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ARTICLE

Long-term effects of average calcineurin inhibitor trough levels (over time) on renal function in a prospectively followed cohort of 150 kidney transplant recipients

Gaetano Ciancio[1,2] | **Jeffrey J. Gaynor**[1,2] | **Giselle Guerra**[1,3] | **Marina M. Tabbara**[1] | **David Roth**[1,3] | **Warren Kupin**[1,3] | **Adela Mattiazzi**[1,3] | **Lissett Moni**[1,2] | **George W. Burke III**[1,2]

[1]Miami Transplant Institute, Leonard M. Miller School of Medicine, University of Miami, Miami, Florida, USA

[2]Department of Surgery, Leonard M. Miller School of Medicine, University of Miami, Miami, Florida, USA

[3]Division of Nephrology, Department of Medicine, Leonard M. Miller School of Medicine, University of Miami, Miami, Florida, USA

Correspondence
Gaetano Ciancio, Department of Surgery and Urology, Miami Transplant Institute, University of Miami Miller School of Medicine and Jackson Memorial Hospital, P.O. Box 012440, Miami, FL 33101, USA.
Email: gciancio@med.miami.edu

Abstract

More favorable clinical outcomes with medium-term follow-up have been reported among kidney transplant recipients receiving maintenance therapy consisting of "reduced-tacrolimus (TAC) dosing," mycophenolate mofetil (MMF), and low-dose corticosteroids. However, it is not clear whether long-term maintenance therapy with reduced-calcineurin inhibitor (CNI) dosing still leads to reduced renal function. A prospectively followed cohort of 150 kidney transplant recipients randomized to receive TAC/sirolimus (SRL) versus TAC/MMF versus cyclosporine microemulsion (CSA)/SRL, plus low-dose maintenance corticosteroids, now has 20 years of post-transplant follow-up. Average CNI trough levels over time among patients who were still alive with functioning grafts at 60, 120, and 180 months post-transplant were determined and ranked from smallest-to-largest for both TAC and CSA. Stepwise linear regression was used to determine whether these ranked average trough levels were associated with the patient's estimated glomerular filtration rate (eGFR) at those times, particularly after controlling for other significant multivariable predictors. Experiencing biopsy-proven acute rejection (BPAR) and older donor age were the two most significant multivariable predictors of poorer eGFR at 60, 120, and 180 months post-transplant ($p < 000001$ and 0.000003 for older donor age at 60 and 120 months; $p = 0.00008$ and <0.000001 for previous BPAR at 60 and 120 months). Assignment to CSA also implied a significantly poorer eGFR (but with less magnitudes of effect) in multivariable analysis at 60 and 120 months ($p = 0.01$ and 0.002). Higher ranked average CNI trough levels had no association with eGFR at any timepoint in either

Abbreviations: BPAR, biopsy-proven acute rejection; CAI, chronic allograft injury; CNI, calcineurin inhibitor; Cr, creatinine; CSA, cyclosporine microemulsion; DD, deceased donor; eGFR, estimated glomerular filtration rate; MMF, mycophenolate mofetil; NODAT, new onset diabetes after transplantation; SE, standard error; SRL, sirolimus; TAC, tacrolimus; TG, transplant glomerulopathy.

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univariable or multivariable analysis ($p > 0.70$). Long-term maintenance therapy with reduced-CNI dosing does not appear to cause reduced renal function.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Numerous studies in the past reported that among kidney (and other organ) transplant recipients who received maintenance immunosuppression of "moderate-to-high" daily dosing with either cyclosporine microemulsion or tacrolimus (TAC), chronic calcineurin inhibitor (CNI) toxicity was a common occurrence, contributing to reduced kidney graft survival (and reduced renal function among all patients). More recent reports in kidney transplantation have shown among patients who received maintenance therapy consisting of "reduced-TAC dosing," mycophenolate mofetil, and low-dose corticosteroids, more favorable renal function and graft survival outcomes were observed.

WHAT QUESTION DID THIS STUDY ADDRESS?

Would long-term maintenance therapy that included reduced-CNI dosing, say for at least 10 years post-kidney transplant, still lead to reduced renal function (and thus, reduced graft survival), implying that no long-term CNI dosing is safe?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

In a prospectively followed cohort of 150 adult, primary kidney transplant recipients with 20 years of post-transplant follow-up, higher average CNI trough levels were not associated with any reduced long-term renal function (as determined by estimated glomerular filtration rate). These results were consistent across various time periods, including average CNI trough levels determined during the first 0–60, 0–120, and 0–180 months post-transplant. Average TAC trough levels for most of the patients receiving TAC during these time periods ranged between 5.44 and 7.73 ng/mL. Thus, long-term maintenance therapy with reduced-CNI dosing, particularly for TAC, does not appear to cause reduced renal function.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Long-term use (for 10–20 years post-kidney transplant) of a reduced-TAC dosing strategy, along with mycophenolate acid and low-dose corticosteroids, provides one gold standard for maintenance immunosuppression in kidney transplantation.

INTRODUCTION

Numerous studies in the past reported that among kidney (and other organ) transplant recipients who received maintenance immunosuppression of "moderate-to-high" daily dosing with either cyclosporine microemulsion (CSA) or tacrolimus (TAC), chronic calcineurin inhibitor (CNI) toxicity was a common occurrence, contributing to reduced kidney graft survival (and reduced renal function among all patients).[1–14] Since then, the important randomized trial in kidney transplantation of Ekberg et al. at 12–36 months post-transplant,[15,16] along with other kidney transplant studies,[17–24] reported that among patients who received three-drug maintenance therapy consisting of "reduced-TAC dosing," mycophenolate mofetil (MMF), and low-dose corticosteroids, more favorable results for the following outcomes were observed: renal function, freedom-from-the occurrence of a first biopsy-proven acute rejection (BPAR), death-censored graft survival during the first year post-transplant, and (death-censored) graft failure due to chronic allograft injury/transplant glomerulopathy (CAI/TG). For all intents and purposes, "reduced-TAC dosing" refers to having a target TAC trough level beyond 12 months post-transplant of 4–8 ng/mL, and "reduced-CSA dosing" refers to having a target CSA trough level beyond 12 months post-transplant of 100–150 ng/mL.

The ultimate goal in using "reduced-CNI dosing" over a long post-transplant time period is to achieve low BPAR and favorable graft survival rates while

simultaneously maintaining favorable renal function and minimizing the incidence of chronic CNI toxicity. Determination of such long-term effects of using "reduced-CNI dosing" would obviously be obtained by prognostic modeling of patient-specific cumulative exposure to this type of dosing, that is, testing the prognostic effect(s) of patient-specific cumulative CNI trough levels over time post-transplant. One lingering question has remained, "Would long-term maintenance therapy that included reduced-CNI dosing, say for at least 10 years post-kidney transplant, still lead to reduced renal function (and thus, reduced graft survival), implying that no long-term CNI dosing is safe?"

Using a prospectively followed cohort of 150 adult, primary kidney transplant recipients with 20 years of post-transplant follow-up who were randomized in a single-center study to receive maintenance therapy with TAC/sirolimus (SRL) versus TAC/MMF versus CSA/SRL ($N = 50$ patients per arm), with all patients scheduled to receive low-dose maintenance corticosteroids, we determined the average CNI trough levels during the first 60, 120, and 180 months post-transplant (thus, time-adjusted cumulative CNI trough levels) among patients who were still alive with functioning grafts at those times, respectively. We then correlated these average trough levels over time with the patient's renal function, as determined by the estimated glomerular filtration rate (eGFR) at 60, 120, and 180 months post-transplant, and after controlling for other significant predictors of renal function (using stepwise linear regression). The results of this observational study are reported here.

MATERIALS AND METHODS

As previously reported,[18,19] between May 2000 and December 2001, 150 recipients between 14 and 78 years of age, of either deceased donor (DD) or non-HLA identical living donor first kidney transplants, were randomized immediately before transplant into one of three study groups (the center institutional review board approved the protocol; patients gave written informed consent prior to enrollment): TAC/SRL, TAC/MMF, and CSA/SRL. All clinical and research activities adhered to the ethical principles (as revised in 2013) of the Helsinki Declaration. In each arm, the CNI was not started until renal function had improved (serum creatinine [Cr] <4 mg/dL absent dialysis). Target TAC trough levels were 6–10 ng/mL during the first 6 months post-transplant and 4–8 ng/mL thereafter. Target CSA trough levels were 150–250 ng/mL during the first 6 months post-transplant and 100–200 ng/mL thereafter. In both SRL arms, a loading dose of SRL (4 mg) was given after surgery, with a target trough level of 6–10 ng/mL. Planned MMF dosing was 1 g twice daily, maintained as tolerated. All patients received daclizumab induction, low-dose maintenance corticosteroids, and non-immunosuppressive adjunctive therapy, as previously reported.[18–22]

Patients were followed for the development of BPARs, infections that required hospitalization, viral infections, new onset diabetes after transplant (NODAT), cardiovascular events (nonfatal or fatal), malignancies, protocol violations, graft failure (return to permanent dialysis, graft nephrectomy, or re-transplantation, whichever occurred first), and death-with-a-functioning graft. Delayed graft function was defined as a requirement for dialysis during the first week post-transplant. Renal function was determined by eGFR using the Chronic Kidney Disease Epidemiology Collaboration formula.[25] Of note, analysis of eGFR at a given timepoint post-transplant was based on using patients who were still alive with a functioning graft at that time. BPAR was defined as a rise of 0.3 mg/dL or greater from the nadir Cr, accompanied by a confirmatory kidney transplant biopsy within 24 h of initiation of anti-rejection therapy. Grading of BPAR and CAI (interstitial fibrosis/tubular atrophy) was performed according to the Banff classification.[26] Histologic diagnosis of acute antibody mediated rejection was determined according to more recent Banff criteria.[27] NODAT was defined according to the most recent American Diabetes Association criteria[28] in patients without a pre-operative history of diabetes mellitus. Protocol violation was defined as the patient discontinuing one or both of the assigned maintenance drugs for at least 1 year or the addition of an unassigned maintenance drug.

All DD kidneys received hypothermic machine perfusion using Water's MOX 100 pulsatile preservation machine with Belzer's MPS perfusion fluid.[29]

As we recently reported the 18-year results of this randomized trial,[22] which included analyses of all clinical outcomes, our intention here was to focus strictly on the long-term impact (with 20 years of post-transplant follow-up) of using a reduced-CNI dosing strategy on clinical outcomes.

Determining the average TAC and CSA trough levels at 60, 120, and 180 months post-transplant

TAC and CSA trough levels that were measured at 1, 2, 3, 6, 9, 12, 18, and 24 months post-transplant, plus annual measurements at 36 months, 48 months, ..., through 180 months post-transplant (i.e., at 21 distinct time points) were utilized. Denoting TAC_i as the TAC trough level measured at i months post-transplant (similarly for CSA),

the weighted sum of the TAC levels during 0–60 months post-transplant was determined (similarly for CSA) as: $Sum\_TAC_{0–60} = TAC_1 + TAC_2 + TAC_3 + (3 \times TAC_6) + (3 \times TAC_9) + (3 \times TAC_{12}) + (6 \times TAC_{18}) + (6 \times TAC_{24}) + (12 \times TAC_{36}) + (12 \times TAC_{48}) + (12 \times TAC_{60})$. The weighted sum of the TAC levels during 0–120 months post-transplant was determined (similarly for CSA) as: $Sum\_TAC_{0–120} = Sum\_TAC_{0–60} + (12 \times TAC_{72}) + (12 \times TAC_{84}) + (12 \times TAC_{96}) + (12 \times TAC_{108}) + (12 \times TAC_{120})$. The weighted sum of the TAC levels during 0–180 months post-transplant was determined (similarly for CSA) as: $Sum\_TAC_{0–180} = Sum\_TAC_{0–120} + (12 \times TAC_{132}) + (12 \times TAC_{144}) + (12 \times TAC_{156}) + (12 \times TAC_{168}) + (12 \times TAC_{180})$. The average TAC levels at 60, 120, and 180 months post-transplant were then calculated as $Avg\_TAC_{0–60} = (Sum\_TAC_{0–60}/60)$, $Avg\_TAC_{0–120} = (Sum\_TAC_{0–120}/120)$, and $Avg\_TAC_{0–180} = (Sum\_TAC_{0–180}/180)$. $Avg\_CSA_{0–60}$, $Avg\_CSA_{0–120}$, and $Avg\_CSA_{0–180}$ were similarly calculated. If patients were missing any CNI determinations (or discontinued the CNI), then the weighted sums and average trough levels were calculated based on determinations that were available and dividing by the length of time in which determinations were available.

Statistical analysis

In order to provide information about patients who were no longer available for analysis, log-rank test comparisons of graft survival (death-uncensored) among the three study groups was performed over the 20-year post-transplant period, with actuarial graft survival curves generated using the Kaplan–Meier method. Standard one-way analysis of variance *F*-tests were used in comparing the mean eGFR across the three study groups at 60, 120, and 180 months post-transplant. Two-sample *t*-tests were used in comparing the mean Avg_TAC between study groups TAC/SRL versus TAC/MMF at 60, 120, and 180 months post-transplant. Last, the primary statistical analysis for this observational study utilized stepwise linear regression in determining the significant multivariable predictors of eGFR at 60, 120, and 180 months post-transplant, respectively. In addition to considering baseline variables for their associations with eGFR at the three time points, zero–one variables that identified whether the patient had developed a first BPAR (and NODAT) during 0–60, 0–120, and 0–180 months post-transplant, respectively, were considered for their predictive value in the linear regression analyses. In order to include patients who received TAC (all TAC/SRL and TAC/MMF patients, plus a few CSA/SRL patients who were switched from CSA to TAC) along with patients who received CSA (only CSA/SRL patients) in the same analysis, for each of the three time periods, the Avg_TAC and Avg_CSA values were ranked separately from smallest-to-largest (note: for 11 patients in group CSA/SRL who switched from CSA to TAC, in a given time period each patient had a single value for either Avg_TAC or Avg_CSA, but not both, depending on when the switch from TAC to CSA was made). Thus, the ranked values for Avg_TAC and Avg_CSA (with higher ranked values indicating higher actual values for Avg_TAC and Avg_CSA) were combined into a single variable as rank {Avg CNI level} during 0–60, 0–120, and 0–180 months post-transplant, maximizing the available sample size in each analysis. Finally, in order to consider shorter time intervals, rank{Avg CNI level} during 60–120 months (i.e., cumulative CNI level during the 60-month period prior to 120 months post-transplant) was tested for its association with eGFR at 120 months, and rank{Avg CNI level} during 120–180 and 60–180 months (i.e., cumulative CNI levels during the 60- and 120-month periods prior to 180 months post-transplant) were tested for their associations with eGFR at 180 months.

RESULTS

Comparison of graft survival (death uncensored) by treatment arm

Comparisons of the hazard rate of developing graft loss (death uncensored) by treatment arm during 0–60, 0–120, 0–180, and 0–240 months post-transplant, using an intent-to-treat analysis, are shown in Table 1. There were no significant differences at any of these timepoints (p = 0.56, 0.24, 0.77, and 0.83, respectively). Graft survival estimates ± SE at each of these times by treatment arm are shown in Table 1. For example, actuarial graft survival at 180 months post-transplant for the TAC/SRL, TAC/MMF, and CSA/SRL arms was 39.0% ± 7.3%, 42.4% ± 7.3%, and 39.2% ± 7.4%, respectively.

Of note, the risk set (number of patients who were alive with a functioning graft) for each treatment arm at 60 months post-transplant was 35, 34, and 39, respectively (see footnote "b" in Table 1). Similarly, the risk set for each treatment arm at 120 months post-transplant was 19, 27, and 30, respectively, and the risk set for each treatment arm at 180 months post-transplant was 13, 17, and 16, respectively.

Comparisons of mean eGFR ± SE (mL/min per 1.73 m^2) by treatment arm

Comparisons of mean eGFR ± SE (mL/min per 1.73 m^2) by treatment arm are shown in Table 2. The average of

TABLE 1 Comparisons of the hazard rate of developing graft loss (death uncensored) by treatment arm.

Outcome	Group A: TAC/SRL (*N* = 50)	Group B: TAC/MMF (*N* = 50)	Group C: CSA/SRL (*N* = 50)	Log rank test *p*-value[a]
Graft loss (death uncensored)[b]				
During first 60 months	15 (70.0% ± 6.5%)	14 (71.7% ± 6.4%)	10 (79.8% ± 5.7%)	0.56
During first 120 months	26 (46.7% ± 7.2%)	20 (58.7% ± 7.1%)	17 (65.0% ± 6.9%)	0.24
During first 180 months	29 (39.0% ± 7.3%)	27 (42.4% ± 7.3%)	28 (39.2% ± 7.4%)	0.77
During first 240 months	34 (23.6% ± 7.0%)	36 (17.8% ± 6.2%)	34 (23.9% ± 6.7%)	0.83

Note: Number of patients with the event (Kaplan–Meier [actuarial] [death uncensored] graft survival estimates at 60 120, 180, and 240 months post-transplant ± SE), respectively.

Abbreviations: CSA, cyclosporine microemulsion; MMF, mycophenolate mofetil; SRL, sirolimus; TAC, tacrolimus.

[a]The *p* values listed in this column represent the results of the log-rank test (with 2 degrees of freedom) for any differences among the three treatment groups' hazard rates of graft loss (death uncensored).

[b]The risk set of patients (who were still alive with a functioning graft) at 60 months post-transplant was 35, 34, and 39 in groups A, B, and C, respectively. The risk set of patients (who were still alive with a functioning graft) at 120 months post-transplant was 19, 27, and 30 in groups A, B, and C, respectively. The risk set of patients (who were still alive with a functioning graft) at 180 months post-transplant was 13, 17, and 16 in groups A, B, and C, respectively. Last, the risk set of patients (who were still alive with a functioning graft) at 240 months post-transplant was 7, 5, and 8 in groups A, B, and C, respectively. Patients without graft loss who were also not in the risk set at a particular timepoint were lost to follow-up.

TABLE 2 Comparisons of mean eGFR ± SE (mL/min per 1.73 m^2) by treatment arm.

Post-tx month	*Group A: N*	Group A: TAC/SRL	*Group B: N*	Group B: TAC/MMF	*Group C: N*	Group C: CSA/SRL	*p* value[a]
60	35	59.8 ± 3.9	32[b]	62.4 ± 4.0	39	51.5 ± 3.0	0.08
120	19	65.7 ± 4.9	22[b]	65.3 ± 4.2	30	50.5 ± 5.0	0.04
180	13	60.2 ± 7.8	15[b]	58.6 ± 6.4	14[b]	58.5 ± 7.4	0.98

Abbreviations: CSA, cyclosporine microemulsion; eGFR, estimated glomerular filtration rate; MMF, mycophenolate mofetil; SRL, sirolimus; TAC, tacrolimus; tx, transplant.

[a]The *p* values listed in this column represent the results of the overall *F*-test (with 2 degrees of freedom in the numerator) for any differences among the three group-specific means. Significant pairwise comparisons of the means (using *t*-tests) include group A versus C at month 120 ($p = 0.03$); and group B versus C at months 60 and 120 ($p = 0.03$ and 0.03, respectively. Significant comparisons of the average of the groups A and B means versus the group C mean occurred at months 60 and 120 ($p = 0.03$ and 0.01, respectively).

[b]In group B, there were 2, 5, and 2 patients who were alive with a functioning graft at 60, 120, and 180 months post-transplant but had no laboratory tests available at those times (i.e., had missing eGFR and TAC trough levels), respectively. In group C, there were two patients who were alive with a functioning graft at 180 months post-transplant but had no laboratory tests available at that time (i.e., had missing eGFR and CSA trough levels).

the mean eGFRs for TAC/SRL and TAC/MMF was significantly higher than the mean eGFR for CSA/SRL at both 60 and 120 months post-transplant ($p = 0.03$ and 0.01, respectively), with no significant differences among the three eGFR means at 180 months post-transplant ($p = 0.98$). For example, the mean eGFR ± SE for the three treatment arms at 120 months post-transplant was 65.7 ± 4.9 ($N = 19$), 65.3 ± 4.2 ($N = 22$), and 50.5 ± 5.0 ($N = 30$), respectively.

Of note, there were a few patients that had no laboratory values available (for serum creatinine and immunosuppression trough levels) at certain timepoints, even though we knew that they were still alive with a functioning graft at those times. Specifically, no laboratory values were available for two, five, and two patients in the TAC/MMF arm at 60, 120, and 180 months post-transplant, respectively. In addition, no laboratory values were available for two patients in the CSA/SRL arm at 180 months post-transplant. Thus, as shown in Table 2, the total sample size of patients who were still alive with a functioning graft and had laboratory values available at 60, 120, and 180 months post-transplant was 106, 71, and 42 patients, respectively.

Percentages of patients alive with a functioning graft who were taking TAC, CSA, SRL, and MMF at 60, 120, and 180 months post-transplant

Percentages of patients (alive with a functioning graft) who were taking TAC, CSA, SRL, and MMF at 60, 120, and 180 months post-transplant are shown in Table 3. Although the great majority of patients in the TAC/MMF arm remained on both TAC and MMF over time, a large minority (25.6%–36.7%) of the CSA/SRL patients

TABLE 3 Percentages of patients alive with a functioning graft who were taking TAC, CSA, SRL, and MMF at 60, 120, and 180 months post-transplant.

(i) Percentage taking TAC				
Post-tx month	**Group A: TAC/SRL**	**Group B: TAC/MMF**	**Group C: CSA/SRL**	***p* value[a]**
60	88.6% (31/35)	90.6% (29/32)	25.6% (10/39)	0.78
120	73.7% (14/19)	90.9% (20/22)	36.7% (11/30)	0.14
180	69.2% (9/13)	93.3% (14/15)	35.7% (5/14)	0.10
(ii) Percentage taking CSA				
Post-tx month	**Group A: TAC/SRL**	**Group B: TAC/MMF**	**Group C: CSA/SRL**	
60	0.0% (0/35)	0.0% (0/32)	46.2% (18/39)	
120	0.0% (0/19)	0.0% (0/22)	36.7% (11/30)	
180	0.0% (0/13)	0.0% (0/15)	42.9% (6/14)	
(iii) Percentage taking SRL				
Post-tx month	**Group A: TAC/SRL**	**Group B: TAC/MMF**	**Group C: CSA/SRL**	***p*-Value[b]**
60	57.1% (20/35)	12.5% (4/32)	48.7% (19/39)	0.47
120	57.9% (11/19)	9.1% (2/22)	56.7% (17/30)	0.93
180	53.8% (7/13)	6.7% (1/15)	35.7% (5/14)	0.34
(iv) Percentage taking MMF				
Post-tx month	**Group A: TAC/SRL**	**Group B: TAC/MMF**	**Group C: CSA/SRL**	
60	37.1% (13/35)	90.6% (29/32)	71.8% (28/39)	
120	52.6% (10/19)	100.0% (22/22)	70.0% (21/30)	
180	61.5% (8/13)	100.0% (15/15)	71.4% (10/14)	

Abbreviations: CSA, cyclosporine microemulsion; MMF, mycophenolate mofetil; SRL, sirolimus; TAC, tacrolimus; tx, transplant.

[a]The *p* values listed in this column represent chi-squared test results comparing the group A versus B percentages at 60, 120, and 180 months post-transplant, respectively.

[b]The *p* values listed in this column represent chi-squared test results comparing the group A versus C percentages at 60, 120, and 180 months post-transplant, respectively.

had switched from CSA to TAC. In addition, a majority of the TAC/SRL and CSA/SRL patients began taking MMF (mostly in place of SRL, but also in place of a CNI).

Distributions of the average CNI trough levels (ng/mL) over 0–60, 0–120, and 0–180 months post-transplant by treatment arm

Distributions of the average CNI trough levels (ng/mL) over 0–60, 0–120, and 0–180 months post-transplant by treatment arm appear in Table 4. The medians (interquartile ranges [IQRs]) of the average TAC trough level during 0–60 months post-transplant among patients in the three treatment arms were 6.80 (IQR 6.49–7.27), 7.01 (IQR 6.68–7.85), and 7.10 (IQR 5.89–7.73) ng/mL, respectively. As the length of follow-up increased from 0–60 months to 0–120 months and 0–180 months post-transplant, slight decreases in the median and IQR values for average TAC trough levels were observed. Combining patients across the three treatment arms, the percentage with an average TAC trough level during 0–60 months post-transplant of less than 6.0, 6.0–6.99, 7.0–7.99, and greater than or equal to 8.0 ng/mL was 10.4% (8/77), 48.1% (37/77), 31.2% (24/77), and 10.4% (8/77), respectively. Similarly, the percentage with an average TAC trough level during 0–120 months post-transplant of less than 6.0, 6.0–6.99, 7.0–7.99, and greater than or equal to 8.0 ng/mL was 26.0% (13/50), 48.0% (24/50), 20.0% (10/50), and 6.0% (3/50), respectively. Last, the percentage with an average TAC trough level during 0–180 months post-transplant of less than 6.0, 6.0–6.99, 7.0–7.99, and greater than or equal to 8.0 ng/mL was 37.5% (12/32), 53.1% (17/32), 9.4% (3/32), and 0.0% (0/32), respectively.

The median (IQR) of the average CSA trough level during 0–60 months post-transplant (comprising only group CSA/SRL patients) was 150.7 (IQR 135.6–160.6) ng/mL. As the length of follow-up increased from 0–60 months to 0–120 months and 0–180 months post-transplant, slight decreases in the median and IQR values for average CSA trough levels were also observed. The percentage with an average CSA trough level during 0–60 months post-transplant of less than 125.0, 125.0–149.99, 150.0–174.99, and greater than or equal to 175.0 ng/mL was 13.8% (4/29), 34.5% (10/29), 37.9% (11/29),

TABLE 4 Distributions of the average CNI trough levels (ng/mL) over 0–60, 0–120, and 0–180 months post-transplant by treatment arm.

Drug	During post-tx months	Mean ± SE (median, interquartile range)						p value[a]
		Group A		Group B		Group C		
		N	TAC/SRL	N	TAC/MMF	N	CSA/SRL	
TAC level:	0–60	35	6.75 ± 0.14 [6.80, 6.49–7.27]	32	7.27 ± 0.17 [7.01, 6.68–7.85]	10[b]	6.88 ± 0.36 [7.10, 5.89–7.73]	0.02
	0–120	19	6.26 ± 0.21 [6.11, 5.70–6.73]	22	6.60 ± 0.24 [6.65, 6.21–6.97]	9[b]	6.56 ± 0.34 [6.78, 6.45–7.22]	0.30
	0–180	13	5.92 ± 0.27 [6.03, 5.44–6.49]	15	6.46 ± 0.14 [6.40, 5.88–6.89]	4[b]	5.93 ± 0.29 [6.00, 5.45–6.40]	0.09
CSA level:	0–60					29	154.2 ± 5.9 [150.7, 135.6–160.6]	
	0–120					21	136.1 ± 4.9 [133.5, 123.4–143.9]	
	0–180					10	134.8 ± 5.1 [133.5, 127.1–139.6]	

Abbreviations: CNI, calcineurin inhibitor; CSA, cyclosporine microemulsion; MMF, mycophenolate mofetil; SE, standard error; SRL, sirolimus; TAC, tacrolimus; tx, transplant.

[a]The *p* values listed in this column represent *t*-test results comparing the group A versus B average TAC trough levels during 0–60, 0–120, and 0–180 months post-transplant, respectively.

[b]A total of 11 patients had switched from CSA to TAC, 6 during the first 0–6 months post-transplant, 3 during the first 6–12 months post-transplant, and 2 beyond 12 months post-transplant.

and 13.8% (4/29), respectively. Similarly, the percentage with an average CSA trough level during 0–120 months post-transplant of less than 125.0, 125.0–149.99, 150.0–174.99, and greater than or equal to 175.0 ng/mL was 28.6% (6/21), 57.1% (12/21), 9.5% (2/21), and 4.8% (1/21), respectively. Last, the percentage with an average CSA trough level during 0–180 months post-transplant of less than 125.0, 125.0–149.99, 150.0–174.99, and greater than or equal to 175.0 ng/mL was 20.0% (2/10), 60.0% (6/10), 20.0% (2/10), and 0.0% (0/10), respectively. In summary, most of the patients who received TAC had long-term average TAC trough levels between 5.44–7.85 ng/mL, and most of the patients who received CSA had long-term average CSA trough levels between 123.4 and 160.6 ng/mL.

Multivariable linear regression results for eGFR at 60, 120, and 180 months post-transplant

Stepwise linear regression results for eGFR at both 60 months ($N = 106$) and 120 months ($N = 71$) post-transplant were similar in that three significant multivariable predictors of a lower eGFR were found (Tables 5 and 6): older donor age ($p < 0.000001$ at 60 months; $p = 0.000003$ at 120 months), previous occurrence of a first BPAR ($p = 0.00008$ at 60 months; $p < 0.000001$ at 120 months), and assignment to group CSA/SRL ($p = 0.01$ at 60 months; $p = 0.002$ at 120 months). Tables 5 and 6 show that the univariable tests of association of rank (Avg CNI level during 0–60 months} with eGFR at 60 months and rank {Avg CNI level during 0–120 months} with eGFR at 120 months were not statistically significant ($p = 0.76$ and 0.82, respectively). Furthermore, Tables 5 and 6 show that after controlling for the significant effects of older donor age, previous occurrence of a first BPAR, and assignment to group CSA/SRL, the multivariable tests of association of rank (Avg CNI level during 0–60 months} with eGFR at 60 months and rank {Avg CNI level during 0–120 months} with eGFR at 120 months were also not statistically significant ($p = 0.93$ and 0.79, respectively). The model coefficient for the effect of rank (Avg CNI level} was positive for both eGFR at 60 and 120 months (Tables 5 and 6), indicating that although clearly non-significant, higher average CNI levels were associated with more a favorable eGFR. Stepwise linear regression results for eGFR at 180 months ($N = 42$) post-transplant found two significant multivariable predictors of a lower eGFR (Table 7): older donor age ($p = 0.02$) and previous occurrence of a first BPAR ($p = 0.01$). The univariable test of association of rank (Avg CNI level during 0–180 months} with eGFR at 180 months was not significant

TABLE 5 Stepwise linear regression results for eGFR at 60 months post-transplant ($N = 106$).

Variable	% With characteristic if categorical/Median [interquartile range] if continuous	Univariable *p* value	Multivariable *p* value	Model[a] Coeff ± SE
Group A	33.0% (35/106)	0.46		
Group B	30.2% (32/106)	0.13		
Group C	36.8% (39/106)	0.03	(√) 0.01	−9.360 ± 3.553
Recipient age (years)	48.3 [34.6–58.6]	0.10		
Male recipient	67.9% (72/106)	0.41		
African–American recipient	17.0% (18/106)	0.03		
Hispanic recipient	38.7% (41/106)	0.45		
Recipient BMI	25.2 [22.4–28.1]	0.80		
Pretransplant CVD	17.0% (18/106)	0.47		
Pretransplant diabetes	12.3% (13/106)	0.64		
DD recipient	81.1% (86/106)	0.64		
Nonstandard DD recipient	13.2% (14/106)	0.08		
Donor age (years)	36.5 [23.0–47.0]	0.000001	(√) <0.000001	−0.770 ± 0.126
# HLA mismatches	3.5 [3.0–4.0]	0.31		
First BPAR ≤60 months	16.0% (17/106)	0.001	(√) 0.00008	−19.212 ± 4.672
NODAT ≤60 months	26.4% (28/106)	0.41		
Rank {Avg CNI level: 0–60 months}	0.51 [0.26–0.76]	0.76		

Multivariable linear regression model for eGFR at 60 mo post-transplant ($N = 106$), considering the effect of rank {avg CNI level during 0–60 months}

Variable	Multivariable *p* value	Model[b] Coeff ± SE
Group C	0.01	−9.373 ± 3.573
Donor age (years)	<0.000001	−0.770 ± 0.126
First BPAR ≤60 months	0.0001	−19.135 ± 4.776
Rank {Avg CNI level: 0–60 months}	0.93	0.528 ± 6.050

Note: (√) Represents selection into the multivariable linear regression model.

Abbreviations: Avg, average; BMI, body mass index; BPAR, biopsy-proven acute rejection; CNI, calcineurin inhibitor; Coeff, coefficient; CVD, cardiovascular disease; DD, deceased donor; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; NODAT, new onset diabetes after transplant.

[a]The 3 variables selected into the multivariable model were defined as follows: group C = {1 if recipient was randomized into group C, 0 otherwise}; donor age (years) (continuous variable); and first BPAR ≤60 months = {1 if the patient experienced a first BPAR during the first 60 months post-transplant, 0 otherwise}. The estimated intercept term ± SE for the multivariable model was: 91.508 ± 5.108, and the coefficient of multiple determination was 0.37.

[b]The 4 variables included in this multivariable model were defined as follows: group C = {1 if recipient was randomized into group C, 0 otherwise}; donor age (years) (continuous variable); first BPAR ≤60 months ={1 if the patient experienced a first BPAR during the first 60 months post-transplant, 0 otherwise}; and rank {Avg CNI level: 0–60 months} = rank of the average CNI trough level during 0–60 months post-transplant. The estimated intercept term ± SE for the multivariable model was: 91.254 ± 5.898, and the coefficient of multiple determination was 0.37.

($p = 0.97$; Table 7), and the multivariable test of association of rank {Avg CNI level during 0–180 months} with eGFR at 180 months was also not significant ($p = 0.72$; Table 7). The model coefficient for the effect of rank (Avg CNI level} was positive for eGFR at 180 months (Table 7), again indicating that although clearly nonsignificant, higher average CNI levels were associated with a more favorable eGFR.

Of note, when considering shorter time intervals, nonsignificant associations were also found for rank {Avg CNI level during 60–120 months} with eGFR at 120 months, and for rank {Avg CNI level during 120–180 months} and rank {Avg CNI level during 60–180 months} with eGFR at 180 months (results not shown).

Using the intercept terms and model coefficients for the three linear regression models in Tables 5–7, for a recipient of a 35-year-old donor who was not assigned to group CSA/SRL and did not develop a first BPAR, the fitted eGFR at 60, 120, and 180 months post-transplant was 64.558, 69.973, and 64.263 mL/min per 1.73 m^2, respectively. Thus, the model

TABLE 6 Stepwise linear regression results for eGFR at 120 months post-transplant ($N = 71$).

Variable	% With characteristic if categorical/Median [interquartile range] if continuous	Univariable *p* value	Multivariable *p* value	Model[a] Coeff ± SE
Group A	26.8% (19/71)	0.18		
Group B	31.0% (22/71)	0.16		
Group C	42.3% (30/71)	0.01	(√) 0.002	−14.394 ± 4.449
Recipient age (years)	48.4 [34.5–58.4]	0.51		
Male recipient	62.0% (44/71)	0.17		
African-American recipient	12.7% (9/71)	0.50		
Hispanic recipient	40.8% (29/71)	0.58		
Recipient BMI	25.1 [22.4–28.1]	0.94		
Pretransplant CVD	14.1% (10/71)	0.73		
Pretransplant diabetes	8.5% (6/71)	0.51		
DD recipient	77.5% (55/71)	0.51		
Nonstandard DD recipient	14.1% (10/71)	0.05		
Donor age (years)	36.0 [22.0–46.0]	0.002	(√) 0.000003	−0.869 ± 0.170
# HLA mismatches	4.0 [3.0–5.0]	0.53		
First BPAR ≤120 months	14.1% (10/71)	0.001	(√)<0.000001	−35.217 ± 6.504
NODAT ≤120 months	35.2% (25/71)	0.08		
Rank {Avg CNI level: 0–120 months}	0.52 [0.26–0.76]	0.82		

Multivariable linear regression model for eGFR at 120 months post-transplant ($N = 71$), considering the effect of rank {Avg CNI Level during 0–120 months}

Variable	Multivariable *p* value	Model[b] Coeff ± SE
Group C	0.002	−14.506 ± 4.500
Donor age (years)	0.000003	−0.872 ± 0.172
First BPAR ≤120 months	0.000001	−35.234 ± 6.550
Rank {Avg CNI level: 0–120 months}	0.79	2.080 ± 7.707

Note: (√) Represents selection into the multivariable linear regression model.

Abbreviations: Avg, average; BMI, body mass index; BPAR, biopsy-proven acute rejection; CNI, calcineurin inhibitor; Coeff, coefficient; CVD, cardiovascular disease; DD, deceased donor; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; NODAT, new onset diabetes after transplant.

[a]The 3 variables selected into the multivariable model were defined as follows: group C = {1 if Recipient was Randomized into Group C, 0 otherwise}; donor age (years) (continuous variable); and first BPAR ≤120 months = {1 if the patient experienced a first BPAR during the first 120 months post-transplant, 0 otherwise}. The estimated intercept term ±SE for the multivariable model was: 100.388 ± 6.774, and the coefficient of multiple determination was 0.46.

[b]The 4 variables included in this multivariable model were defined as follows: group C = {1 if recipient was randomized into group C, 0 otherwise}; donor age (years) (continuous variable); first BPAR ≤120 months = {1 if the patient experienced a first BPAR during the first 120 months post-transplant, 0 otherwise}; and rank {Avg CNI level: 0–120 months} = rank of the average CNI trough level during 0–120 months post-transplant. The estimated intercept term ±SE for the multivariable model was: 99.457 ± 7.644, and the coefficient of multiple determination was 0.46.

coefficients for donor age (year) and previous occurrence of a first BPAR, as shown in Table 5, indicate the following similar decreases in eGFR (mL/min per 1.73 m^2) at 60 months post-transplant: 19.25 (25 × 0.770) for an increase in donor age of 25 years (to 60 years) versus 19.212 for a previous occurrence of first BPAR. Assignment to group CSA/SRL would reduce eGFR (mL/min per 1.73 m^2) by a lesser amount (i.e., 9.360). The model coefficients for donor age (year) and previous occurrence of a first BPAR, as shown in Table 6, indicate the following decreases in eGFR (mL/min per 1.73 m^2) at 120 months post-transplant: 21.725 (25 × 0.869) for an increase in donor age of 25 years, and 35.217 for a previous occurrence of first BPAR. Assignment to group CSA/SRL would reduce eGFR (mL/min per 1.73 m^2) by a lesser amount (i.e., 14.394). Last, the model coefficients for donor age (year) and previous occurrence of a first BPAR, as shown in Table 7, indicate the following decreases in eGFR (mL/min per 1.73 m^2) at 180 months post-transplant: 17.675 (i.e., 25 × 0.707) for an increase in donor age of 25 years, and 25.097 for a previous occurrence of first BPAR.

TABLE 7 Stepwise linear regression results for eGFR at 180 months post-transplant ($N = 42$).

Variable	% With characteristic if categorical/Median [interquartile range] if continuous	Univariable *p* value	Multivariable *p* value	Model[a] Coeff ± SE
Group A	31.0% (13/42)	0.85		
Group B	35.7% (15/42)	0.93		
Group C	33.3% (14/42)	0.93		
Recipient age (years)	46.2 [33.0–55.7]	0.09		
Male recipient	64.3% (27/42)	0.33		
African-American recipient	14.3% (6/42)	0.95		
Hispanic recipient	47.6% (20/42)	0.36		
Recipient BMI	25.1 [22.4–28.1]	0.90		
Pretransplant CVD	11.9% (5/42)	0.51		
Pretransplant diabetes	4.8% (2/42)	0.53		
DD recipient	78.6% (33/42)	0.41		
Nonstandard DD recipient	11.9% (5/42)	0.83		
Donor age (years)	35.0 [22.0–46.0]	0.09	(√) 0.02	−0.707 ± 0.280
# HLA mismatches	3.5 [3.0–5.0]	0.32		
First BPAR ≤180 months	21.4% (9/42)	0.05	(√) 0.01	−25.097 ± 9.219
NODAT ≤180 month	31.0% (13/42)	0.07		
Rank {Avg CNI level: 0–180 months}	0.52 [0.28–0.78]	0.97		

Multivariable linear regression model for eGFR at 180 months post-transplant ($N = 42$), considering the effect of rank {Avg CNI level during 0–180 months}

Variable	Multivariable *p*-value	Model[b] Coeff ± SE
Donor age (years)	0.02	−0.729 ± 0.290
First BPAR ≤180 months	0.01	−25.113 ± 9.323
Rank {Avg CNI level: 0–180 months}	0.72	4.775 ± 13.095

Note: (√) Represents selection into the multivariable linear regression model.

Abbreviations: Avg, average; BMI, body mass index; BPAR, biopsy-proven acute rejection; CNI, calcineurin inhibitor; Coeff, coefficient; CVD, cardiovascular disease; DD, deceased donor; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; NODAT, new onset diabetes after transplant.

[a]The 3 variables selected into the multivariable model were defined as follows: group C = {1 if recipient was randomized into group C, 0 otherwise}; donor age (years) (continuous variable); and first BPAR ≤180 months = {1 if the patient experienced a first BPAR during the first 180 months post-transplant, 0 otherwise}. The estimated intercept term ± SE for the multivariable model was: 89.008 ± 11.045, and the coefficient of multiple determination was 0.22.

[b]The 4 variables included in this multivariable model were defined as follows: donor age (years) (continuous variable); first BPAR ≤180 months = {1 if the patient experienced a first BPAR during the first 180 months post-transplant, 0 otherwise}; and rank {Avg CNI level: 0–180 mo} = rank of the average CNI trough level during 0–180 months post-transplant. The estimated intercept term ± SE for the multivariable model was: 87.263 ± 12.152, and the coefficient of multiple determination was 0.22.

Graft failure-due-to chronic CNI toxicity

It should be noted that although protocol biopsies were not routinely performed, clinically indicated biopsies that were performed over time found that only one patient had developed biopsy-proven chronic CNI toxicity (at 86 months post-transplant). This patient was assigned to group C and had received CSA/SRA. This was the only patient in the cohort (1/150) who was determined to have experienced graft failure-due-to chronic CNI toxicity (at 94 months post-transplant).

DISCUSSION

This study shows that in a prospectively followed cohort of 150 adult, primary kidney transplant recipients with 20 years of post-transplant follow-up, higher average CNI trough levels (within our observed range of levels) were not associated with any reduced long-term renal function (as determined by eGFR). These results were consistent across various time periods, including average CNI trough levels determined during the first 0–60, 0–120, and 0–180 months (as well as during 60–120, 120–180, and

60–180 months) post-transplant. The average TAC and CSA trough levels for most of the patients receiving TAC and CSA during these time periods ranged between 5.44–7.85 ng/mL and 123.4–160.6 ng/mL, respectively. Thus, long-term use of reduced-CNI dosing appears to have successfully avoided reduced renal function due to chronic CNI toxicity in nearly all of the patients.

The reported randomized trial results of Ekberg et al.[15,16] support our findings in that reduced-TAC dosing with target TAC trough levels of 4–8 ng/mL combined with mycophenolate acid and low-dose corticosteroids significantly lowered the hazard rate of first BPAR combined with providing more favorable renal function through 36 months post-transplant. Whereas the achieved CSA trough levels of 100–150 ng/mL, as reported by Ekberg et al.,[15,16] may result in less CAI/TG occurrence in comparison with previous studies that used higher CSA dosing, their clinical trial reporting through 36 months post-transplant was simply not long enough to be able to make such a determination. Although other studies have reported more favorable renal function when using TAC versus CSA,[5,14,30–33] even the results, as reported by Silva et al.,[32,33] were simply not long enough (at 4 years post-transplant) to be able to draw long-term conclusions.

Interestingly, our results also show that experiencing a first BPAR and older donor age are the two most significant multivariable predictors of poorer renal function (determined via eGFR) at 60, 120, and 180 months post-transplant. Whereas the unfavorable influence of both factors are known, this study shows the continual long-term importance of avoiding BPAR in order to maintain a more favorable renal function. Although the unfavorable influence of using CSA (vs. TAC) was also a significant multivariable predictor of poorer renal function in our analysis, its influence was less strong in comparison with the multivariable effects of experiencing a first BPAR and receiving an older donor age allograft. Thus, immunosuppression approaches that lower the hazard rate of first BPAR occurrence should also achieve more favorable long-term renal function as well.

Last, one clear study limitation is the fact that with a larger sample size, there would likely be numerous contributing factors beyond those reported here that would significantly affect renal function in a post-kidney transplant setting. Although the multivariable linear regression results reported for eGFR in this study at 60, 120, and 180 months post-transplant were based on relatively small sample sizes (N = 106, 71, and 42, respectively), and despite the lack of available long-term protocol biopsies, our study is somewhat unique in that results at 20 years post-kidney transplant of a prospectively followed single-center cohort are being reported here, particularly long-term renal function as measured by eGFR. The clear lack of any noticeable unfavorable effect of higher average CNI trough levels (within our observed range of levels) on long-term renal function, we believe, overcomes the main study limitation of possibly having low statistical power to detect small-to-moderate differences in such clinical outcomes. We hope that these study results will encourage others to follow and subsequently report such long-term results (at 10–20 years post-transplant) of their kidney transplant clinical trials, as the importance of understanding predictors of long-term results must be based on observed clinical outcomes of patients followed for this type of study length.

AUTHOR CONTRIBUTIONS

G.C., J.J.G., G.G., M.M.T., D.R., W.K., A.M., L.T., and G.W.B. designed the research. G.C., J.J.G., G.G., M.M.T., D.R., W.K., A.M., L.T., and G.W.B. performed the research. G.C., J.J.G., and L.T. analyzed the data. G.C. and J.J.G. wrote the manuscript.

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ORCID

Adela Mattiazzi https://orcid.org/0000-0002-3388-5648

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