Results of ASERTAA, a Randomized Prospective Crossover Pharmacogenetic Study of Immediate-Release Versus Extended-Release Tacrolimus in African American Kidney Transplant Recipients

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Background: Differences in tacrolimus dosing across ancestries is partly attributable to polymorphisms in *CYP3A5* genes that encode tacrolimus-metabolizing cytochrome P450 3A5 enzymes. The *CYP3A5*1* allele, preponderant in African Americans, is associated with rapid metabolism, subtherapeutic concentrations, and higher dose requirements for tacrolimus, all contributing to worse outcomes. Little is known about the relationship between *CYP3A5* genotype and the tacrolimus pharmacokinetic area under the curve (AUC) profile in African Americans or whether pharmacogenetic differences exist between conventional twice-daily, rapidly absorbed, immediate-release tacrolimus (IR-Tac) and once-daily extended-release tacrolimus (LifeCycle Pharma Tac [LCPT]) with a delayed absorption profile.

Study Design: Randomized prospective crossover study.

Setting & Participants: 50 African American maintenance kidney recipients on stable IR-Tac dosing.

Intervention: Recipients were randomly assigned to continue IR-Tac on days 1 to 7 and then switch to LCPT on day 8 or receive LCPT on days 1 to 7 and then switch to IR-Tac on day 8. The LCPT dose was 85% of the IR-Tac total daily dose.

Outcomes: Tacrolimus 24-hour AUC ($AUC_{0\text{-}24}$), peak and trough concentrations (C_{max} and C_{min}), time to peak concentration, and bioavailability of LCPT versus IR-Tac, according to *CYP3A5* genotype.

Measurements: *CYP3A5* genotype, 24-hour tacrolimus pharmacokinetic profiles.

Results: ~80% of participants carried the *CYP3A5*1* allele (*CYP3A5* expressers). There were no significant differences in $AUC_{0\text{-}24}$ or C_{min} between *CYP3A5* expressers and nonexpressers during administration of either IR-Tac or LCPT. With IR-Tac, tacrolimus C_{max} was 33% higher in *CYP3A5* expressers compared with nonexpressers ($P = 0.04$): With LCPT, this difference was 11% ($P = 0.4$).

Limitations: This was primarily a pharmacogenetic study rather than an efficacy study; the follow-up period was too short to capture clinical outcomes.

Conclusions: Achieving therapeutic tacrolimus trough concentrations with IR-Tac in most African Americans results in significantly higher peak concentrations, potentially magnifying the risk for toxicity and adverse outcomes. This pharmacogenetic effect is attenuated by delayed tacrolimus absorption with LCPT.

Trial Registration: Registered at ClinicalTrials.gov, with study number NCT01962922.

Complete author and article information provided before references.

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Individuals of African ancestry accounted for one-third of US deceased donor kidney recipients in 2015 despite constituting 13% of the population.[1] Because African Americans are inadequately represented in most immunosuppression trials,[2,3] findings from such studies cannot necessarily be extrapolated to this subpopulation. Rates of rejection[4] and transplant loss[4–7] are greater in African Americans compared with Americans of European ancestry due to immunologic and nonimmunologic factors.[8] Recently, the contribution of genetics to these disparate outcomes has become an area of focus.[9,10] Among contemporary therapies, the widely used tacrolimus[11,12] exemplifies a drug for which patient genotype affects dosing.

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Factors that affect tacrolimus pharmacokinetics include sex, ethnicity, concomitant medications, and genetic polymorphisms.[13–16] Although tacrolimus is metabolized via CYP3A4 and CYP3A5 enzymes primarily in the gut and liver, the intrinsic tacrolimus clearance capacity of CYP3A5 predominates over CYP3A4.[17] Loss-of-function alleles *CYP3A5*6*, *CYP3A5*7* (found only in individuals of African ancestry), and *CYP3A5*3* (present in most Americans of European ancestry and Asians) result in marked diminution of CYP3A5 enzyme activity (*CYP3A5* nonexpressers).[18] The *CYP3A5*1* allele, found predominantly but not exclusively in individuals of black African descent,[18] encodes CYP3A5 enzymes that are associated with rapid tacrolimus disposition (*CYP3A5* expressers), leading to subtherapeutic concentrations and increased dose requirements.[19]

In the Deterioration of Kidney Allograft Function (DeKAF) Study, the *CYP3A5*1* allele was found to be the most important allele associated with subtherapeutic tacrolimus concentrations in African Americans,[15,20] supporting tacrolimus underexposure as an inferior transplant outcome determinant in this population.[21]

Pharmacogenetic studies of tacrolimus in African Americans (such as DeKAF) have been limited by: (1) tacrolimus assays being performed at individual centers rather than a centralized laboratory, (2) lack of standardized tacrolimus dosing,[22] and (3) measurement of trough concentrations rather than steady-state pharmacokinetic area-under-the-curve (AUC) profiles.[15,20] This latter limitation is especially important because investigations into the association between pharmacokinetic profile and adverse effects of calcineurin inhibitors suggest that their toxicities[23-25] are related to peak concentration (C_{max}), with improvement when the dose is reduced or withdrawn.[26-28]

CYP3A5 enzyme activity is greatest in the foregut and progressively decreases downstream through the bowel.[29] Conventional twice-daily tacrolimus (ie, immediate-release tacrolimus [IR-Tac]) undergoes immediate capsular release and rapid absorption in the proximal small bowel, leading to peak blood concentrations 90 to 120 minutes after administration (t_{max}). LCPT (originally LifeCycle Pharma Tacrolimus [Envarsus XR in the United States]) is a once-daily tacrolimus formulation with similar efficacy and safety to IR-Tac. LCPT's drug delivery technology results in delayed tacrolimus absorption throughout the gastrointestinal tract,[30,31] leading to longer t_{max} and increased bioavailability compared to IR-Tac. Studies have demonstrated that LCPT has a lower dose requirement than IR-Tac to achieve similar tacrolimus trough concentrations.[32,33] Whereas Clinical Pharmacogenetics Implementation Consortium guidelines for *CYP3A5* genotype and tacrolimus dosing are available for IR-Tac, there are currently no guidelines for once-daily tacrolimus. Given the pharmacokinetic differences of LCPT compared to IR-Tac, it is unlikely that the same recommendations are applicable.[34]

The purpose of this study was to advance understanding of the differences in tacrolimus exposure between African American *CYP3A5* expressers and *CYP3A5* nonexpressers using steady-state 24-hour pharmacokinetic profiling and to explore the hypothesis that pharmacogenetic differences between *CYP3A5* expressers and nonexpressers would be attenuated by delayed tacrolimus absorption with LCPT compared to immediate absorption with IR-Tac.

Methods

Study Design and Objectives

ASERTAA (A Study of Extended Release Tacrolimus in African Americans) was an open-label, prospective, randomized, 2-sequence, 3-period, crossover, pharmacogenetic study conducted at the University of Pennsylvania, University of Illinois, and Washington University School of Medicine (St. Louis) between November 25, 2013, and July 30, 2015 (Fig 1). The main study objective was to compare steady-state pharmacokinetics of once-daily LCPT tablets (dosed 15% lower than total daily IR-Tac dose) with evenly divided twice-daily IR-Tac capsules (Prograf [Astellas Pharma US, Inc] or its generic formulations [predominately Sandoz, Dr Reddy, and Accord formulations], for which the systemic exposure differs minimally compared to Prograf[35-37]) in stable African American kidney recipients, according to *CYP3A5* genotype. Secondary objectives were to confirm the total daily dose reduction in the LCPT group following conversion from IR-Tac and compare the safety and short-term efficacy of the 2 formulations. After completing the pharmacokinetic phase, patients had an option to enter a 5-month extended-use phase with their second assigned treatment.

Eligible patients were randomly assigned in a 1:1 ratio using a fixed-block randomization scheme, generated by an independent statistician before study initiation, to one of 2 sequences (Fig 1): sequence I: patients continued their current IR-Tac dose until study day 7, then switched to LCPT; sequence II: patients started on LCPT at 15% lower total daily dose than IR-Tac until study day 7, then switched to IR-Tac at its previous twice-daily dose. Each participant received the second assigned treatment from days 8 to 21. Twenty-four–hour pharmacokinetic profiles were obtained at days 7, 14, and 21. No immunosuppression dose adjustment was permitted during the pharmacokinetic phase. Patients continued concomitant immunosuppression (mycophenolate mofetil/mycophenolate sodium and corticosteroids) throughout the study per each institution's standard of care. Safety assessments were completed approximately 30 days after administration of the last study treatment for all patients. The study was reviewed and approved by the institutional review board (approval numbers: University of Pennsylvania: 818642; University of Illinois: 2014-0494; and Washington University: 201406026) in each center. This study was conducted in accordance with the Declaration of Helsinki; all participants provided informed consent.

Participants

Male or female deceased or living donor kidney recipients aged 18 to 70 years of African ancestry were invited to participate. Participants were at least 6 months posttransplantation (2 exceptions were granted: 1 patient, 5.9 months, and another, 4.7 months posttransplantation), with therapeutic tacrolimus concentrations (per center practice) on a stable IR-Tac dose and formulation. Eight patients at enrollment were taking medications known to have drug-drug interactions with tacrolimus and were required to continue the same dose of these medications (diltiazem hydrochloride, n = 1; azithromycin, n = 6; and amiodarone, n = 1) during the pharmacokinetics

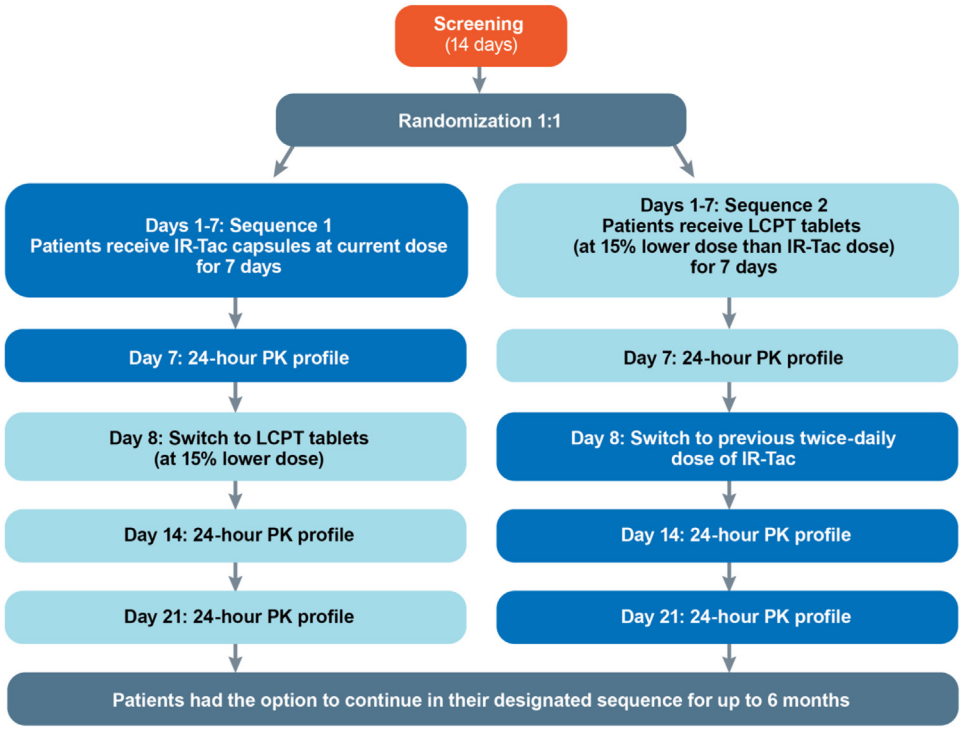

Figure 1. Study design. Abbreviations and definitions: IR-Tac, immediate-release tacrolimus; LCPT, extended-released tacrolimus (originally LifeCycle Pharma Tacrolimus); PK, pharmacokinetic.

study. Participants were not permitted to start new medications or products known to affect tacrolimus blood concentrations.

Study exclusion criteria included acute rejection within 3 months before enrollment, donor-specific antibody positivity, BK viremia, or estimated glomerular filtration rate ≤ 25 mL/min/1.73m^2.

Bioanalytic Methods

The central laboratory (University of Pennsylvania) conducted tacrolimus whole blood concentration analyses on all study samples according to principles of Good Laboratory Practice. The analysis of tacrolimus was performed with high-performance liquid chromatography followed by tandem mass spectrometry detection.

Genotyping

After DNA extraction, polymerase chain reaction–based genotypes for each of the candidate single-nucleotide polymorphisms (SNPs) were generated by TaqMan SNP genotyping assay performed within the Molecular Core facilities of the University of Pennsylvania. A detailed description of genotyping methods can be found in the supplementary material (Item S1). There were no undetermined genotypes in this analysis.

Participants were classified as nonexpressers if they possessed 2 variant loss-of-function *CYP3A5* alleles (ie, *CYP3A5* *3, *6, or *7). Individuals with only 1 or none of these variant alleles were presumed to have at least 1 functional *CYP3A5*1* allele and were considered to be expressers. The *CYP3A5* genotypic frequencies of the study population were in Hardy-Weinberg equilibrium.

Study End Points

Pharmacokinetic parameters included AUC from time 0 to 24 hours ($AUC_{0\text{-}24}$), C_{max}, t_{max}, minimum blood concentration observed over the 24-hour interval (C_{min}; also referenced as C_{24} because the value is obtained directly from the observed concentration data at the 24-hour nominal time point), predose concentration, percent peak-to-trough fluctuation of the drug concentration over the dosing interval (%Fluctuation = $100 \times [(C_{max} - C_{min})/C_{avg}]$, and percent concentration swing at the steady state (%Swing = $100 \times [(C_{max} - C_{min})/C_{min}]$; peak to trough ratio). Pharmacokinetic sampling times were predose and 0.50, 1.00, 1.50, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, 13.00, 14.00, 16.00, 18.00, and 24.00 hours postdose.

Safety end points included incidence and severity of treatment-emergent adverse events. Incidences of biopsy-proven or clinical rejection and deaths were captured for all randomly assigned participants. For safety measurements, laboratory specimens were analyzed at the local laboratory; the investigator classified each result as either clinically significant or not clinically significant.

Statistical Analysis

SAS software (version 9.3; SAS Institute Inc) was used to carry out the statistical analysis. A detailed description

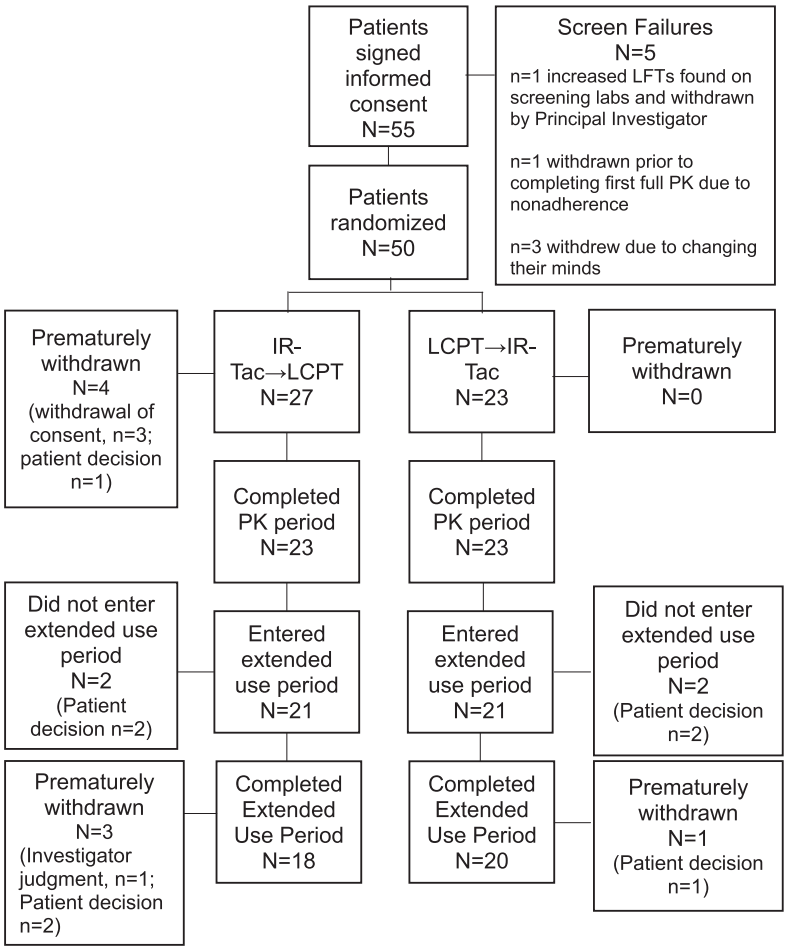

Figure 2. Patient attrition flow diagram. Abbreviations and definitions: IR-Tac, immediate-release tacrolimus; LCPT, extended-release tacrolimus (originally LifeCycle Pharma Tacrolimus); LFT, liver function test; PK, pharmacokinetic.

of analytical methods used in this study is provided in Item S1. All *P* values from inferential tests were reported as is without adjustment for multiple comparisons.

Sample Size Determination

Sample size determination was not based on formal statistical assumptions. In order to provide a descriptive evaluation of the pharmacokinetics of tacrolimus from LCPT and IR-Tac in the study population, a sample size of up to 72 male and female African American kidney recipients on stable immunosuppression regimens was planned.

Results

Participants

Fifty patients were randomly assigned and treated (n = 27 in sequence I [IR-Tac → LCPT], n = 23 in sequence II [LCPT → IR-Tac]); 46 patients completed the entire pharmacokinetics study of three 24-hour assessments (Fig 2); of these, 35 (76%) were *CYP3A5* expressers. Demographic and transplant characteristics across both treatment sequence groups were comparable (Table 1). Twenty-five (54%) participants had preexisting diabetes. Forty-two participants entered the extension phase (21 individuals per treatment group); 18 and 20 individuals in the LCPT and IR-Tac groups, respectively, completed this phase (Fig 2).

Overall Pharmacokinetics of LCPT and IR-Tac

Consistent with the study conversion protocol, the mean ± standard deviation total daily dose was 9.17 ± 4.02 mg for IR-Tac and 7.78 ± 3.44 mg for LCPT (Table 2). During both treatments, tacrolimus $AUC_{0\text{-}24}$ was maintained without dose adjustments for the entire pharmacokinetics phase. Overall, the estimated conversion ratio of 0.85 from IR-Tac to LCPT resulted in higher exposure in terms of C_{min} and $AUC_{0\text{-}24}$, with a ratio of geometric means of 112.8% ($P = 0.02$) and 112.6% ($P = 0.01$), respectively. IR-Tac was characterized by higher C_{max} and t_{max} of 1.1 hours, in contrast to LCPT, for which C_{max} was lower and t_{max} was 5.0 hours ($P < 0.001$ vs IR-Tac for C_{max} and t_{max}; Fig 3). C_{max} was ~30% lower during LCPT treatment than with IR-Tac ($P < 0.001$, ratio of geometric means of 71.7%, 90% confidence interval [CI], 64.8%-79.3%). Peak-to-trough fluctuation was reduced by ~40% ($P < 0.001$) and bioavailability of LCPT was 32% higher than for IR-Tac ($P < 0.001$). Differences in $AUC_{0\text{-}24}$, C_{min}, and C_{max} persisted after dose normalization. Estimated intra-individual coefficients of variation for $AUC_{0\text{-}24}$, C_{max}, and C_{min} for LCPT and IR-Tac were all <30%, the US

Table 1. Summary of Demographics and Baseline Characteristics by *CYP3A5* Genotype and Treatment Sequence in Patients From the Pharmacokinetic Population

Parameter	CYP3A5 Genotype		Treatment Sequence (PK Population)			
	Expresser (N = 35)	Nonexpresser (N = 11)	IR-Tac → LCPT (N = 23)	LCPT → IR-Tac (N = 23)	Study (N = 46)	Safety Population (N = 50)
Treatment sequence						
LCPT → IR-Tac	20 (57.1%)	3 (27.3%)	0 (0%)	23 (100.0%)	23 (50.0%)	23 (46%)
IR-Tac → LCPT	15 (42.9%)	8 (72.7%)	23 (100.0%)	0 (0%)	23 (50.0%)	27 (54%)
Age, y, mean ± SD	48.5 ± 10.85	54.0 ± 10.12	48.3 ± 9.87	51.4 ± 11.72	49.8 ± 10.83	49.8 ± 10.38
Black or African American ancestry	35 (100.0%)	11 (100.0%)	23 (100.0%)	23 (100.0%)	46 (100.0%)	50 (100%)
Sex						
Female	16 (45.7%)	3 (27.3%)	10 (43.5%)	9 (39.1%)	19 (41.3%)	21 (42.0%)
Male	19 (54.3%)	8 (72.7%)	13 (56.5%)	14 (60.9%)	27 (58.7%)	29 (58.0%)
Time from KTx to first dose of any study drug, mo	31.6 ± 28.68	44.2 ± 38.75	25.8 ± 26.97	43.5 ± 33.49	34.6 ± 31.38	32.8 ± 30.83
Kidney donor type						
Deceased	26 (74.3%)	8 (72.7%)	18 (78.3%)	16 (69.6%)	34 (73.9%)	36 (72.0%)
Living	9 (25.7%)	3 (27.3%)	5 (21.7%)	7 (30.4%)	12 (26.1%)	14 (28.0%)
Prior kidney transplant						
Yes	4 (11.4%)	1 (9.1%)	3 (13.0%)	2 (8.7%)	5 (10.9%)	6 (12.0%)
No	31 (88.6%)	10 (90.9%)	20 (87.0%)	21 (91.3%)	41 (89.1%)	44 (88.0%)
CYP3A5 genotype						
*1/*1	12 (34.3%)	0 (0%)	4 (17.4%)	8 (34.8%)	12 (26.1%)	13 (26.0%)
*1/*3	17 (48.6%)	0 (0%)	7 (30.4%)	10 (43.5%)	17 (37.0%)	19 (38.0%)
*1/*6	6 (17.1%)	0 (0%)	4 (17.4%)	2 (8.7%)	6 (13.0%)	6 (12.0%)
*3/*3	0 (0%)	4 (36.4%)	4 (17.4%)	0 (0%)	4 (8.7%)	4 (8.0%)
*3/*6	0 (0%)	6 (54.5%)	3 (13.0%)	3 (13.0%)	6 (13.0%)	6 (12.0%)
*6/*6	0 (0%)	1 (9.1%)	1 (4.3%)	0 (0%)	1 (2.2%)	1 (2.0%)
Missing						1 (2.0%)
Baseline weight, kg	88.5 ± 25.03	93.8 ± 19.25	88.1 ± 19.27	91.4 ± 27.75	89.7 ± 23.68	89.4 ± 23.75
Baseline BMI, kg/m^2	30.3 ± 6.85	31.2 ± 5.44	30.0 ± 4.80	31.0 ± 7.92	30.5 ± 6.50	30.3 ± 6.49
Screening trough concentration, ng/mL	6.7 ± 1.82	6.5 ± 2.14	6.9 ± 2.16	6.4 ± 1.55	6.7 ± 1.88	6.6 ± 1.82

Note: Values for continuous variables are given as count (percentage); for categorical variables, as mean ± SD.
Abbreviations and definitions: BMI, body mass index; KTx, kidney transplant; IR-Tac, immediate-release tacrolimus; LCPT, extended-release tacrolimus (originally LifeCycle Pharma Tacrolimus); PK, pharmacokinetics; SD, standard deviation.

Food and Drug Administration (FDA) threshold for a highly variable drug.[38]

Effect of CYP3A5 Genotype on Tacrolimus Total Daily Dose

The IR-Tac total daily dose was 10.1 mg/d among *CYP3A5* expressers and 6.3 mg/d for nonexpressers ($P < 0.01$; Table 3; Fig 4). Among *CYP3A5* expressers, tacrolimus daily dose requirements were higher in participants with 2 variant alleles (*CYP3A5*1*1) than in *CYP3A5*1*6 heterozygotes.

Effect of CYP3A5 Genotype on the Pharmacokinetics of IR-Tac and LCPT

During treatment with IR-Tac, there were no significant differences in overall $AUC_{0\text{-}24}$ or C_{min} between *CYP3A5* expressers and nonexpressers (Tables 3 and 4). However, C_{max} for *CYP3A5* expressers (25.51 ng/mL) was 33.9% higher (90% CI, 6.2%-68.8%; $P = 0.04$) than for nonexpressers (19.50 ng/mL; Fig 5A; Tables 3 and 4). When participants were treated with LCPT, there were no significant differences in $AUC_{0\text{-}24}$, C_{max}, or C_{min} between *CYP3A5* expressers and nonexpressers (Fig 5B; Tables 3 and 4).

Pharmacokinetics of IR-Tac and LCPT Stratified by CYP3A5 Genotype

Among *CYP3A5* expressers, LCPT C_{max} was 31.4% lower ($P < 0.001$) than that of IR-Tac; LCPT $AUC_{0\text{-}24}$ was 12.2% higher ($P = 0.04$) than that of IR-Tac (Table 5). Among *CYP3A5* nonexpressers, C_{max} and $AUC_{0\text{-}24}$ were similar between IR-Tac and LCPT, although C_{min} was significantly higher for LCPT than IR-Tac, reflecting our underestimation of the correct conversion ratio between tacrolimus formulations. On a milligram-to-milligram basis, bioavailability was increased in *CYP3A5* expressers and nonexpressers by 32.6% and 35.8% (data on file), respectively, with LCPT compared to IR-Tac.

ABCB1 Genotype

Forty-one percent of participants carried the C3435T variant allele of *ABCB1* (adenosine triphosphate–binding

Table 2. Summary of Pharmacokinetic Parameters and Comparisons With Observed and Dose-Normalized Data for Tacrolimus in Patients From the Pharmacokinetic Population

	Observed Result		Comparisons of LCPT vs IR-Tac[a]: RGM (90% CI)
	LCPT	IR-Tac	
TDD, mg/d[b]	7.78 ± 3.44	9.17 ± 4.02	
Observed parameters			
$AUC_{0\text{-}24}$,[c] h × ng/mL	255.82 (36.2)	226.73 (31.9)	112.6 (104.6 to 121.1); $P = 0.01$
C_{max}, ng/mL[c]	17.11 (40.1)	23.92 (41.2)	71.7 (64.8 to 79.3); $P < 0.001$
C_{min}, ng/mL[c]	7.35 (37.9)	6.50 (32.9)	112.8 (104.0 to 122.3); $P = 0.02$
t_{max}, h[d]	5.00 (1.00, 16.00)	1.13 (0.50, 14.0)	$P < 0.001$
Fluctuation, %[b]	94.21 ± 38.781	192.05 ± 77.145	−97.94 (−120.9 to −74.9); $P < 0.001$
C_{max}/C_{min}[b]	2.46 ± 0.760	3.94 ± 1.468	−1.479 (−1.901 to −1.056); $P < 0.001$
Dose-normalized parameters			
$AUC_{0\text{-}24\_D}$,[c] h×ng/mL/mg	36.37 (50.6)	27.36 (43.5)	132.6 (123.4 to 142.6); $P < 0.001$
C_{max_D},[c] ng/mL/mg	2.43 (43.0)	2.89 (44.6)	84.5 (76.5 to 93.4); $P = 0.01$
C_{min_D},[c] ng/mL/mg	1.05 (61.5)	0.78 (50.0)	132.8 (122.6 to 144.0); $P < 0.001$

Note: n = 46.
Abbreviations and definitions: $AUC_{0\text{-}24}$, area under the concentration-time curve from time 0 to 24 hours; CI, confidence interval; C_{max}, maximum observed concentration, peak; C_{min}, minimum blood concentration observed over the 24-hour interval (0-24 hours); this parameter is also referenced as C_{24} because the value is directly taken from the observed concentration data at the 24-hour nominal time point; IR-Tac, immediate-release tacrolimus; LCPT, extended-release tacrolimus (originally LifeCycle Pharma Tacrolimus); RGM, ratio of geometric means; TDD, total daily dose; t_{max}, time to maximum observed concentration.
[a]Treatment effect *P* value was calculated from the analysis used analysis of covariance models that included fixed effects of treatment, sequence, and period. Statements of RANDOM and REPEATED (effects) were used for the repeated measures in this 3-period partial replicated design. Estimates were based on the FA0(2) covariance structure and restricted maximum likelihood estimation method.
[b]Data presented are arithmetic mean ± standard deviation and differences in least squares mean (95% CI).
[c]Data presented are geometric means (% coefficient of variation of geometric mean) and ratios of geometric means (90% CI).
[d]Data presented are median (min, max). *P* value was from 2-sided Wilcoxon 2-sample rank sum test (t-approximation).

cassette subfamily B member 1: referred to as *ABCB1* AA or AG genotypes). Only 2 patients were homozygous for the C3435T variant allele and were not included in further analyses. Neither heterozygous nor homozygous *ABCB1* C3435T variant allele carriers had increased tacrolimus dose requirements. Among both *ABCB1* C3435T variant allele carriers and noncarriers, $AUC_{0\text{-}24}$ was significantly higher for LCPT than for IR-Tac. Within treatment groups, the number of *ABCB1* variant alleles did not significantly affect the weight-adjusted total daily dose or individually

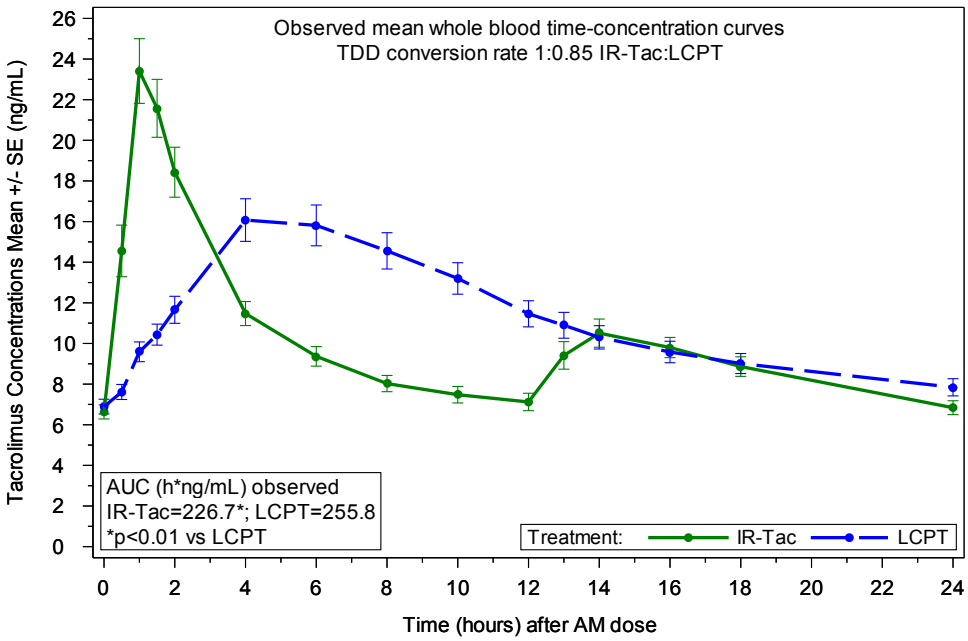

Figure 3. Observed mean tacrolimus whole blood time-concentration curves for immediate-release tacrolimus (IR-Tac) and LCPT (extended-release tacrolimus; originally LifeCycle Pharma Tacrolimus). Abbreviations: AUC, area under the curve; SE, standard error; TDD, total daily dose.

Table 3. Observed Pharmacokinetic Parameters by Treatment and *CYP3A5* Expresser Type From the Pharmacokinetic Population

	CYP3A5 Expresser (n = 35)		*CYP3A5* Nonexpresser (n = 11)	
PK Parameter	LCPT	IR-Tac	LCPT	IR-Tac
TDD,[a] mg/d	8.55 ± 3.42	10.09 ± 3.97	5.34 ± 2.18	6.27 ± 2.61[b]
Weight-normalized TDD,[a] mg/kg	0.103 ± 0.048	0.121 ± 0.056	0.058 ± 0.024	0.068 ± 0.029
$AUC_{0\text{-}24}$,[c] h × ng/mL	256.60 (34.9)	230.34 (26.5)	253.35 (42.0)	215.63 (47.5)
C_{max},[c] ng/mL	17.30 (39.0)	25.51 (37.4)	16.51 (45.4)	19.50 (47.3)[d]
C_{min},[c] ng/mL	7.23 (34.9)	6.52 (27.9)	7.78 (48.1)	6.41 (48.2)
C_0, ng/mL	6.32 (34.7)	6.26 (31.4)	7.04 (44.0)	6.24 (50.5)

Note: n = 46.
Abbreviations and definitions: $AUC_{0\text{-}24}$, area under the concentration-time curve from time 0 to 24 hours; C_{max}, maximum observed concentration, peak; C_{min}, minimum blood concentration observed over the 24-hour interval (0-24 hours); this parameter is also referenced as C_{24} because the value is directly taken from the observed concentration data at the 24-hour nominal time point; IR-Tac, immediate-release tacrolimus; LCPT, extended-release tacrolimus (originally LifeCycle Pharma Tacrolimus); PK, pharmacokinetics; TDD, total daily dose.
[a]Data presented are arithmetic mean and standard deviation.
[b]$P < 0.02$ for IR-Tac, nonexpresser versus expresser.
[c]Data presented are geometric mean (% geometric coefficient of variation).
[d]$P = 0.04$ for IR-Tac, nonexpresser versus expresser.

measured pharmacokinetic parameters for tacrolimus (Table S1). Within an *ABCB1* genotype (GG vs AG), the pattern of treatment differences was consistent with patterns observed in the general transplantation population.

Safety

Duration of treatment and total dose exposure were comparable between treatment sequence groups. The mean duration of treatment was 138.8 days (range, 8-233 days) for sequence I and 166.6 days (range, 21-240 days) for sequence II. Safety analysis of the 50 randomly assigned and dosed patients showed that mean dose exposures were 1,107.58 (range, 60.0-2,893.0) mg for sequence I and 1,448.07 (124.0-2,679.5) mg for sequence II.

No deaths or acute rejections occurred in study participants. The 2 treatment arms were comparable in treatment-emergent adverse events during both the pharmacokinetic and extended-use phases of the study. During the extended-use phase, 7 patients experienced a total of 11 serious adverse events, 5 events in 3 LCPT-treated patients and 6 events in 4 patients using IR-Tac.

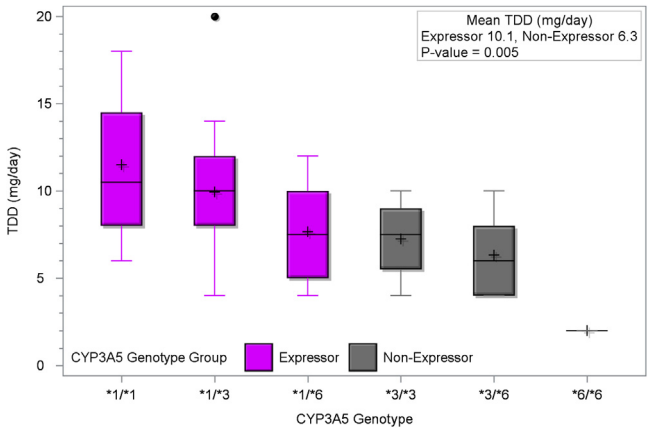

Figure 4. Tacrolimus total daily dose (TDD) by *CYP3A5* genotype.

Adverse Events According to CYP3A5 Genotype

An ad hoc analysis according to genotype of all adverse events observed during the entire study was performed and showed no statistically significant differences in adverse events (Table S2). No patients reported infectious episodes during the pharmacokinetics portion of the trial.

Discussion

This randomized, prospective, multicenter, crossover, pharmacogenetic study of tacrolimus is the first such investigation conducted exclusively in African American recipients. In this cohort, for which the 51% *CYP3A5**1 allelic frequency was similar to that in the general African American population,[18] there are several notable findings. First, achievement of a therapeutic tacrolimus trough concentration with IR-Tac was found to result in a 33% higher peak concentration among *CYP3A5* expressers compared with nonexpressers, an effect attenuated with LCPT. Second, LCPT has increased bioavailability compared to IR-Tac regardless of *CYPA3A5* genotype. Third, with conversion from IR-Tac to LCPT, a total daily dose reduction of 20% is generally appropriate to achieve equivalent exposure.

Modified-release drugs are commonly developed to attenuate fluctuation and reduce dosing administration frequency.[39] The 2 available once-daily tacrolimus formulations, LCPT and extended release tacrolimus (ER-Tac; Astagraf XL; Astellas Pharma US, Inc) are not bioequivalent to one another[40] or to IR-Tac.[41-45] Although conversion from IR-Tac to ER-Tac frequently necessitates a dose escalation to achieve similar trough concentrations and $AUC_{0\text{-}24}$,[13,46] conversion of ER-Tac or IR-Tac to LCPT has been demonstrated to require dose reductions of about 36% and 20%, respectively.[40] Until now, African American–specific tacrolimus 24-hour pharmacokinetic data

Table 4. Comparisons of *CYP3A5* Expresser and Nonexpresser Within a Treatment From the Pharmacokinetic Population

	LCPT		IR-Tac	
	RGM (90% CI), Expresser to Nonexpresser	*P*[a]	RGM (90% CI), Expresser to Nonexpresser	*P*[a]
$AUC_{0\text{-}24}$, h × ng/mL	105.7 (85.5-130.5)	0.7	108.4 (89.7-131.2)	0.5
C_{max}, ng/mL	111.2 (88.5-139.9)	0.4	133.9 (106.2-168.8)	0.04
C_{min}, ng/mL	94.7 (75.7-118.4)	0.7	102.7 (84.4-125.1)	0.8

Note: n = 46.

Abbreviations and definitions: $AUC_{0\text{-}24}$, area under the concentration-time curve from time 0 to 24 hours; CI, confidence interval; C_{max}, maximum observed concentration, peak; C_{min}, minimum blood concentration observed over the 24-hour interval (0-24 hours); this parameter is also referenced as C_{24} because the value is directly taken from the observed concentration data at the 24-hour nominal time point; IR-Tac, immediate-release tacrolimus; LCPT, extended-release tacrolimus (originally LifeCycle Pharma Tacrolimus); RGM, ratio of geometric means.

[a]*P* value of genotype effect was derived from mixed-effects analysis of covariance models that included fixed effects of genotype and period and random effect of participants (sequence) on log-transformed data; averages from period 2 (day 14) and period 3 (day 21) were calculated for each patient before analysis.

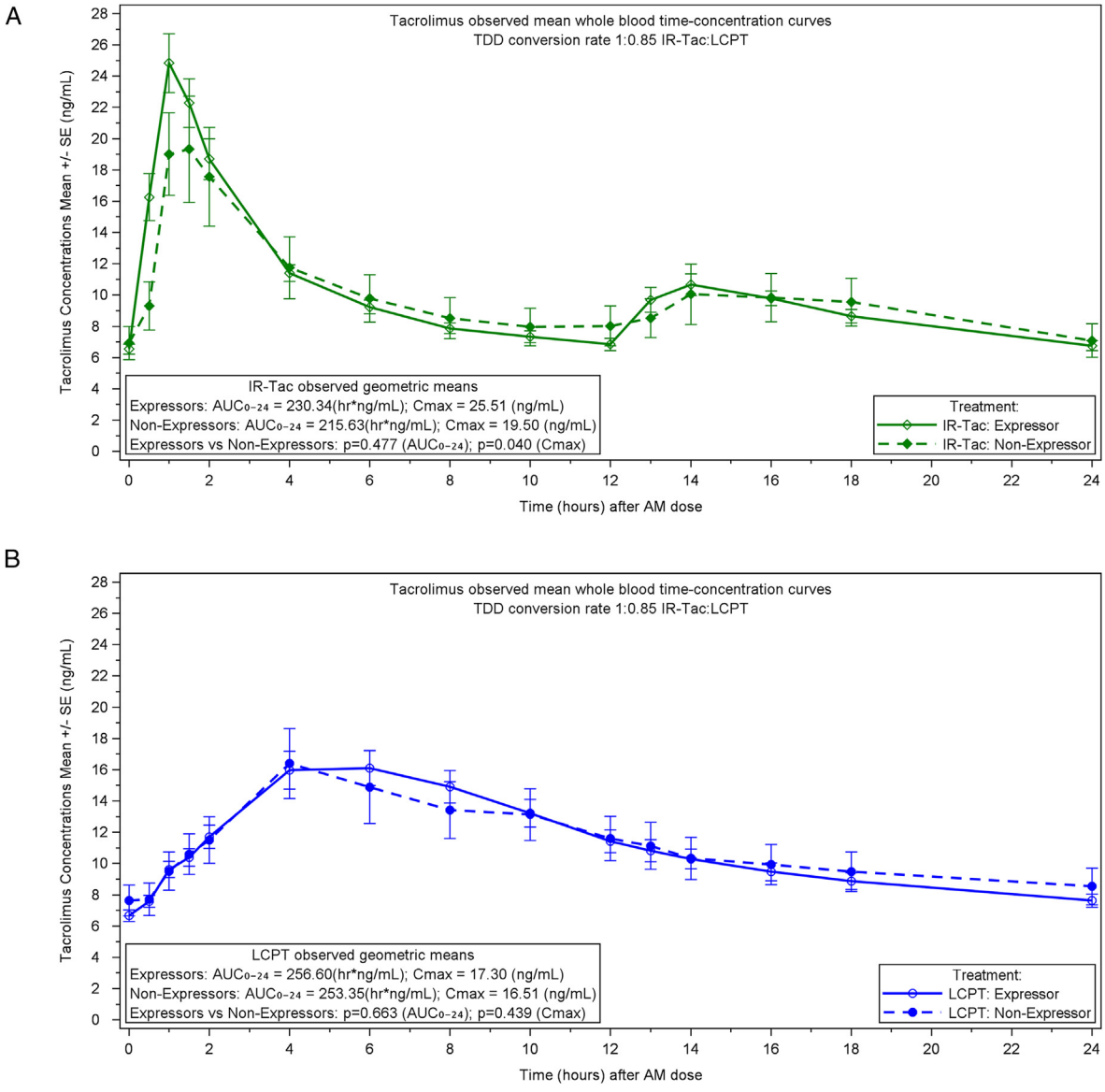

Figure 5. Observed mean tacrolimus whole blood time-concentration curves by *CYP3A5* expresser status for (A) immediate-release tacrolimus (IR-Tac) and (B) LCPT (extended-release tacrolimus; originally LifeCycle Pharma Tacrolimus). Abbreviations: $AUC_{0\text{-}24}$, area under the concentration-time curve from time 0 to 24 hours; C_{max}, maximum observed concentration, peak; SE, standard error; TDD, total daily dose.

Table 5. Comparisons Between Treatments Within *CYP3A5* Expresser and Nonexpresser From the Pharmacokinetic Population

	CYP3A5 Expresser (n = 35)		CYP3A5 Nonexpresser (n = 11)	
PK Parameter	RGM (90% CI), LCPT to IR-Tac	*P*[a]	RGM (90% CI), LCPT to IR-Tac	*P*[a]
$AUC_{0\text{-}24}$, h × ng/mL	112.2 (102.2-123.2)	0.04	116.1 (99.7-135.2)	0.1
C_{max}, ng/mL	68.6 (61.0-77.2)	<0.001	85.2 (64.7-112.3)	0.3
C_{min}, ng/mL	111.1 (100.1-123.2)	0.1	121.8 (104.3-142.2)	0.05

Note: n = 46.

Abbreviations and definitions: $AUC_{0\text{-}24}$, area under the concentration-time curve from time 0 to 24 hours; CI, confidence interval; C_{max}, maximum observed concentration, peak; C_{min}, minimum blood concentration observed over the 24-hour interval (0-24 hours); this parameter is also referenced as C_{24} because the value is directly taken from the observed concentration data at the 24-hour nominal time point; IR-Tac, immediate-release tacrolimus; LCPT, extended-release tacrolimus (originally LifeCycle Pharma Tacrolimus); PK, pharmacokinetics; RGM, ratio of geometric means.

[a]*P* value of treatment effect was derived from mixed-effects analysis of covariance models that included fixed effects of treatment and period and random effect of participant (sequence) on log-transformed data; averages from period 2 (day 14) and period 3 (day 21) were calculated for each patient before analysis.

have been lacking for all tacrolimus formulations, especially important in the context of *CYP3A5* genotype status.

Achieving adequate tacrolimus exposure, reflected by therapeutic trough concentrations, is critical for preventing rejection. In DeKAF, tacrolimus concentrations in African American recipients were subtherapeutic despite 60% higher tacrolimus dosing compared with non–African Americans in whom target concentrations were achieved; *CYP3A5*1* allele was the most important variant associated with measured concentrations.[15,20] Similar to DeKAF, we found that *CYP3A5* expressers required higher tacrolimus dosing than nonexpressers to maintain a therapeutic concentration, attributable to the fact that CYP3A5 enzyme, as a more efficient catalyst of tacrolimus than CYP3A4,[17] contributes to most CYP3A activity in carriers of the *CYP3A5*1* allele. In nonexpressers, the contribution of CYP3A5 enzyme is minimal and tacrolimus is primarily metabolized by CYP3A4.

We also observed that during IR-Tac administration, peak concentrations were 33% higher in *CYP3A5* expressers compared with nonexpressers, a difference not observed during LCPT therapy. We propose that when *CYP3A5* expressers are administered IR-Tac, CYP3A5 enzyme activity, which is highest in the proximal gut, where IR-Tac is absorbed,[29] results in more extensive presystemic metabolism than occurs in nonexpressers (for which CYP3A5 enzymes play a minor role and CYP3A4 predominates). The combined effects of IR-Tac's rapid absorption profile plus a 2-fold higher intrinsic tacrolimus clearance capacity of CYP3A5 enzyme compared to CYP3A4 enzyme leads to higher peak concentrations in expressers in order to achieve adequate drug exposure. In contrast, because CYP3A5 enzyme activity decreases downstream along the bowel, delayed and more distal gastrointestinal release and absorption in *CYP3A5* expressers during LCPT treatment leads to tacrolimus escaping some presystemic metabolism in the proximal gut. As a result, LCPT oral bioavailability is increased in *CYP3A5* expressers and the overall pharmacokinetic profile, including C_{max}, is similar to that in nonexpressers.

Kuypers et al[47] have previously demonstrated that with IR-Tac, tacrolimus C_{max} is inversely correlated with creatinine clearance. Moreover, through routine use of tacrolimus AUC profiling and surveillance kidney transplant biopsies, this same group has shown that persistent high tacrolimus dose requirement (>0.1 mg/kg/d) and the presence of at least 1 *CYP3A5*1* allele is associated with histologic evidence of chronic calcineurin inhibitor–associated nephrotoxicity and worse transplant outcomes.[48] As reviewed recently by Andrews et al,[49] emerging data with LCPT suggest that its lower daily dose requirement and lower C_{max} are associated with less toxicity.[36,50] Although it is possible that tacrolimus C_{max} could be inversely related to rejection in organ recipients, a notion embraced by some during the cyclosporine era,[51,52] this has not been demonstrated in current clinical practice. A recent pooled analysis of 2 phase 3 trials comparing LCPT to IR-Tac further demonstrated lower efficacy failure rates in black kidney recipients treated with LCPT.[53]

It is notable that the use of *CYP3A5* genotype–based tacrolimus dosing has not shown an impact on transplant or patient outcomes in contemporary European trials.[54-57] Because patients of African ancestry constituted <8% of participants in these trials, these study findings may not be generalizable to the US transplantation population, where African Americans make up one-third of deceased donor kidney recipients.[12]

Toward the end of our study, the *CYP3A5*7* allele, largely confined to individuals originating from the Niger-Congo region (range, 0%-22%),[58] was demonstrated to affect tacrolimus metabolism.[20] Because failure to perform *CYP3A5*7* genotyping may therefore result in misclassification,[59] we performed an ad hoc analysis in our study participants. Only 1 individual was a *CYP3A5*7* allele expresser. Although this resulted in reclassification from *1/*1 to *1/*7, the participant remained classified as a *CYP3A5* expresser by our study definition and study results were not affected.

Strengths of this study include a multicenter, prospective, randomized, crossover trial design and an exclusively African American population, consistently underrepresented in clinical transplantation studies. Additional strengths include a centralized laboratory for genotype and pharmacokinetic sample testing, incorporation of steady-state 24-hour pharmacokinetic AUC profiling rather than trough concentrations typically used in most contemporary studies, and a study design that precluded

changes in immunosuppression dosing or addition of medications known to interfere with tacrolimus blood concentrations during the entire pharmacokinetic study phase.

Limitations include that this was a small sample, primarily a pharmacokinetic study rather than clinical efficacy study, and that the follow-up period was too short to capture meaningful clinical outcomes. Because participants were from the East Coast and Midwest, the study findings may not be generalizable to African American populations elsewhere in the United States.

In conclusion, our study demonstrates that with the use of IR-Tac, achievement of therapeutic tacrolimus concentrations in most African Americans resulted in much higher peak concentrations, with potential for enhanced toxicity and adverse outcomes. With LCPT, the shape of the pharmacokinetics profile was not affected by *CYP3A5* genotype, and tacrolimus exposure was maintained at ~80% of the IR-Tac total daily dose. Results from this study additionally indicate that the pharmacokinetics of LCPT is less influenced by *CYP3A5* genotype in African Americans, and LCPT has distinctive pharmacogenetic differences compared to IR-Tac in this population. Studies are ongoing to determine whether these pharmacogenetic differences represent an opportunity for LCPT to optimize immunosuppression management in African American patients and thereby narrow health outcome disparities in kidney transplantation.

Supplementary Material

Item S1: Detailed description of the genotyping and analytical methods used.

Table S1: Comparisons of observed pharmacokinetic parameters between *MDR1* GG and AG genotypes within a treatment in patients from the PK PP population (n = 44).

Table S2: Treatment-emergent adverse events during the study (PK portion and extended-use phase).

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References

1. Health Resources and Services Administration, US Department of Health & Human Services. Transplant: Ethnicity by Donor Type, Transplant Year (2014-2015) https://optn.transplant.hrsa.gov/data/view-data-reports/build-advanced/. Accessed October 21, 2016.
2. Branson RD, Davis KJ, Butler KL. African Americans' participation in clinical research: importance, barriers, and solutions. *Am J Surg*. 2007;193(1):32-39.
3. Blosser CD, Huverserian A, Bloom RD, et al. Age, exclusion criteria, and generalizability of randomized trials enrolling kidney transplant recipients. *Transplantation*. 2011;91(8):858-863.
4. Narayanan M, Pankewycz O, Shihab F, et al. Long-term outcomes in African American kidney transplant recipients under contemporary immunosuppression: a four-yr analysis of the Mycophenolic acid Observational REnal transplant (MORE) study. *Clin Transplant*. 2014;28(2):184-191.
5. Laging M, Kal-van Gestel JA, van de Wetering J, et al. Understanding the influence of ethnicity and socioeconomic factors

on graft and patient survival after kidney transplantation. *Transplantation*. 2014;98(9):974-978.
6. Taber DJ, Douglass K, Srinivas T, et al. Significant racial differences in the key factors associated with early graft loss in kidney transplant recipients. *Am J Nephrol*. 2014;40(1):19-28.
7. Malek SK, Keys BJ, Kumar S, Milford E, Tullius SG. Racial and ethnic disparities in kidney transplantation. *Transpl Int*. 2011;24(5):419-424.
8. Gordon EJ, Ladner DP, Caicedo JC, Franklin J. Disparities in kidney transplant outcomes: a review. *Semin Nephrol*. 2010;30(1):81-89.
9. Williams WW, Delmonico FL. The end of racial disparities in kidney transplantation? Not so fast! *J Am Soc Nephrol*. 2016;27(8):2224-2226.
10. Williams WW, Pollak MR. Health disparities in kidney disease—emerging data from the human genome. *N Engl J Med*. 2013;369(23):2260-2261.
11. Axelrod DA, Naik AS, Schnitzler MA, et al. National variation in use of immunosuppression for kidney transplantation: a call for evidence-based regimen selection. *Am J Transplant*. 2016;16(8):2453-2462.
12. Hart A, Smith JM, Skeans MA, et al. Kidney. *Am J Transplant*. 2016;16(suppl 2):11-46.
13. Niioka T, Satoh S, Kagaya H, et al. Comparison of pharmacokinetics and pharmacogenetics of once- and twice-daily tacrolimus in the early stage after renal transplantation. *Transplantation*. 2012;94(10):1013-1019.
14. Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: part I. *Clin Pharmacokinet*. 2010;49(3):141-175.
15. Jacobson PA, Oetting WS, Brearley AM, et al. Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. *Transplantation*. 2011;91(3):300-308.
16. Srinivas TR, Meier-Kriesche HU, Kaplan B. Pharmacokinetic principles of immunosuppressive drugs. *Am J Transplant*. 2005;5(2):207-217.
17. 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.
18. Dirks NL, Huth B, Yates CR, Meibohm B. Pharmacokinetics of immunosuppressants: a perspective on ethnic differences. *Int J Clin Pharmacol Ther*. 2004;42(12):701-718.
19. Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet*. 2001;27(4):383-391.
20. Oetting WS, Schladt DP, Guan W, et al. Genomewide association study of tacrolimus concentrations in African American kidney transplant recipients identifies multiple CYP3A5 alleles. *Am J Transplant*. 2015;16(2):574-582.
21. Taber DJ, Gebregziabher MG, Srinivas TR, et al. African-American race modifies the influence of tacrolimus concentrations on acute rejection and toxicity in kidney transplant recipients. *Pharmacotherapy*. 2015;35(6):569-577.
22. Sanghavi K, Brundage RC, Miller MB, et al. Genotype-guided tacrolimus dosing in African-American kidney transplant recipients. *Pharmacogenomics J*. 2015;17(1):61-68.
23. Perico N, Ruggenenti P, Gaspari F, et al. Daily renal hypoperfusion induced by cyclosporine in patients with renal transplantation. *Transplantation*. 1992;54(1):56-60.
24. Italia JL, Bhatt DK, Bhardwaj V, Tikoo K, Kumar MN. PLGA nanoparticles for oral delivery of cyclosporine: nephrotoxicity and pharmacokinetic studies in comparison to Sandimmune Neoral®. *J Control Release*. 2007;119(2):197-206.
25. Binet I, Wallnöfer A, Weber C, Jones R, Thiel G. Renal hemodynamics and pharmacokinetics of bosentan with and without cyclosporine A. *Kidney Int*. 2000;57(1):224-231.
26. Bechstein WO. Neurotoxicity of calcineurin inhibitors: impact and clinical management. *Transpl Int*. 2000;13(5):313-326.
27. Eidelman BH, Abu-Elmagd K, Wilson J, et al. Neurologic complications of FK 506. *Transplant Proc*. 1991;23(6):3175-3178.
28. Abouljoud MS, Kumar MSA, Brayman KL, et al. Neoral® rescue therapy in transplant patients with intolerance to tacrolimus. *Clin Transplant*. 2002;16(3):168-172.
29. Thörn M, Finnström N, Lundgren S, Rane A, Lööf L. Cytochromes P450 and MDR1 mRNA expression along the human gastrointestinal tract. *Br J Clin Pharmacol*. 2005;60(1):54-60.
30. Gavhanea YN, Yadavb AV. Loss of orally administered drugs in GI tract. *Saudi Pharm J*. 2012;20(4):331-344.
31. Nigro V, Glicklich A, Weinberg J. Improved bioavailability of MELTDOSE once-daily formulation of tacrolimus (LCP-Tacro) with controlled agglomeration allows for consistent absorption over 24 hrs: a scintigraphic and pharmacokinetic evaluation. Presented at: American Transplant Congress, May 19, 2013, Seattle, WA. Abstract number: B1034.
32. Bunnapradist S, Ciechanowski K, West-Thielke P, et al. Conversion from twice-daily tacrolimus to once-daily extended release tacrolimus (LCPT): the phase III randomized MELT Trial. *Am J Transplant*. 2013;13(3):760-769.
33. Budde K, Bunnapradist S, Grinyo JM, et al. Once daily LCP-Tacro MeltDose® tacrolimus vs. twice daily tacrolimus in de novo kidney transplants: one-year results of phase 3, double-blind, randomized trial. *Am J Transplant*. 2014;14:2796-2806.
34. 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.
35. Trofe-Clark J, Gabardi S, McDevitt-Potter L, Alloway RR. Immunosuppression, generic drugs and the FDA. *Am J Transplant*. 2012;12(3):792-793.
36. Davit BM, Nwakama PE, Buehler GJ, et al. Comparing generic and innovator drugs: a review of 12 years of bioequivalence data from the United States Food and Drug Administration. *Ann Pharmacother*. 2009;43(10):1583-1597.
37. Yu Y, Teerenstra S, Neef C, Burger D, Maliepaard M. Investigation into the interchangeability of generic formulations using immunosuppressants and a broad selection of medicines. *Eur J Clin Pharmacol*. 2015;71(8):979-990.
38. Davit BM, Chen M-L, Conner DP, et al. Implementation of a reference-scaled average bioequivalence approach for highly variable generic drug products by the US Food and Drug Administration. *AAPS J*. 2012;14(4):915-924.
39. Endrenyi L, Tothfalusi L. Metrics for the evaluation of bioequivalence of modified-release formulations. *AAPS J*. 2012;14(4):813-819.
40. Tremblay S, Nigro V, Weinberg J, Woodle ES, Alloway RR. A steady-state head-to-head pharmacokinetic comparison of all FK-506 (tacrolimus) formulations (ASTCOFF): an open-label, prospective, randomized, two-arm, three-period crossover study. *Am J Transplant*. 2017;17(2):432-442.
41. Silva HT, Yang HC, Abouljoud M, et al. One-year results with extended-release tacrolimus/MMF, tacrolimus/MMF and cyclosporine/MMF in de novo kidney transplant recipients. *Am J Transplant*. 2007;7(3):595-608.
42. Krämer BK, Charpentier B, Bäckman L, et al. Tacrolimus once daily (ADVAGRAF) versus twice daily (PROGRAF) in de novo

renal transplantation: a randomized phase III study. *Am J Transplant*. 2010;10(12):2632-2643.
43. de Jonge H, Kuypers DR, Verbeke K, Vanrenterghem Y. Reduced C0 concentrations and increased dose requirements in renal allograft recipients converted to the novel once-daily tacrolimus formulation. *Transplantation*. 2010;90(5):523-529.
44. Alloway R, Steinberg S, Khalil K, et al. Conversion of stable kidney transplant recipients from a twice daily Prograf-based regimen to a once daily modified release tacrolimus-based regimen. *Transplant Proc*. 2005;37(2):867-870.
45. Slatinska J, Rohal T, Wohlfahrtova M, Viklicky O. Long-term follow-up of stable kidney transplant recipients after conversion from tacrolimus twice daily immediate release to tacrolimus once-daily prolonged release: a large single-center experience. *Transplant Proc*. 2013;45(4):1491-1496.
46. Barraclough K, Isbel N, Johnson D, Campbell S, Staatz C. Once- versus twice-daily tacrolimus: are the formulations truly equivalent? *Drugs*. 2011;71(12):1561-1577.
47. Kuypers DJ, Claes K, Evenepoel P, et al. Time-related clinical determinants of long-term tacrolimus pharmacokinetics in combination therapy with mycophenolic acid and corticosteroids. *Clin Pharmacokinet*. 2004;43(11):741-762.
48. Kuypers DRJ, Naesens M, de Jonge H, et al. Tacrolimus dose requirements and CYP3A5 genotype and the development of calcineurin inhibitor-associated nephrotoxicity in renal allograft recipients. *Ther Drug Monit*. 2010;32(4):394-404.
49. Andrews LM, De Winter BCM, Van Gelder T, Hesselink DA. Consideration of the ethnic prevalence of genotypes in the clinical use of tacrolimus. *Pharmacogenomics*. 2016;17(16):1737-1740.
50. Langone A, Steinberg SM, Gedaly R, et al. Switching STudy of Kidney TRansplant PAtients with Tremor to LCP-TacrO (STRATO): an open-label, multicenter, prospective phase 3b study. *Clin Transplant*. 2015;29(9):796-805.
51. Levy G, Thervet E, Lake J, Uchida K; group obotC. Patient management by Neoral C2 monitoring: an international consensus statement. *Transplantation*. 2002;73(9 suppl):S12-S18.
52. International Neoral Renal Transplantation Study Group. Randomized, international study of cyclosporine microemulsion absorption profiling in renal transplantation with basiliximab immunoprophylaxis. *Am J Transplant*. 2002;2(2):157-166.
53. Bunnapradist S, Rostaing L, Alloway RR, et al. LCPT once-daily extended-release tacrolimus tablets versus twice-daily capsules: a pooled analysis of two phase 3 trials in important de novo and stable kidney transplant recipient subgroups. *Transpl Int*. 2016;29(5):603-611.
54. Yaowakulpatana K, Vadcharavivad S, Ingsathit A, et al. Impact of CYP3A5 polymorphism on trough concentrations and outcomes of tacrolimus minimization during the early period after kidney transplantation. *Eur J Clin Pharmacol*. 2016;72(3):277-283.
55. Shuker N, Bouamar R, van Schaik RHN, et al. A randomized controlled trial comparing the efficacy of Cyp3a5 genotype-based with body-weight-based tacrolimus dosing after living donor kidney transplantation. *Am J Transplant*. 2016;16(7):2085-2096.
56. De Meyer M, Haufroid V, Kanaan N, et al. Pharmacogenetic-based strategy using de novo tacrolimus once daily after kidney transplantation: prospective pilot study. *Pharmacogenomics*. 2016;17(9):1019-1027.
57. Pallet N, Etienne I, Buchler M, et al. Long-term clinical impact of adaptation of initial tacrolimus dosing to CYP3A5 genotype. *Am J Transplant*. 2016;16(9):2670-2675.
58. Bains RK, Kovacevic M, Plaster CA, et al. Molecular diversity and population structure at the cytochrome P450 3A5 gene in Africa. *BMC Genet*. 2013;14(1):1-18.
59. Macphee IAM, Fredericks S, Tai T, et al. Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome p4503A5 and p-glycoprotein correlate with dose requirement. *Transplantation*. 2002;74(11):1486-1489.