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

Jennifer Trofe-Clark, Daniel C. Brennan, Patricia West-Thielke, Michael C. Milone, Mary Ann Lim, Robin Neubauer, Vincenza Nigro, and Roy D. Bloom

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 (AUC0-24), peak and trough concentrations (Cmax and Cmin), 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 AUC0-24 or Cmin between CYP3A5 expressers and nonexpressers during administration of either IR-Tac or LCPT. With IR-Tac, tacrolimus Cmax 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.

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

Factors that affect tacrolimus pharmacokinetics include sex, ethnicity, concomitant medications, and genetic polymorphisms. 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. 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). The CYP3A5*1 allele, found predominantly but not exclusively in individuals of black African descent, encodes CYP3A5 enzymes that are associated with rapid tacrolimus disposition (CYP3A5 expressers), leading to subtherapeutic concentrations and increased dose requirements.
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, supporting tacrolimus underexposure as an inferior transplant outcome determinant in this population.

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, and (3) measurement of trough concentrations rather than steady-state pharmacokinetic area-under-the-curve (AUC) profiles. This latter limitation is especially important because investigations into the association between pharmacokinetic profile and adverse effects of calcineurin inhibitors suggest that their toxicities are related to peak concentration (C\(_{\text{max}}\)), with improvement when the dose is reduced or withdrawn.

CYP3A5 enzyme activity is greatest in the foregut and progressively decreases downstream through the bowel. 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\(_{\text{max}}\)). 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, leading to longer t\(_{\text{max}}\) 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. 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.

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) 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...
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/1.73m².

**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 (AUC0-24), Cmax, tmax, minimum blood concentration observed over the 24-hour interval (Cmin; also referenced as C24 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 × [(Cmax - Cmin)/Cavg]), and percent concentration swing at the steady state (%Swing = 100 × [(Cmax - Cmin)/Cmin]; 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
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 AUC0-24 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 Cmin and AUC0-24, 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 Cmax and tmax of 1.1 hours, in contrast to LCPT, for which Cmax was lower and tmax was 5.0 hours (P < 0.001 vs IR-Tac for Cmax and tmax; Fig 3). Cmax 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 AUC0-24, Cmax, and Cmin for LCPT and IR-Tac were all <30%, the US
Food and Drug Administration (FDA) threshold for a highly variable drug.\textsuperscript{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\textscript{0-24}, C\textsubscript{max}, or C\textsubscript{min} between CYP3A5 expressers and nonexpressers (Tables 3 and 4). However, C\textsubscript{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\textscript{0-24}, C\textsubscript{max}, or C\textsubscript{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\textsubscript{max} was 31.4% lower ($P < 0.001$) than that of IR-Tac; LCPT AUC\textsubscript{0-24} was 12.2% higher ($P = 0.04$) than that of IR-Tac (Table 5). Among CYP3A5 nonexpressers, C\textsubscript{max} and AUC\textsubscript{0-24} were similar between IR-Tac and LCPT, although C\textsubscript{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
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, AUC0-24 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

| Table 2. Summary of Pharmacokinetic Parameters and Comparisons With Observed and Dose-Normalized Data for Tacrolimus in Patients From the Pharmacokinetic Population |
|-----------------|-----------------|-----------------|
| **Observed Result** | **IR-Tac** | **Comparisons of LCPT vs IR-Tac**: |
| TDD, mg/d<b> | 7.78 ± 3.44 | 9.17 ± 4.02 |
| **Observed parameters** | | |
| AUC0-24, h × ng/mL | 255.82 (36.2) | 226.73 (31.9) | 112.6 (104.6 to 121.1); P = 0.01 |
| Cmax, ng/mL | 17.11 (40.1) | 23.92 (41.2) | 71.7 (64.8 to 79.3); P < 0.001 |
| Cmin, ng/mL | 7.35 (37.9) | 6.50 (32.9) | 112.8 (104.0 to 122.3); P = 0.02 |
| tmax, h<b> | 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 |
| Cmax/Cmin<sup>b</sup> | 2.46 ± 0.760 | 3.94 ± 1.468 | -1.479 (-1.901 to -1.056); P < 0.001 |
| **Dose-normalized parameters** | | |
| AUC0-24_D, h × ng/mL/mg | 36.37 (50.6) | 27.36 (43.5) | 132.6 (123.4 to 142.6); P < 0.001 |
| Cmax_D, ng/mL/mg | 2.43 (43.0) | 2.89 (44.6) | 84.5 (76.5 to 93.4); P = 0.01 |
| Cmin_D, 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: AUC0-24, area under the concentration-time curve from time 0 to 24 hours; CI, confidence interval; Cmax, maximum observed concentration, peak; Cmin, minimum blood concentration observed over the 24-hour interval (0-24 hours); this parameter is also referenced as C24 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; tmax, time to maximum observed concentration.

<sup>a</sup>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.

<sup>b</sup>Data presented are arithmetic mean ± standard deviation and differences in least squares mean (95% CI).
<sup>c</sup>Data presented are geometric means (% coefficient of variation of geometric mean) and ratios of geometric means (90% CI).
<sup>d</sup>Data presented are median (min, max). P value was from 2-sided Wilcoxon 2-sample rank sum test (t-approximation).

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.
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.

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, 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 CYP3A5 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. 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 or to IR-Tac. Although conversion from IR-Tac to ER-Tac frequently necessitates a dose escalation to achieve similar trough concentrations and AUC_{0-24}, conversion of ER-Tac or IR-Tac to LCPT has been demonstrated to require dose reductions of about 36% and 20%, respectively. Until now, African American–specific tacrolimus 24-hour pharmacokinetic data

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

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>CYP3A5 Expresser (n = 35)</th>
<th>CYP3A5 Nonexpresser (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCPT</td>
<td>IR-Tac</td>
</tr>
<tr>
<td>TDD, mg/d</td>
<td>8.55 ± 3.42</td>
<td>10.09 ± 3.97</td>
</tr>
<tr>
<td>Weight-normalized TDD, mg/kg</td>
<td>0.103 ± 0.048</td>
<td>0.121 ± 0.056</td>
</tr>
<tr>
<td>AUC_{0-24}, h × ng/mL</td>
<td>256.60 (34.9)</td>
<td>230.34 (26.5)</td>
</tr>
<tr>
<td>C_{max}, ng/mL</td>
<td>17.30 (39.0)</td>
<td>25.51 (37.4)</td>
</tr>
<tr>
<td>C_{min}, ng/mL</td>
<td>7.23 (34.9)</td>
<td>6.52 (27.9)</td>
</tr>
<tr>
<td>C_{0}, ng/mL</td>
<td>6.32 (34.7)</td>
<td>6.26 (31.4)</td>
</tr>
</tbody>
</table>

Note: n = 46.

Abbreviations and definitions: AUC_{0-24}, 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.

aData presented are arithmetic mean and standard deviation.

bP < 0.02 for IR-Tac, nonexpresser versus expresser.

cData presented are geometric mean (% geometric coefficient of variation).

dP = 0.04 for IR-Tac, nonexpresser versus expresser.
Table 4. Comparisons of CYP3A5 Expresser and Nonexpresser Within a Treatment From the Pharmacokinetic Population

<table>
<thead>
<tr>
<th></th>
<th>LCPT (90% CI), Expresser to Nonexpresser</th>
<th>P ( ^{a} )</th>
<th>IR-Tac (90% CI), Expresser to Nonexpresser</th>
<th>P ( ^{a} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{0-24}), h × ng/mL</td>
<td>105.7 (85.5-130.5)</td>
<td>0.7</td>
<td>108.4 (89.7-131.2)</td>
<td>0.5</td>
</tr>
<tr>
<td>C(_{\text{max}}), ng/mL</td>
<td>111.2 (88.5-139.9)</td>
<td>0.4</td>
<td>133.9 (106.2-168.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>C(_{\text{min}}), ng/mL</td>
<td>94.7 (75.7-118.4)</td>
<td>0.7</td>
<td>102.7 (84.4-125.1)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Note: \( n = 46. \)

Abbreviations and definitions: AUC\(_{0-24}\), area under the concentration-time curve from time 0 to 24 hours; CI, confidence interval; C\(_{\text{max}}\), maximum observed concentration, peak; C\(_{\text{min}}\), 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\(_{0-24}\), area under the concentration-time curve from time 0 to 24 hours; C\(_{\text{max}}\), maximum observed concentration, peak; SE, standard error; TDD, total daily dose.
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 CYP3A3 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 Cmax, is similar to that in nonexpressers.

Kuypers et al 37 have previously demonstrated that with IR-Tac, tacrolimus Cmax 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. 45 As reviewed recently by Andrews et al, 49 emerging data with LCPT suggest that its lower daily dose requirement and lower Cmax are associated with less toxicity. 15,60 Although it is possible that tacrolimus Cmax 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. 15

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. 14,57 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. 21 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

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

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>CYP3A5 Expresser (n = 35)</th>
<th>CYP3A5 Nonexpresser (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–24, h × ng/mL</td>
<td>112.2 (102.2-123.2)</td>
<td>116.1 (99.7-135.2)</td>
</tr>
<tr>
<td>Cmin, ng/mL</td>
<td>68.6 (61.0-77.2)</td>
<td>85.2 (64.7-112.3)</td>
</tr>
<tr>
<td>Cmax, ng/mL</td>
<td>111.1 (100.1-123.2)</td>
<td>121.8 (104.3-142.2)</td>
</tr>
</tbody>
</table>

Note: n = 46. Abbreviations and definitions: AUC0–24, area under the concentration-time curve from time 0 to 24 hours; CI, confidence interval; Cmax, maximum observed concentration, peak; Cmin, minimum blood concentration observed over the 24-hour interval (0-24 hours); this parameter is also referenced as C24 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.

*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.

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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).

Article Information

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**Support**: This study was funded by Veloxis Pharmaceuticals, including institutional funding to Dr Trofe-Clark and Ms Neubauer. Medical writing assistance was provided by Kristin Kiattler, PhD, Evidera, and was funded by Veloxis; the final decisions on the primary and secondary outcomes, the relevant data to be reported in the manuscript, and the main points to be communicated in the manuscript, particularly in the conclusions drawn, were made by Dr Bloom. As an investigator-initiated (Drs Bloom and Trofe-Clark) Veloxis-sponsored phase 3b clinical trial, Veloxis participated in the planning, protocol development, site selection, data analysis, preparation of publication, etc. This study was also submitted to the FDA for review and commentary before implementation because this study had the potential for change to the Envarsus XR label, which is the case because results of this study were included in the most recent revision of the Envarsus XR Product Information in June 2016. Statistical support was provided by Wei Du, PhD.

**Financial Disclosure**: Dr Trofe-Clark: paid speaker in a Veloxis Renal Transplant Expert Advisory Board Meeting in November 2015. Dr Brennan: grants/research support: Alexion, Astellas, Bristol-Meyers Squibb, CareDx, Oxford Immunotec, Shire, Pfizer, Veloxis; consultant: Alexion, CareDx, Immucor, Sanofi, Veloxis; honoraria/speakers bureau: Alexion, Astellas, Novartis, Sanofi, Veloxis; other: royalties from UpToDate. Dr West-Thielke: Veloxis (grant support, advisory boards, and speakers bureau); Astellas (grant support, advisory boards). Ms Nigro: employee of Veloxis pharmaceuticals. Dr Bloom: grants/research support: Alexion, CareDx, Shire, Veloxis; consultant: Abbvie, CareDx, Glaxo Smith Kline, Merck, Spark Therapeutics, Veloxis; board position: Public Policy Board, American Society of Nephrology; other: royalties from UpToDate. The remaining authors declare that they have no other relevant financial interests.

**Peer Review**: Received January 23, 2017. Evaluated by 4 external peer reviewers and a statistician, with editorial input from an Acting Editor-in-Chief (Editorial Board Member Thomas D. Nolin, PharmD, PhD). Accepted in revised form July 20, 2017. The involvement of an Acting Editor-in-Chief to handle the peer-review and decision-making processes was to comply with *AJKD’s* procedures for potential conflicts of interest for editors, described in the Information for Authors & Journal Policies.

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