²² Specifically, the study examined two methods (M1 and M2) to optimize model-based prediction performance, as well as two dosing adjustment strategies (D1 and D2). Last, the study also identified potential covariates that are likely to be clinically relevant to adult patients receiving HCT and to future tacrolimus precision dosing efforts.

Incorporating a single steady-state trough concentration value into the M2 model showed better model-predictions of the observed first steady-state tacrolimus trough concentrations than M1. Overall, M1 was more beneficial for patients with initial subtherapeutic steady-state trough concentrations, whereas M2 was more beneficial for patients with initial supratherapeutic steady-state trough concentrations. Although M2 demonstrated a larger magnitude of overall improvement, M1 can be potentially more beneficial to help patients reach target range from the subtherapeutic state, which is more concerning for aGVHD incidence. In addition to generating model-predicted tacrolimus steady-state trough concentrations, two different dosing simulation strategies were investigated. Dosing simulations concluded that the D2 strategy, which adjusted dosing based on the observed trough concentrations rather than the D1 strategy that used model-predicted concentrations, resulted in a greater proportion of patients achieving target range tacrolimus trough concentrations (5–10 ng/ml).

The study also evaluated the two significant covariates identified in the Campagne et al. model (CYP3A5 metabolizer phenotype and TBW) against the post-dose adjustment tacrolimus steady-state trough concentrations, and concluded that applying the previously published population PK model provided more benefit to CYP3A5 PM patients than IM or EM patients, and in patients who weighed less than 90 kg. A stepwise covariate multiple linear regression was used because it can incorporate each covariate while adjusting for

TABLE 2 Covariate evaluation

Term	Degree of freedom	Sum of squares	F ratio	p value
CYP3A5 metabolizer phenotype	2	33.58	62.41	< 0.0001
TBW	1	1.68	6.24	0.01
Diagnosis that led to HCT	4	2.94	2.73	0.03
HLA status	2	2.64	4.90	0.01
Conditioning regimen	1	10.17	37.80	<0.0001

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CYP3A5, cytochrome P450 isoform 5; HLA, human leukocyte antigen; TBW, total body weight.

Analysis of variance and a stepwise approach were used to evaluate covariates. Covariates that were tested, but not significant ($p > 0.05$) and therefore not included in the final model, included baseline liver function tests (ALT, AST, and total bilirubin), baseline serum creatine, hemoglobin levels, source of transplanted cells, and Karnofsky performance status score.

other covariates. The results of the stepwise covariate analyses showed that, in addition to CYP3A5 metabolizer phenotype and TBW, HLA match and conditioning regimen were statistically significant and we believe to be potentially clinically relevant, and therefore should be considered in future studies to further refine tacrolimus dosing.

Because the dose adjustment methods utilized in this research were based on the assumption of linear PK for tacrolimus, it can be hypothesized that a TBW of 90 kg may represent the threshold for nonlinear tacrolimus PK. Studies have shown that in patients with obesity, Phase 1 cytochrome P450 and Phase 2 metabolic processes are increased, thereby increasing tacrolimus bioinactivation and clearance.^{32,33} The fact that the ratio between lean body weight and adipose tissue decreases in patients with obesity also partially supports this hypothesis.³⁴ In these patients, lipophilic drugs like tacrolimus ($\log P = 5.59$), with a reported volume of distribution greater than 40 L, could readily distribute into the adipose tissue and increase half-life.^{35–37} Thus, in patients heavier than 90 kg, additional modified dose adjustments might be required to correct for this nonlinear PK.

In addition to CYP3A5 metabolizer phenotype and TBW, MAC versus RIC conditioning regimen and HLA match were significantly associated with the observed tacrolimus steady-state trough concentrations. MAC regimens have previously been independently associated with supratherapeutic tacrolimus trough concentrations,⁹ which could be caused by the damage exerted to the gut mucosal barrier and potential hepatotoxicity that could affect tacrolimus PK.³⁸ Interestingly, HLA match was also significantly associated with tacrolimus concentrations. Specifically, full HLA matches between the recipient and donor (e.g., 8/8 or 10/10) was associated with lower tacrolimus concentrations than mismatch of 1–2 HLAs. Although perfect HLA matching has previously been associated with lower aGVHD risk,^{39,40} and suboptimal tacrolimus concentrations can lead to the increased aGVHD risk,^{10,30,41} a direct mechanistic relationship between HLA typing and tacrolimus PK remains unknown.

Previously published data suggest that using an existing published population PK model could help predict PK parameter estimates in their specific population of interest.^{20,42,43} For instance, Tasa et al. concluded that the inclusion of a single PK sample per patient improved the overall model predictions of the observed data.⁴³ Their observation was consistent with our observations that M2 better predicted observed concentrations than M1. Together with Tasa et al., this study has demonstrated that including one initial steady-state trough concentration has potential for future research and clinical utility because the methodologies proposed by this study can be easily applied by clinicians after the first tacrolimus steady-state trough is obtained and a dose adjustment calculation is performed. Future research should expand on

these results and evaluate the effects that subsequent trough concentration collection has on model predictability.

In addition to the three CYP3A5 SNPs, three ABCB1 SNPs and two CYP3A4 SNPs were also genotyped. However, no significant associations were identified among these SNPs and tacrolimus steady-state trough concentrations in a previously published study,⁹ and were also not determined to be significant covariates in the present study. Similarly, the Campagne et al. study did not detect significant associations between the ABCB1 and CYP3A4 SNPs, and were not included in their final model either.²² Aside from these SNPs, previous publications have suggested that germline variants in other genes (e.g., *POR*, *PPARA*, and *CYB5R2*) could potentially impact tacrolimus PK/PD.^{44–46} Thus, future studies should evaluate variants in these genes individually, as well as in polygenic models, during covariate analyses.

There were notable limitations associated with the study. First, only a single tacrolimus PK sample was collected from each patient. The overall model predictions could improve if additional tacrolimus samples were collected. Nevertheless, this study evaluated the difference between two model-prediction methods: no real-world patient drug concentration data (M1) versus one actual PK sample collected per patient (M2), and concluded that an additional PK sample was beneficial to model predictability. Another limitation was that a tacrolimus population PK model developed originally for kidney transplant was applied to adult patients receiving allogeneic HCT. Baseline SCr was significantly higher in the Campagne et al. study, which was expected of the patients who underwent kidney transplantation (Table 1). However, this baseline demographic difference should not affect the model performance because renal elimination of tacrolimus only counts for less than 1% of total clearance and SCr was not included as a model covariate.^{37,47}

In conclusion, this study identified an efficient and feasible method to derive model-predicted steady-state tacrolimus concentrations. This study only used one trough sample per patient and did not attempt to refit the model due to sparse data. Ultimately, these data should be prospectively validated in future studies to examine the inclusion of more than one trough concentration. A new study to fully characterize the tacrolimus PK profile in adult patients receiving allogeneic HCT is currently underway, where we prospectively enroll patients receiving allogeneic HCT, conduct intense PK sampling, and evaluate both PD end points of tacrolimus response (e.g., *IL2* expression and NFAT nuclear localization) and genetic variants beyond ABCB1 and CYP3A4/5 (ClinicalTrials.gov Identifier: NCT04645667). The population PK model generated here will then be the starting point to perform model-based dose adjustment for each patient. With additional intensely sampled tacrolimus concentrations, model predictions will be more accurate so that ideally a starting oral dose

of tacrolimus will yield more trough concentrations within the target range by the date of allogeneic HCT.

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CONFLICT OF INTEREST

All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. J.Z., J.R.P., P.M.A., D.L.W., and D.J.C. designed the research. J.Z., C.D.T., G.F., J.A.M., T.P., D.L.W., and D.J.C. performed research and analyzed data. O.C., O.S., T.W., and D.E.M. contributed new reagents/analytical tools.

REFERENCES

- Przepiorka D, Devine SM, Fay JW, Uberti JP, Wingard JR. Practical considerations in the use of tacrolimus for allogeneic marrow transplantation. *Bone Marrow Transplant.* 1999;24(10):1053-1056.
- Azzi JR, Sayegh MH, Mallat SG. Calcineurin inhibitors: 40 years later. can't live without. *J Immunol.* 2013;191(12):5785-5791.
- Kelly PA, Burckart GJ, Venkataramanan R. Tacrolimus: a new immunosuppressive agent. *Am J Health Syst Pharm.* 1995;52(14):1521-1535.
- Astellas Pharma US. Prograf (tacrolimus) [package insert]. 2012.
- Brunet M, van Gelder T, Åsberg A, et al. Therapeutic drug monitoring of tacrolimus-personalized therapy: second consensus report. *Ther Drug Monit.* 2019;41(3):261-307.
- Kuypers D, Claes K, Evenepoel P, Maes B, Vanrenterghem Y. Clinical efficacy and toxicity profile of tacrolimus and mycophenolic acid in relation to combined long-term pharmacokinetics in de novo renal allograft recipients. *Clin Pharmacol Ther.* 2004;75(5):434-447.
- Leino AD, King EC, Jiang W, et al. Assessment of tacrolimus inpatient variability in stable adherent transplant recipients: establishing baseline values. *Am J Transplant.* 2019;19(5):1410-1420.
- Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet.* 2004;43(10):623-653.
- Zhu J, Patel T, Miller JA et al. Influence of germline genetics on tacrolimus pharmacokinetics and pharmacodynamics in allogeneic hematopoietic stem cell transplant patients. *Int J Mol Sci.* 2020;21(3):858.
- Hamadeh IS, Zhang Q, Steuerwald N et al. Effect of CYP3A4, CYP3A5, and ABCB1 polymorphisms on intravenous tacrolimus exposure and adverse events in adult allogeneic stem cell transplant patients. *Biol Blood Marrow Transplant.* 2019;25(4):656-663.
- Khaled SK, Palmer JM, Herzog J et al. Influence of absorption, distribution, metabolism, and excretion genomic variants on tacrolimus/sirolimus blood levels and graft-versus-host disease after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 2016;22(2):268-276.
- 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(1):19-24.
- Lunenburg CATC, van der Wouden CH, Nijenhuis M et al. Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of DPYD and fluoropyrimidines. *Eur J Hum Genet.* 2020;28(4):508-517.
- Woillard J-B, Mourad M, Neely M, et al. Tacrolimus updated guidelines through popPK modeling: how to benefit more from CYP3A pre-emptive genotyping prior to kidney transplantation. *Front Pharmacol.* 2017;8:358.
- Dai Y, Hebert MF, Isoherranen N et al. Effect of cyp3a5 polymorphism on tacrolimus metabolic clearance in vitro. *Drug Metab Dispos.* 2006;34(5):836-847.
- Hesselink DA, Bouamar R, Elens L, van Schaik RHN, van Gelder T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. *Clin Pharmacokinet.* 2014;3(2):123-139.
- Lamba J, Hebert JM, Schuetz EG, Klein TE, Altman RB. PharmGKB summary. *Pharmacogenet Genomics.* 2012;22(7):555-558.
- Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: part II. *Clin Pharmacokinet.* 2010;49(4):207-221.
- Zhao W, Elie V, Roussey G et al. Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. *Clin Pharmacol Ther.* 2009;86(6):609-618.
- Zhao C-Y, Jiao Z, Mao J-J, Qiu X-Y. External evaluation of published population pharmacokinetic models of tacrolimus in adult renal transplant recipients. *Br J Clin Pharmacol.* 2016;81(5):891-907.
- Moes DJAR, van der Bent SAS, Swen JJ et al. Population pharmacokinetics and pharmacogenetics of once daily tacrolimus formulation in stable liver transplant recipients. *Eur J Clin Pharmacol.* 2016;72(2):163-174.
- Campagne O, Mager DE, Brazeau D, Venuto RC, Tornatore KM. Tacrolimus population pharmacokinetics and multiple CYP3A5 genotypes in black and white renal transplant recipients. *J Clin Pharmacol.* 2018;58(9):1184-1195.
- Zhu L, Wang H, Sun X et al. The population pharmacokinetic models of tacrolimus in Chinese adult liver transplantation patients. *J Pharm (Cairo).* 2014;2014:713650.
- Han Y, Zhou H, Cai J et al. Prediction of tacrolimus dosage in the early period after heart transplantation: a population pharmacokinetic approach. *Pharmacogenomics.* 2019;20(1):21-35.
- Fredj NB, Woillard JB, Debord J et al. Modeling of tacrolimus exposure in kidney transplant according to posttransplant time based on routine trough concentration data. *Exp Clin Transplant.* 2016;14(4):394-400.
- Zhang Y, Yang J, Zhu LQ, Wang N. Effects on pharmacokinetics of tacrolimus in liver transplant patients. *SM J Pharmac Ther.* 2016;2(1):20-23.

27. Chinen J, Buckley RH. Transplantation immunology: Solid organ and bone marrow. *J Allergy Clin Immunol.* 2010;125(2 suppl. 2):1-26.
28. Dickinson AM, Norden J, Li S et al. Graft-versus-leukemia effect following hematopoietic stem cell transplantation for leukemia. *Front Immunol.* 2017;8:496.
29. Mori T, Kato J, Shimizu T, et al. Effect of early posttransplantation tacrolimus concentration on the development of acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation from unrelated donors. *Biol Blood Marrow Transpl.* 2012;18:229-234.
30. Ganetsky A, Shah A, Miano TA, et al. Higher tacrolimus concentrations early after transplant reduce the risk of acute GvHD in reduced-intensity allogeneic stem cell transplantation. *Bone Marrow Transplant.* 2016;51(4):568-572.
31. Suzuki O, Dong OM, Howard RM, Wiltshire T. Characterizing the pharmacogenome using molecular inversion probes for targeted next-generation sequencing. *Pharmacogenomics.* 2019;20(14):1005-1020.
32. Brill MJE, Diepstraten J, van Rongen A et al. Impact of obesity on drug metabolism and elimination in adults and children. *Clin Pharmacokinet.* 2012;51:277-304.
33. Hanley M, Abernathy D, Greenblatt D. Effect of obesity on the pharmacokinetics of drugs in humans. *Clin Pharmacokinet.* 2010;49:71-87.
34. Barras M, Legg A. Drug dosing in obese adults. *Aust Prescr.* 2017;40(5):189-193.
35. Patel P, Patel H, Panchal S, Mehta T. Formulation strategies for drug delivery of tacrolimus: an overview. *Int J Pharm Investig.* 2012;2(4):169-175.
36. Antignac M, Barrou B, Farinotti R, Lechat P, Urien S. Population pharmacokinetics and bioavailability of tacrolimus in kidney transplant patients. *Br J Clin Pharmacol.* 2007;64(6):750-757.
37. Sikma MA, Van Maarseveen EM, Van De Graaf EA, et al. Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation. *Am J Transplant.* 2015;15(9):2301-2313.
38. Johansson JE, Brune M, Ekman T. The gut mucosa barrier is preserved during allogeneic, haemopoietic stem cell transplantation with reduced intensity conditioning. *Bone Marrow Transplant.* 2001;28(8):737-742.
39. Kanda J. Effect of HLA mismatch on acute graft-versus-host disease. *Int J Hematol.* 2013;98(3):300-308.
40. Petersdorf EW. Role of major histocompatibility complex variation in graft-versus-host disease after hematopoietic cell transplantation. *F1000Res.* 2017;6(617):1-12.
41. Hagen PA, Adams W, Smith S, Tsai S, Stiff P. Low mean post-transplantation tacrolimus levels in weeks 2–3 correlate with acute graft-versus-host disease in allogeneic hematopoietic stem cell transplantation from related and unrelated donors. *Bone Marrow Transplant.* 2019;54(1):155-158.
42. Mao JJ, Jiao Z, Yun HY, et al. External evaluation of population pharmacokinetic models for ciclosporin in adult renal transplant recipients. *Br J Clin Pharmacol.* 2018;84(1):153-171.
43. Tasa T, Kalamees R, Vilo J, Lutsar I, Metsvaht T. External evaluation of population pharmacokinetic models for vancomycin in neonates [published online ahead of print October 2018]. *bioRxiv.* <https://doi.org/10.1101/458125>.
44. Elens L, Hesselink DA, Bouamar R et al. Impact of POR*28 on the pharmacokinetics of tacrolimus and cyclosporine A in renal transplant patients. *Ther Drug Monit.* 2014;36(1):71-79.
45. Kurzawski M, Malinowski D, Dziejwanowski K, Drożdżika M. Impact of PPARA and POR polymorphisms on tacrolimus pharmacokinetics and new-onset diabetes in kidney transplant recipients. *Pharmacogenet Genomics.* 2014;24(8):397-400.
46. Dorr CR, Wu B, Rimmel RP, et al. Phenotype sampling and next generation sequencing. *Pharmacogenomics J.* 2019;19(4):375-389.
47. Marfo K, Altshuler J, Lu A. Tacrolimus pharmacokinetic and pharmacogenomic differences between adults and pediatric solid organ transplant recipients. *Pharmaceutics.* 2010;2(3):291-299.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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