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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

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This two-sequence, three-period crossover study is the first pharmacokinetic (PK) study to compare all three innovator formulations of tacrolimus (twice-daily immediate-release tacrolimus capsules [IR-Tac]; once-daily extended-release tacrolimus capsules [ER-Tac]; novel once-daily tacrolimus tablets [LCPT]). Stable renal transplant patients were dosed with each drug for 7 days, and blood samples were obtained over 24 h. Thirty subjects were included in the PK analysis set. A conversion factor of 1:1:0.80 for IR-Tac: ER-Tac:LCPT was used; no dose adjustments were permitted during the study. The median (interquartile range) total daily dose was 6.0 (4.0–8.0) mg for IR-Tac and ER-Tac and 4.8 (3.3–6.3) for LCPT. Significantly higher exposure on a per milligram basis, lower intra-day fluctuation and prolonged time (T_{max}) to peak concentration (C_{max}) were found for LCPT versus IR-Tac or ER-Tac. ER-Tac showed no differences versus IR-Tac in exposure, C_{max}, T_{max} or fluctuation. The observed exposure of IR-Tac was used to normalize exposure for LCPT and ER-Tac, resulting in the following recommended total daily dose conversion rates: IR-Tac:ER-Tac, +8%; IR-Tac:LCPT, −30%; ER-Tac:LCPT, −36%. After exposure normalization, C_{max} was ~17% lower for LCPT than for IR-Tac or ER-Tac; C_{min} was ~6% lower for LCPT compared with IR-Tac and 3% higher compared with ER-Tac.

Abbreviations: AE, adverse event; ANCOVA, analysis of covariance; ASTCOFF, a steady-state pharmacokinetic comparison of all FK-506 formulations; AUC, area under the curve; BMI, body mass index; BPAR, biopsy-proven acute rejection; C_0, predose concentration; C_{avg}, average concentration; CI, confidence interval; C_{max}, peak concentration; C_{min}, minimum concentration; DBS, dried blood spot; eGFR, estimated glomerular filtration rate; ER-Tac, extended-release tacrolimus; GI, gastrointestinal; IQR, interquartile range; IR-Tac, immediate-release tacrolimus; LC-MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; LCPT, once-daily MeltDose tacrolimus tablets; LSM, least square means; MDRD-4, Modification of Diet in Renal Disease; PK, pharmacokinetics; RGM, ratio of geometric means; SAE, serious adverse event; SD, standard deviation; TDD, total daily dose; TEAE, treatment-emergent adverse event; T_{max}, time to maximum observed concentration

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Introduction

Tacrolimus is an integral part of most kidney transplant patients' immunosuppression drug therapy, with over 90% of kidney transplant recipients being discharged with a tacrolimus-containing regimen (1). Three innovator formulations are currently available: traditional twice-daily immediate-release tacrolimus capsules (IR-Tac; Prograf, Astellas Pharma US, Inc., Northbrook, IL), and two once-daily formulations: a once-daily MeltDose tablet formulation (LCPT; Envarsus XR [Envarsus in Europe]; Veloxis Pharmaceuticals, Inc., Edison, NJ) and a once-daily extended-release tacrolimus capsule (ER-Tac; Astagraf XL; Astellas Pharma US, Inc., Northbrook, IL).

The pharmacokinetics (PK), efficacy and safety of both once-daily formulations have been compared individually with those of IR-Tac in clinical studies in *de novo* and

conversion studies of renal transplant recipients (2–6). Comparisons of ER-Tac with IR-Tac have shown variable effects of formulation on peak concentration (C_{max}) and time of peak concentration (T_{max}) but have generally shown that the minimum concentration (C_{min}) and 24-h area under the curve (AUC_{24}) are lower for ER-Tac on a milligram to milligram basis (7,8). Clinical studies of *de novo* and stable transplant patients have consistently shown that ER-Tac requires a higher dose than IR-Tac to achieve similar trough and exposure levels (7,9–12).

Although both LCPT and ER-Tac have once-daily dosing, important differences in their formulations affect their PK. The extended release of tacrolimus offered by ER-Tac is a result of adding ethylcellulose, which acts to slow down the diffusion rate of tacrolimus, leading to a prolonged release (13). In contrast, LCPT's development was focused on MeltDose technology (Veloxis Pharmaceuticals, Inc., Edison, NJ), which improves the solubility of tacrolimus, and therefore bioavailability, by dispersing tacrolimus in a polymeric matrix (14). This results in a more distal distribution of tacrolimus in the gut (15). This formulation has also been shown to promote a more rapid attainment of therapeutic tacrolimus systemic exposure compared with that of IR-Tac and requires a lower total daily dose to achieve therapeutic exposure levels (16,17). LCPT is also associated with less fluctuation between maximum ("peak") exposure and trough and with a lower peak exposure level (18). From a clinical perspective, LCPT is noninferior in terms of efficacy (composite endpoint, including graft loss, death, biopsy-proven acute rejection [BPAR] and loss to follow-up) when compared to IR-Tac with a similar safety profile (17).

However, a direct head-to-head PK comparison of all three formulations has not yet been conducted. Therefore, the objective of this study was to compare the steady-state PK profile of LCPT with IR-Tac and ER-Tac in stable kidney transplant recipients to provide clinicians with dose conversion strategies between formulations.

Materials and Methods

Study design

This steady-state pharmacokinetic comparison of all FK-506 formulations (ASTCOFF) study was an open-label, randomized, two-sequence, three-period crossover trial (Figure 1; ClinicalTrials.gov NCT02339246). The primary objective of the study was to evaluate the PK profile of LCPT in stable renal transplant patients compared with the profiles of IR-Tac and ER-Tac, which in turn allows for the generation of clinical recommendations for converting patients from one formulation to another.

Secondary study objectives included evaluating the daily tacrolimus trough level during each 7-day crossover period, evaluating the labeled conversion factors when switching from the twice-daily IR-Tac formulation to once-daily ER-Tac or LCPT, and safety. The conversion factors used in the study were based on the Food and Drug Administration labeling for converting patients from IR-Tac to LCPT and on the European Medicines Agency labeling for converting patients from IR-Tac to ER-Tac, because conversion labeling is not available in the United States. Sample size calculations determined that at least 14 subjects per sequence were required to have 85% power to show that, by following the labeled conversation rate, the ratio of geometric means (RGM) of AUC_{0-24} between LCPT and IR-Tac would be bioequivalent based on the equivalence criteria of 0.80–1.25, inclusive, and based on two 1-sided t-tests, each at 5% significance level and assuming a standard deviation of 0.22 (ln scale). This was a single-center study that received institutional review board approval (IRB# 2014–7906); recruitment took place at two centers: the Christ Hospital and the University of Cincinnati medical center. This study was conducted in accordance with the Declaration of Helsinki.

Eligible patients were randomized in a 1:1 fashion to one of the two treatment sequences. Study personnel involved in trial operations remained blinded to the randomization list.

Inclusion and exclusion criteria

Eligible patients had to be 18 years or older, have received a first or second renal transplant at least 6 months prior to study entry, be on a stable (no tacrolimus dose change in the 7 days prior to screening) immunosuppressive regimen consisting of any twice-daily tacrolimus and mycophenolate with or without prednisone, have a BMI ≥19 kg/m^2 and not be scheduled to start any new medications or agents that could interfere with tacrolimus blood levels during the study. Patients with an episode of rejection within 3 months of screening, having received another organ than a kidney, with an estimated glomerular filtration rate (eGFR) of ≤25 mL/min/1.73 m^2, with severe gastroparesis or gastrointestinal conditions that could interfere with tacrolimus absorption, and pregnant or lactating women were excluded. Any patient omitting a tacrolimus dose within 48 h of PK measurements was also excluded from the PK analysis.

Intervention

After randomization, each patient received IR-Tac (Prograf, the reference drug for both sequences, Astellas Pharma US, Inc., Northbrook, IL) followed by either LCPT followed by ER-Tac, or ER-Tac followed by LCPT, depending on the randomization sequence. At day 1 of each crossover period, patients were switched to a new formulation using a milligram to

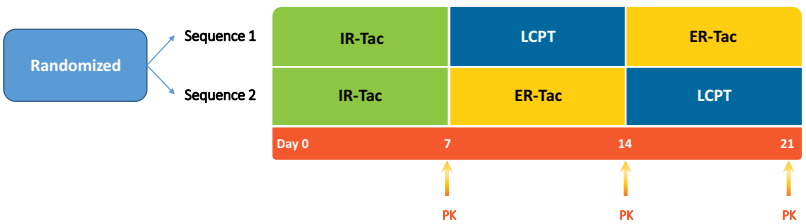

Figure 1: Study design. ER-Tac, extended-release tacrolimus; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; PK, pharmacokinetic profiling.

milligram total daily dose conversion factor of 1:1 for IR-Tac to ER-Tac, and 1:0.80 for IR-Tac or ER-Tac to LCPT, representing a 20% lower total daily dose. Doses were rounded to take into account the availability of dosage strengths. No immunosuppressant dose titrations (tacrolimus, mycophenolate or prednisone if present) were allowed during the study period.

Patients were required to remain fasting for at least 8 h prior to study drug administration and for 3 h following administration on the days of PK measurements. Dosing times were preassigned and remained constant throughout the study. To measure patient adherence, prior to taking their morning dose of tacrolimus, patients had to perform a fingerstick and apply their blood to a protein saver card with a predefined surface area; the protein saver card was returned to the investigators at every PK visit for trough tacrolimus measurement via dried blood spot (DBS) analysis by a central laboratory via tandem mass spectrometry (19–21). Patients were provided with a daily diary to record dosing times and daily trough tacrolimus measurement times and to document any delays or omissions in study doses. In addition, a whole blood sample for trough level at every PK visit was also obtained and analyzed by a local laboratory with an immunoassay (Abbott Architect platform, Abbott Laboratories, IL) for the purpose of safety assessment. All patients received study drug under the direct supervision of the investigators.

Safety laboratory assessments were obtained at screening and at every PK visit and were all analyzed at the clinical laboratory. Concomitant medications were reviewed at each study visit.

Pharmacokinetic profiles

Twenty-four-hour PK collections were performed at the end of each 1-week period; a total of 17 or 21 time points were sampled over 24 h. Blood samples for tacrolimus were drawn as follows: IR-Tac sampling strategy: predose concentration (C_0) and then 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 18, 20, and 24 h; LCPT and ER-Tac: C_0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 16, 18, 21, and 24 h. The additional time points for the IR-Tac PK sampling were designed to better characterize the 12- to 24-h PK profile of the evening dose.

The following PK parameters were *a priori* defined for analysis: area under the concentration–time curve from time 0 to 24 h (AUC_{0-24}), maximum (peak) observed concentration (C_{max}), time to maximum observed concentration (T_{max}), minimum blood concentration observed over the 24-h interval (C_{min}; the value was taken from the observed concentration data at the 24-h nominal time point), predose (nominal time = 0) concentration (C_0), average concentration (C_{avg}) and percent peak-to-trough fluctuation of the drug concentration over the dosing interval (0–24) at the steady state (% fluctuation; calculated as $100*[(C_{max} - C_{min})/C_{avg}]$).

Bioanalytic methods

The central laboratory used (Lambda Therapeutics Research Inc., Toronto, Canada) conducted the tacrolimus whole blood level analyses according to principles of Good Laboratory Practice. The bioanalytical validated methods for assessing tacrolimus whole blood concentrations were analyzed by tandem mass spectrometry (liquid chromatography–mass spectrometry/mass spectrometry [LC-MS/MS]). In brief, tacrolimus was extracted from whole blood and separated via high-performance liquid chromatography and detected by using a TSQ Quantum tandem mass spectrometer (ThermoScientific, Waltham, MA).

Dried blood spot analysis was performed using previously validated and described technique (19–22). Following extraction of tacrolimus from the DBS card, concentrations were evaluated by LC-MS/MS on a platform consisting of Agilent components (Agilent, Santa Clara, CA) in combination with AB Sciex (AB Sciex, Foster City, CA) mass spectrometers at iC42 Clinical Research and Development (University of Colorado, CO).

Study drug

Study drugs were provided by the study sponsor, Veloxis Pharmaceuticals; both IR-Tac and ER-Tac were acquired from commercial supply. All acquired bottles were from a single lot for each product. All dosage strengths available were allowed during the study (0.5, 1, 5 mg for IR-Tac and 1, 5 mg for ER-Tac). For LCPT, tablets had to remain in their original packaging throughout the study, and dosage strengths included 0.75, 1, and 4 mg tablets. At every PK visit, patients had to return unused study drug and bottles for pill counting.

Safety assessments

Safety parameters included incidence of treatment emergent adverse events (TEAEs), serious adverse events (SAEs), graft failure, BPAR and death; changes in safety laboratory tests; changes in vital signs; and tacrolimus trough level determined by the local clinical laboratory for safety assessment. Patient eGFR was assessed by the Modification of Diet in Renal Disease (MDRD-4) (23) and 24-h urine collection at every PK visit to calculate creatinine clearance.

Adherence assessment

Drug accountability and adherence evaluation were performed at each study visit by reviewing the patients' daily diary and by pill counting. Adherence was primarily assessed by comparing the number of total doses taken with the total number of prescribed doses based on pill counts. *Ad hoc* analyses of adherence were performed by comparing the time when patients took their dose to the prescribed time, allowing for a 30-min window on non-PK days, and analysis of patient diaries.

Statistical analysis

All patients treated with study drug were included in the safety analysis; patients who successfully completed the 3-week crossover periods were included in the PK analysis. The actual time of observed concentration-time data was used to derive PK parameters via WinNonlin version 6.3 (Certara USA, Princeton, NJ) based on noncompartmental analysis and linear trapezoidal linear interpolation calculation methods. All PK parameters (except T_{max}) were evaluated using a mixed effect analysis of covariance (ANCOVA) model in which period, sequence and formulation were fixed effects and patient within a sequence was a random effect. Natural logarithm transformation was performed for AUC, C_{max}, C_{avg} and C_0, and no transformation for other parameters was used for observed and dose-corrected analyses. Time to maximal concentration was analyzed using the Wilcoxon signed-rank test. Pearson linear correlation coefficients between AUC_{0-24} and C_{min} were also estimated. Clinical safety parameters were tabulated for each treatment without statistical inferential testing.

One of the objectives of this study was to provide guidance to clinicians with regard to dose conversion rates when switching patients from one formulation to another to achieve similar exposure. As such, the exposure normalization analysis methodology examined the relative bioavailability of the employed dose conversion factor (80% for LCPT and 100% for ER-Tac when converting from IR-Tac) in this study based on the equal exposure principle (exposure is expressed as AUC_{0-24}) before and after conversion from IR-Tac to LCPT or ER-Tac. The factors were applied to AUC_{0-24}, C_{max} and C_{min} for each patient, and ANCOVA models were used to confirm whether the RGM for AUC_{0-24} was approximately 100% postnormalization.

All p-values from inferential tests were reported as is without adjustment for multiple comparisons.

Results

Patient disposition

A total of 32 patients were screened for participation in the study between January 23, 2015, and February 27, 2015, and 31 were randomized, with 1 patient being a screen failure. Sixteen patients were randomized to the IR-Tac/LCPT/ER-Tac arm and 15 to the IR-Tac/ER-Tac/LCPT arm. All 31 randomized patients completed the study and are included in the safety analyses. One patient was not adherent with the study drug dosing requirement based on pill count while on LCPT treatment and was excluded from PK analysis for missing 5 doses (Figure 2). Baseline characteristics were similar across the groups, with a mean age of 50.1 versus 46.3 years; 56% versus 60% male; 81.3% versus 66.7% Caucasian; and with a mean time since transplant of 7 versus 5.2 years for the IR-Tac/LCPT/ER-Tac group compared to the IR-Tac/ER-Tac/LCPT group, respectively (Table 1). Based on the 30 subjects in the PK analysis, the median (interquartile range) total daily dose was 6.0 (4.0–8.0) mg for IR-Tac and ER-Tac and 4.8 (3.3–6.3) for LCPT.

Observed PK data

Figure 3(A) displays the whole blood concentrations of tacrolimus for each formulation, and Table 2 displays the observed PK parameters. When using labeled conversion rate (1:1 for IR-Tac to ER-Tac and 1:0.80 for IR-Tac to LCPT), the AUC_{0-24} was significantly greater for LCPT compared with IR-Tac (RGM: 117.0%; $p = 0.002$) and ER-Tac (RGM: 125.7%; $p < 0.001$). The intraday peak-to-trough fluctuation was approximately 30% lower for LCPT compared with IR-Tac (least square means [LSM] difference: −29.0%; $p = 0.004$) and ER-Tac (LSM difference: −35.3%; $p < 0.001$). The T_{max} was significantly longer at 5.9 h for LCPT compared with IR-Tac and ER-Tac (1.9 and 1.5 h, respectively; $p < 0.001$). Conversely, T_{max} between IR-Tac and ER-Tac did not differ statistically ($p = 0.669$). The C_{min} was significantly lower for ER-Tac (5.1 ng/mL) compared with both LCPT (6.8 ng/mL, $p < 0.001$) and IR-Tac (6.1 ng/mL, $p = 0.001$). There were no other statistically significant differences between IR-Tac and ER-Tac, nor were there significant period or sequence effects (Table 2). Overall, LCPT had a greater relative bioavailability with an increase of ~50% ($p < 0.001$) compared with both IR-Tac and ER-Tac on a milligram to milligram basis.

An *ad hoc* subgroup analysis to evaluate the differences in PK parameters among formulations between African Americans (n = 7) and non–African Americans (n = 23) was performed. Total median daily doses of all formulations were approximately twice as high for African Americans when compared with doses of non–African Americans (6.25, 8 and 8 mg vs. 3.25, 4 and 4 mg for LCPT, ER-Tac and IR-Tac, respectively). The overall differences in PK parameters among formulations for African Americans were similar to the main analysis, suggesting that LCPT provided a higher AUC_{0-24} (RGM 138.2, $p < 0.01$, and 149.1, $p = 0.02$), C_{max} was similar despite a higher AUC_{0-24} (RGM 107.4, $p = 0.54$, and 109.8, $p = 0.22$), C_{min} was higher (RGM 125.3, $p = 0.02$, and 151.0, $p < 0.01$), T_{max} was delayed (RGM 2.48, $p = 0.22$, and 2.55, $p < 0.01$) and intraday peak-to-trough

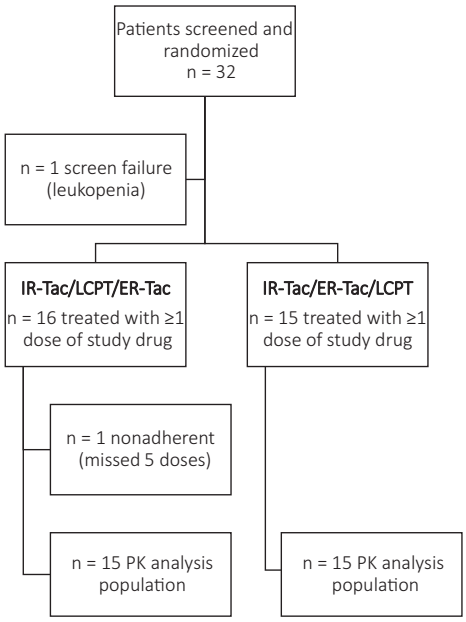

Figure 2: Patient attrition. ER-Tac, extended-release tacrolimus; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; PK, pharmacokinetics.

Table 1: Patient characteristics

	All patients (n = 31)	IR-Tac–LCPT–ER-Tac (n = 16)	IR-Tac–ER-Tac–LCPT (n = 15)
Age (years), mean (SD)	48.3 (12.1)	50.1 (11.0)	46.3 (13.3)
Male sex, n (%)	18 (58.1)	9 (56.3)	9 (60.0)
Race, n (%)			
Caucasian	23 (74.2)	13 (81.3)	10 (66.7)
African American	7 (22.6)	2 (12.5)	5 (33.3)
Other	1 (3.2)	1 (6.3)	0 (0)
Donor type, n (%)			
Deceased	3 (9.7)	1 (6.3)	2 (13.3)
Living	28 (90.3%)	15 (93.8%)	13 (86.7%)
Years since transplant to study, mean (min–max)	6.1 (0.7–14.2)	7.0 (3.2–14.2)	5.2 (0.7–9.2)
Baseline BMI (kg/m^2), mean (SD)	30.4 (4.9)	30.9 (4.8)	29.9 (5.1)

BMI, body mass index; ER-Tac, extended-release tacrolimus; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; SD, standard deviation.

fluctuation was lower (−39.64, p = 0.07, and −54.08, p = 0.02) for LCPT versus IR-Tac and LCPT versus ER-Tac, respectively.

Exposure normalization and dose conversion results

When converting patients from IR-Tac to LCPT, the study dose conversion rate applied resulted in significantly higher overall exposure for LCPT. This difference was not apparent when using the labeled conversion rate between IR-Tac and ER-Tac because the RGM of AUC_{0-24} was 93.1% (90% confidence interval [CI] 85.8–101.0).

For these reasons, the exposure normalization and dose conversion analysis was performed. A conversion factor (percentage of total daily dose of reference drug) of −30% yielded comparable AUC_{0-24} when converting from IR-Tac to LCPT (AUC_{0-24} RGM of 102.4% [90% CI 94.4–111.1%, p = 0.627]. A conversion factor of −36% when converting from ER-Tac to LCPT led to a RGM of 100.6% [90% CI 91.3–110.8%, p = 0.924]). A conversion factor of +8% was required to obtain similar exposures when converting from IR-Tac to ER-Tac (RGM of 100.6% [92.7–109.1%, p = 0.908]) (Figure 3B and Table 3). In addition, LCPT C_{max} was reduced by 17% when compared with the C_{max} of both IR-Tac and ER-Tac, with an RGM of approximately 82% (p = 0.002 and 0.006, respectively), whereas C_{max} of ER-Tac and IR-Tac remained similar (p = 0.887).

Correlation between AUC_{0-24} and C_{min}

A robust correlation between AUC_{0-24} and C_{min} was found for all three tacrolimus formulations across the observed range of C_{min} and AUC_{0-24}. Pearson's linear correlation coefficient between $\ln(AUC_{0-24})$ and $\ln(C_{min})$ was 0.92 (p < 0.001) for LCPT, 0.92 (p < 0.001) for ER-Tac and 0.81 (p < 0.001) for IR-Tac.

Adherence assessment

Overall mean (standard deviation [SD]) daily tacrolimus morning trough concentrations were assessed by DBS for days 1–6. Tacrolimus morning trough level assessed by DBS on each day within a period is also presented in Figure 4. To account for a potential carryover effect resulting from the change in tacrolimus formulations immediately following conversion, we removed the first 3 days of the 6-day period from the following calculations. Morning tacrolimus trough concentrations for days 4–6 were 9.73 (3.61) ng/mL for LCPT, 8.13 (3.08) for ER-Tac and 9.57 (3.46) for IR-Tac. There were no statistically significant differences between LCPT and IR-Tac (p = 0.812), but there were differences between LCPT and ER-Tac (p = 0.021) and ER-Tac and IR-Tac (p = 0.036). Tacrolimus morning trough levels assessed by DBS on each day within a period are also presented in Figure 4.

Overall adherence was excellent. An *ad hoc* analysis showed that when actual dosing time based on patient diaries was compared with prescribed dosing time, the proportion of doses taken within plus or minus 30 min of prescribed time was 91.9% (SD 12.8%), 87.1% (SD 14.2%) and 89.9% (SD 15.0%) for LCPT, ER-Tac and IR-Tac, respectively.

Safety

No patients discontinued study drug during the study because of adverse events. No deaths, BPARs, graft losses or SAEs occurred. A summary of the incidence of the most common TEAEs occurring in 5% or more of the patients overall by system organ class and preferred term for the safety set are presented in Table 4. The two most commonly reported system organ classes were "gastrointestinal disorders" and "general disorders and administration site conditions." The most commonly reported adverse events (AEs) were diarrhea, peripheral edema and headache.

Of the 31 patients treated with at least 1 dose of study drug, 15 (48.4%) unique patients had at least 1 TEAE: 6 (19.4%) patients in the LCPT treatment period, 10 (32.3%) patients in the ER-Tac treatment period and 3 (9.7%) patients in the IR-Tac treatment period.

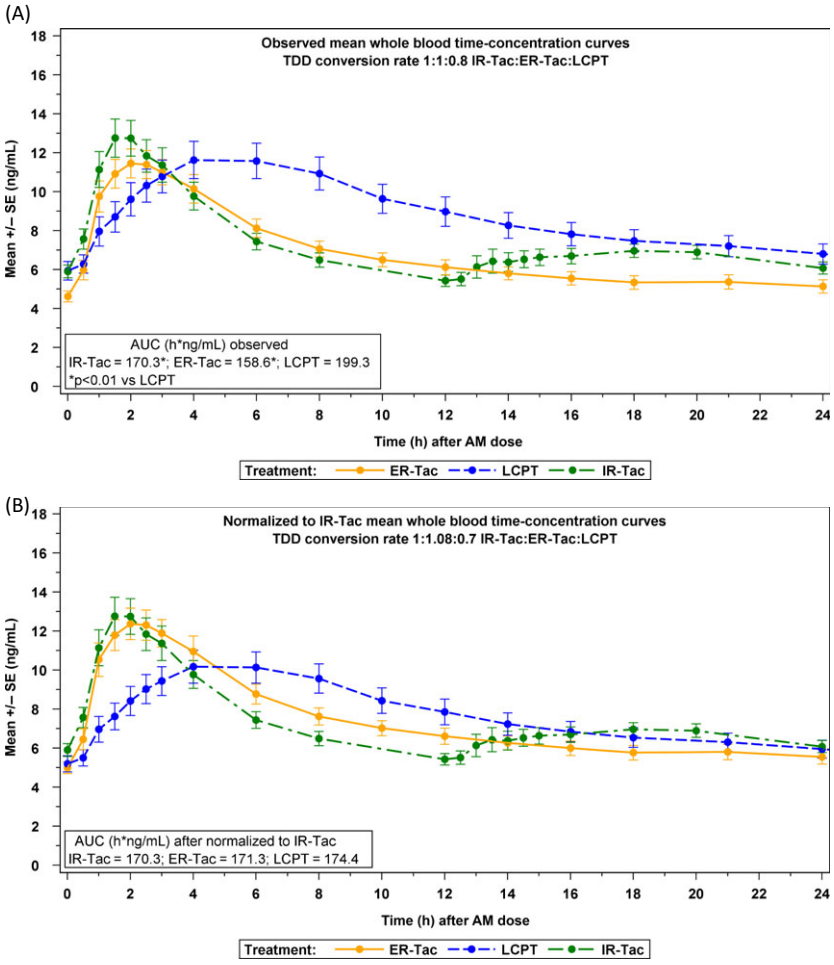

Figure 3: (A) Observed mean whole blood concentrations of tacrolimus based on conversion factors of 1:1:0.80 for IR-Tac:ER-Tac:LCPT (upper panel) versus (B) exposure (AUC)-normalized mean whole blood concentrations of tacrolimus based on conversion factors of 1:1.08:0.70 (lower panel). AUC, area under the curve, ER-Tac, extended-release tacrolimus; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; SE, standard error of the mean; TDD, total daily dose.

Results of renal function assessments are presented in Table 5. There were no statistical differences between groups when assessed by MDRD-4 or 24-h urine collection.

Discussion

ASTCOFF is the first PK study to compare all three innovator tacrolimus formulations. The PK profile of LCPT differed significantly from the profiles of ER-Tac and IR-Tac and is consistent with previous studies (16,17). When comparing the three formulations based on the study conversion factors, overall exposure was higher with LCPT, T_{max} was delayed and C_{max} was similar despite a higher exposure for LCPT. Given the importance of overall exposure to tacrolimus, dose conversion factors were derived on the basis of exposure normalization to IR-Tac. This resulted in a lower and delayed peak with comparable C_{min} for LCPT when compared with IR-Tac and ER-Tac to achieve comparable overall exposure. LCPT showed a higher tacrolimus systemic exposure, greater apparent bioavailability with lower drug dosing compared with the other two tacrolimus formulations. The characteristic high peak in tacrolimus PK following initial dosing of IR-Tac was also observed for ER-Tac, but not for LCPT, when normalizing the PK profiles for exposure. Furthermore, LCPT had less fluctuation between trough and peak exposures than did other formulations and a longer time to maximum concentration, as has been previously reported (18,24). These observations are consistent with the "flatter" PK profile of LCPT as compared with the profiles of the other formulations. The clinical significance of this finding remains to be fully explored but may contribute in decreasing toxicity associated with tacrolimus peak levels, as recently suggested by a study comparing LCPT with IR-Tac in which the

Table 2: Summary of observed pharmacokinetic parameters (n = 30)

	Observed results[1]			Comparisons[2]		
Observed PK parameter	LCPT (L)	ER-Tac (ER)	IR-Tac (IR)	L versus IR (%)	ER versus IR (%)	L versus ER (%)
TDD (mg/day)	4.9 ± 2.3	6.1 ± 2.9	6.1 ± 2.9	–	–	–
Median (IQR)	4.8 (3.3–6.3)	6.0 (4.0–8.0)	6.0 (4.0–8.0)	–	–	–
AUC_{0-24} (h*ng/mL)	213.4 ± 83.1 199.3 (38.6)	165.0 ± 50.0 158.6 (29.1)	176.5 ± 50.8 170.3 (27.2)	117.0 (107.9, 127.0) p = 0.002	93.1 (85.8, 101.0) p = 0.149	125.7 (114.1, 138.5) p < 0.001
C_{max} (ng/mL)	13.9 ± 5.3 12.9 (41.9)	13.2 ± 4.4 12.5 (34.2)	14.5 ± 5.5 13.6 (38.5)	94.7 (85.8, 104.4) p = 0.354	91.8 (83.2, 101.3) p = 0.150	103.1 (92.4, 115.0) p = 0.638
C_{min} (ng/mL)	6.8 ± 2.9 6.3 (42.4)	5.1 ± 1.8 4.9 (33.3)	6.1 ± 1.7 5.9 (27.0)	107.0 (97.6, 117.2) p = 0.223	83.0 (75.7, 90.9) p = 0.001	128.9 (117.4, 141.6) p < 0.001
T_{max} (h)	5.9 (1.5, 14.0)	1.9 (0.9, 5.9)	1.5 (0.9, 20.0)	3.0 (1.6, 4.4) p < 0.001	0.1 (−0.4, 0.5) p = 0.670	3.0 (1.9, 4.0) p < 0.001
Fluctuation (%)	83.6 ± (51.7)	118.9 ± (48.4)	112.6 ± (53.1)	−29.0 (−48.4, −9.6) p = 0.004	6.4 (−13.1, 25.7) p = 0.518	−35.3 (−53.4, −17.3) p < 0.001

Parameter fluctuation: Presented differences in LSM (95% CI) derived from ANCOVA models that included fixed effects of treatment, sequence, period (LCPT vs. ER-Tac analyses) and random effect of subjects (sequence). p-value was from 2-sample t-test.

Parameter T_{max}: Presented median location shifts (95% CI) using Hodges–Lehmann estimation; the Hodges–Lehmann estimates of median location shift ("Comparisons" columns) may not match the difference in observed median values ("Observed Result" columns).

ANCOVA, analysis of covariance; AUC_{0-24}, 24-h area under the curve; CI, confidence interval; C_{max}, maximal concentration; C_{min}, minimal concentration; CV, coefficient of variation; ER-Tac, extended-release tacrolimus; IQR, interquartile range; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; LSM, least square means; PK, pharmacokinetics; RGM, ratio of geometric means; SD, standard deviation; TDD, total daily dose; T_{max}, time to maximal concentration.

[1]Parameter AUC_{0-24}, C_{max}, C_{min}: Presented arithmetic mean ± SD and geometric means (CV% of geometric means); Parameter TDD: Presented arithmetic mean ± SD; T_{max}: Presented median (min, max).

[2]Parameter AUC_{0-24}, C_{max}, C_{min}: Presented RGM in percent (90% CI) derived from ANCOVA models that included fixed effects of treatment, sequence, period (LCPT vs. ER-Tac analyses) and random effect of subjects (sequence). p-value was from 2-sample t-test. Analysis was performed on natural log-transformed data.

Table 3: Recommended dose conversions and resulting normalized PK parameters

Dose conversion factor	LCPT (L) −30%	IR-Tac[1](IR) Reference	RGM[2] and 90% CI L/IR	p-value L versus IR
−30% from IR-Tac to LCPT based on normalized (AUC) exposure				
AUC_{0-24} (h*ng/mL)	174.4	170.3	102.4 (94.4, 111.1)	0.627
C_{max} (ng/mL)	11.3	13.6	82.8 (75.1, 91.4)	0.002
C_{min} (ng/mL)	5.5	5.9	93.6 (85.4, 102.6)	0.233
Dose conversion rate	**ER-Tac (ER) +8%**	**IR-Tac[1](IR) Reference**	**RGM[2] and 90% CI ER/IR**	**p-value ER versus IR**
+8% from IR-Tac to ER-Tac based on normalized (AUC) exposure				
AUC_{24} (h*ng/mL)	171.3	170.3	100.6 (92.7, 109.1)	0.908
C_{max} (ng/mL)	13.5	13.6	99.2 (89.9, 109.4)	0.887
C_{min} (ng/mL)	5.3	5.9	89.6 (81.8, 98.2)	0.050
Dose conversion rate	**LCPT (L) −36%**	**ER-Tac[1](ER) Reference**	**RGM[2] and 90% CI L/ER**	**p-value L versus ER**
−36% from ER-Tac to LCPT based on normalized (AUC) exposure				
AUC_{24} (h*ng/mL)	159.5	158.6	100.6 (91.3, 110.8)	0.924
C_{max} (ng/mL)	10.3	12.5	82.5 (73.9, 92.0)	0.006
C_{min} (ng/mL)	5.0	4.9	103.1 (93.9, 113.2)	0.579

AUC_{0-24}, 24-h area under the curve; CI, confidence interval; C_{max}, maximal concentration; C_{min}, minimal concentration; ER-Tac, extended-release tacrolimus; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; PK, pharmacokinetics; RGM, ratio of geometric means.
[1]The AUC_{0-24} values used as reference were obtained from the observed data.
[2]RGM is expressed as % value.

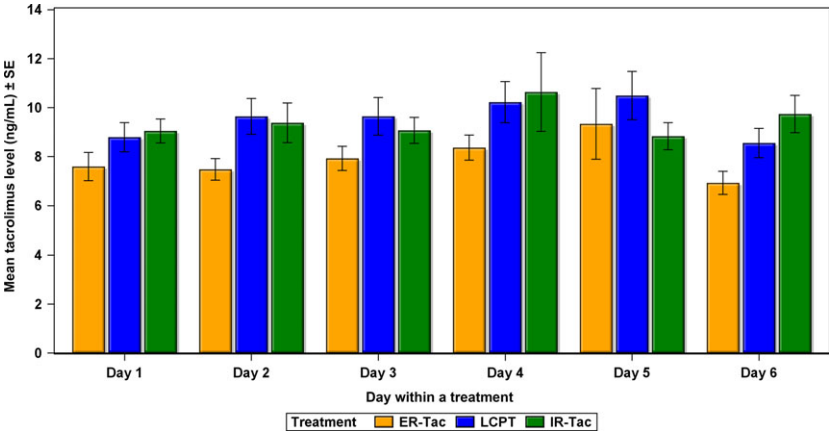

Figure 4: Group mean daily tacrolimus trough level (ng/mL) determined by the dried blood samples in each period. ER-Tac, extended-release tacrolimus; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; SE, standard error of the mean.

severity of tremors was significantly reduced with LCPT with no correlation to C_{min} levels (25).

Contrary to the differences found in most PK parameters for LCPT as compared with ER-Tac and IR-Tac, ER-Tac and IR-Tac tended to be similar to each other in PK profiles. Results from these analyses showed a significantly lower trough for ER-Tac compared with both LCPT and IR-Tac. This observation is consistent with previous reports showing a decrease in overall exposure and C_{min} when converting from IR-Tac to ER-Tac on a 1:1 ratio (12).

Race effects for LCPT have been previously published and are consistent with the *ad hoc* analysis that showed greater per milligram exposure in both African Americans and non–African Americans (18). In the subgroup *ad hoc* analysis, the LCPT followed the same pattern as the overall observed PK in that exposure; relative bioavailability and fluctuation differed for LCPT versus IR-Tac and

Table 4: Overall summary of treatment-emergent adverse events

	Total (n = 31)		LCPT (n = 31)		ER-Tac (n = 31)		IR-Tac (n = 31)	
	Patients (%)	Events	Patients (%)	Events	Patients (%)	Events	Patients (%)	Events
Patients with ≥1 TEAE, n (%)	15 (48.4)	29	6 (19.4)	10	10 (32.3)	16	3 (9.7)	3
TEAE severity, n (%)								
Mild	14 (45.2)	24	6 (19.4)	7	9 (29.0)	14	3 (9.7)	3
Moderate	3 (9.7)	5	1 (3.2)	3	2 (6.5)	2	0	0
Number of TEAE per patient, median (min, max)	0 (0.0, 5.0)	–	0 (0.0, 4.0)	–	0 (0.0, 3.0)	–	0 (1.0)	–
Gastrointestinal disorders, n (%)	6 (19.4)	8	1 (3.2)	1	4 (12.9)	6	1 (3.2)	1
Diarrhea	3 (9.7)	3	0	0	2 (6.5)	2	1 (3.2)	1
Vomiting	2 (6.5)	2	0	0	2 (6.5)	2	0	0
General disorders, n (%)	6 (19.4)	7	3 (9.7)	4	3 (9.7)	3	0	0
Fatigue	2 (6.5)	2	2 (6.5)	2	0	0	0	0
Edema, peripheral	3 (9.7)	3	0	0	3 (9.7)	3	0	0
Infections and infestations, n (%)	3 (9.7)	3	1 (3.2)	1	2 (6.5)	2	0	0
Nasopharyngitis	2 (6.5)	2	0	0	2 (6.5)	2	0	0
Nervous system disorders, n (%)	4 (12.9)	6	1 (3.2)	1	2 (6.5)	3	2 (6.5)	2
Headache	3 (9.7)	3	0	0	1 (3.2)	1	2 (6.5)	2

ER-Tac, extended-release tacrolimus; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; TEAE, treatment-emergent adverse event.

Table 5: Renal function assessments

Parameter	IR-Tac (N = 30)	ER-Tac (N = 30)[1]	LCPT (N = 30)[1]	Treatment effect p-value[2]
Serum creatinine (mg/dL), mean (SD)	1.28 (0.23)	1.30 (0.29)	1.28 (0.26)	0.961
24 h urine total volume (mL), mean (SD)	3019 (1312)	3160 (2831)	3335 (2616)	0.873
24 h creatinine clearance, calculated (mL/min), mean (SD)	77 (32)	74 (33)	76 (32)	0.929
eGFR (mL/min/1.73 m^2)				
Non–African Americans, mean (SD)	n = 23 61 (19.4)	n = 23 61 (20.4)	n = 22 63 (20.1)	0.964
African Americans, mean (SD)	n = 7 72 (17.7)	n = 6 71 (19.0)	n = 7 71 (20.6)	0.981

ER-Tac, extended-release tacrolimus; IR-Tac, immediate-release tacrolimus; LCPT, once-daily, MeltDose tacrolimus; eGFR, glomerular filtration rate, estimated (mL/min/1.73 m); SD, standard deviation.
[1]N = 29 for ER-Tac group for 24-h creatinine clearance; N = 29 for eGFR for LCPT.
[2]p-value from one-way ANOVA with main effect of treatment.

ER-Tac. However, the point estimates and inferential statistics presented here have to be interpreted cautiously because this study was not designed nor initially powered to address this question. These findings may be explained by the increased solubility of LCPT or the delayed and more distal distribution in the gastrointestinal tract of LCPT in contrast with the other formulations (15,26). This distal distribution may allow for partial bypass of presystemic metabolism since proximal and distal portions of the gastrointestinal (GI) tract express different levels of cytochrome p450 3A4 and p-gylcoprotein (27).

Given the novel formulation that represents LCPT, C_{min} to AUC_{0-24} correlations were also performed to allow for comparison of trough sampling across formulations as is currently done in most clinical settings. A robust correlation between AUC_{0-24} and C_{min} was found for all three formulations. However, other limited sampling strategies could be explored in further studies (28).

Adherence as measured by diaries and DBS was excellent. We observed higher DBS trough levels on average (all days) for all formulations as compared to whole blood samples (PK days only). This may be explained by variability in DBS sampling moment, analytical steps required to perform extraction, effect of hematocrit, impact of food on tacrolimus absorption or other unobserved patient-related factors. This observation is consistent with previous reports suggesting that DBS results tend to be higher than when measured by other methods relying on whole blood sampling (29).

Renal function was assessed at every PK visit and did not appear to be influenced by formulation, despite a

higher exposure to LCPT when compared with IR-Tac and ER-Tac. This is contrary to other studies that found an association between tacrolimus exposure and renal function (30,31). Given the short duration of exposure to each formulation, exposure time may not have been sufficient to observe changes in renal function. Nonetheless, the higher overall exposure with LCPT did not affect renal function in this study. Numerical differences in TEAEs among formulations were not considered clinically meaningful.

This trial has many strengths, including a robust randomized crossover design, adequate sample size, advanced analytical techniques, strict PK sampling protocols, predefined feeding schedules, strict adherence monitoring and, most importantly, included a sample of renal transplant recipients as opposed to healthy volunteers. Although sample size was adequate for the primary outcome, it did not allow for full assessment of the impact of recipient genotype on formulation PK. Investigators were not blinded to treatment groups and may therefore have unintentionally introduced biases. Finally, our sample constituted a group of stable renal transplant recipients who were mainly Caucasian males and who may not be representative of other populations. Generalizability to nonfasting conditions and uncontrolled conditions also may not be possible. These questions could be addressed in pragmatic trials.

Conclusion

Results from this comparative PK study of all three innovator tacrolimus formulations, conducted in stable renal transplant recipients, demonstrate that there are significant PK differences between LCPT and both IR-Tac and ER-Tac and that formulations are not interchangeable with LCPT. Based on the results of this study and exposure normalization analysis, a 36% total daily does (TDD) reduction is recommended when converting from ER-Tac to LCPT and a 30% TDD reduction when converting from IR-Tac to LCPT. The results also suggest an 8% TDD increase when converting from IR-Tac to ER-Tac; however, the 8% was not statistically significant and may be within the PK variability range. Although available dosage strengths may limit exact conversion, this information will facilitate achieving target tacrolimus exposure when converting between different formulations; in particular, at hospitals with limited formularies that may not provide all tacrolimus formulations.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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