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ORIGINAL ARTICLE

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Comparison of the effects of standard vs low-dose prolonged-release tacrolimus with or without ACEi/ARB on the histology and function of renal allografts

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Targeting the renin-angiotensin system and optimizing tacrolimus exposure are both postulated to improve outcomes in renal transplant recipients (RTRs) by preventing interstitial fibrosis/tubular atrophy (IF/TA). In this multicenter, prospective, open-label controlled trial, adult de novo RTRs were randomized in a 2 × 2 design to low- vs

Abbreviations: (m)FAS, (modified) full analysis set; (O)AHT, (other) antihypertensive therapy; ACEi, angiotensin-converting enzyme inhibitor; AMR, antibody-mediated rejection; ARB, angiotensin II receptor 1 blocker; $ATIIR_1$, angiotensin II receptor type 1; CI, confidence interval; CNI, calcineurin inhibitor; cPRA, calculated PRA; DGF, delayed graft function; DSA, donor-specific antibody; ECD, extended criteria donor; eGFR, estimated glomerular filtration rate; IF/TA (+i), interstitial fibrosis/tubular atrophy (with inflammation); ITT, intent-to-treat; LOW, low-dose tacrolimus; MMF, mycophenolate mofetil; RAS, renin-angiotensin II system; RTR, renal transplant recipient; SD, standard deviation; STD, standard-dose tacrolimus; Tac, tacrolimus; TCMR/B, T cell–mediated rejection including borderline changes; TCMR, T cell–mediated rejection; TEAE, treatment-emergent adverse event.

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standard-dose (LOW vs STD) prolonged-release tacrolimus and to angiotensin-converting enzyme inhibitors/angiotensin II receptor 1 blockers (ACEi/ARBs) vs other antihypertensive therapy (OAHT). There were 2 coprimary endpoints: the prevalence of IF/TA at month 6 and at month 24. IF/TA prevalence was similar for LOW vs STD tacrolimus at month 6 (36.8% vs 39.5%; *P* = .80) and ACEi/ARBs vs OAHT at month 24 (54.8% vs 58.2%; *P* = .33). IF/TA progression decreased significantly with LOW vs STD tacrolimus at month 24 (mean [SD] change, +0.42 [1.477] vs +1.10 [1.577]; *P* = .0039). Across the 4 treatment groups, LOW + ACEi/ARB patients exhibited the lowest mean IF/TA change and, compared with LOW + OAHT patients, experienced significantly delayed time to first T cell–mediated rejection. Renal function was stable from month 1 to month 24 in all treatment groups. No unexpected safety findings were detected. Coupled with LOW tacrolimus dosing, ACEi/ARBs appear to reduce IF/TA progression and delay rejection relative to reduced tacrolimus exposure without renin-angiotensin system blockade.
ClinicalTrials.gov identifier: NCT00933231.

KEYWORDS
clinical research/practice, clinical trial, graft survival, immunosuppressant - calcineurin inhibitor: tacrolimus, immunosuppression/immune modulation, kidney transplantation/nephrology, organ transplantation in general, patient survival

1 | INTRODUCTION

Renal allograft and patient survival have improved considerably during the initial year posttransplant, whereas longer-term survival has improved more modestly.[1,2] The standard of care for maintenance immunosuppression, used in 93% of centers in the United States[3] and in most centers in Canada,[4] consists of the calcineurin inhibitor (CNI) tacrolimus and mycophenolate mofetil (MMF). Tacrolimus dosing is subject to local practice; some centers use standard tacrolimus dosing, targeting trough concentrations that are generally sufficient to suppress inflammation in early protocol biopsies.[5,6] Others, following the SYMPHONY Study,[7,8] target lower immunosuppressant concentrations (eg, tacrolimus trough values near 5 ng/mL) while maintaining MMF and steroid treatment.

Reduced tacrolimus exposure in renal transplant recipients (RTRs) might be preferred to prevent activation of endogenous viruses, including polyomavirus.[9] Tacrolimus minimization might also be chosen to decrease the risk of CNI nephrotoxicity, such as interstitial fibrosis and tubular atrophy (IF/TA), histologic changes historically associated with graft failure.[10,11] However, evidence from the Long-Term Deterioration of Kidney Allograft Function (DeKAF)[12] and other studies suggests that immunological events account for most allograft losses and have brought into question the association between IF/TA and adverse outcomes.[13-16] Indeed, newer analyses indicate that IF/TA with inflammation (IF/TA+i)[15] is more deleterious to the graft than is IF/TA alone.[17-20] However, little is known about clinical interventions that can prevent or reverse IF/TA+i.

Reduced tacrolimus exposure has been associated with better allograft function, less IF/TA, and reduced prevalence of polyomavirus viremia. However, it has also been associated with a greater incidence of rejection, relative to standard tacrolimus dosing,[6] by permitting allograft-specific T cell activation, T cell–mediated rejection (TCMR), donor-specific antibody (DSA) development[21] and, ultimately, antibody-mediated rejection (AMR).[18,22,23]

Another approach proposed to improve clinical outcomes in RTRs is treatment with blockers of the renin-angiotensin system (RAS), namely antihypertensive therapy (AHT) of the angiotensin-converting enzyme inhibitor or angiotensin II receptor 1 blocker classes (ACEi/ARBs). Independent of their vasodilatory effects, these RAS-targeting AHTs are anti-inflammatory and immunomodulating,[24,25] and they appear to block histopathologic change in renal allografts.[26,27]

As with reduced-dose tacrolimus, clinical evidence supporting the use of ACEi/ARBs in RTRs is ambiguous, although they are used to limit systemic inflammation and renal fibrosis in glomerulonephritis,[28] hypertensive injury, and other pathologic states.[29] In a post hoc analysis of trial data, ACEi/ARB use was independently associated with protection from IF/TA at 24 months.[30] A recent study of RTRs with proteinuria showed that ACEi use had no significant effect on renal function or patient survival.[31] Moreover, despite some promising preclinical[32] and clinical[33,34] findings, ACEi/ARBs have shown no consistent patient or allograft survival benefit after meta-analysis.[35]

This study (FKC-014) was designed to address these uncertainties by assessing the effects on IF/TA prevalence of 2 different interventions: a reduced tacrolimus dosing strategy and use of RAS-blocking AHTs.

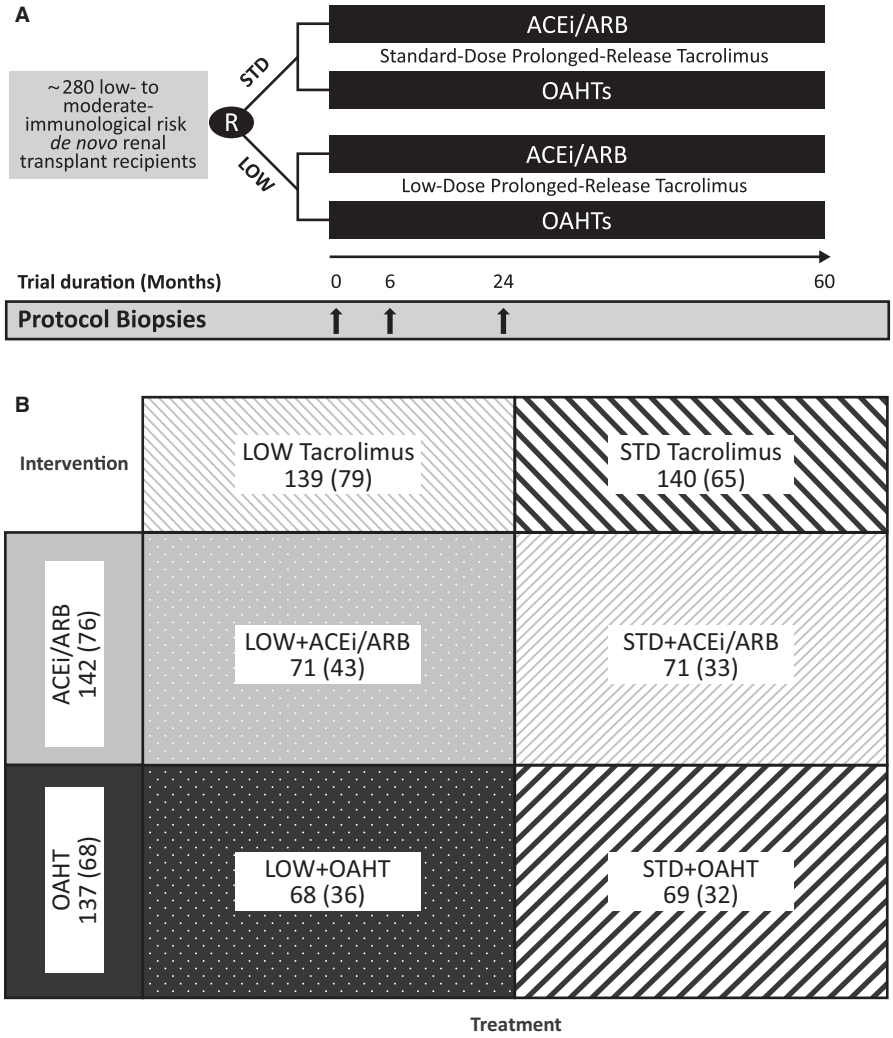


FIGURE 1 Design of FKC-014 (A) and distribution of patients across intervention and treatment groups (B). At randomization, patients were assigned to 1 of 4 possible treatments (LOW + ACEi/ARB; LOW + OAHT; STD + ACEi/ARB; and STD + OAHT), corresponding to 2 tacrolimus interventions (LOW vs STD) and 2 AHT interventions (ACEi/ARB vs OAHT). Patient numbers in (B) correspond to the FAS/SAF and mFAS6/24 populations. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor 1 blocker; FAS, full analysis set; mFAS, modified full analysis set; LOW, low dose; OAHT, other antihypertensive treatment; SAF, safety set; STD, standard dose

2 | MATERIALS AND METHODS

2.1 | Study design

FKC-014 (Figure 1) is a multicenter, prospective, open-label, randomized controlled trial undertaken at 13 sites in Canada and conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, the International Conference on Harmonisation guidelines, and applicable laws and regulations. An independent ethics committee from each study center granted approval before initiation. Written informed consent was obtained from each patient before enrollment into the study.

Patients were randomized 1:1:1:1 by using a 2 × 2 factorial design to receive either low- or standard-dose (LOW or STD interventions, respectively) prolonged-release tacrolimus (Advagraf®, Astellas Pharma Canada, Inc, Markham, ON, Canada)[36] plus either an ACEi or an ARB (ACEi/ARB intervention group) or other (non–ACEi/ARB-based) OAHT (OAHT intervention group), as clinically indicated. Details regarding study inclusion, study procedures, and statistical methods are provided as Supporting Information.

2.2 | Endpoints

There were 2 coprimary endpoints: the prevalence of IF/TA (defined as ci + ct ≥ 2, based on Banff 2007 criteria[37]) at month 6 (in the STD vs LOW intervention groups) and at month 24 (in the ACEi/ARB vs OAHT intervention groups). Secondary endpoints included the progression of IF/TA (defined as the change in ci + ct) from implant to month 6 or month 24 posttransplant and assessment of renal function (Chronic Kidney Disease Epidemiology Collaboration formula), blood pressure, and use of antihypertensive agents throughout the study period. An additional post hoc endpoint was the prevalence of IF/TA+i (Banff ci + ct ≥ 2 and i ≥ 1) at 6 months and 24 months. For the primary and secondary endpoints, treatment effects were assessed in a pairwise fashion between intervention groups; in other analyses, comparisons were made across the 4 treatment groups.

2.3 | Treatments

All patients received basiliximab (Simulect®; Novartis Pharmaceuticals Canada Inc., Dorval, QC, Canada) induction (20 mg, 2 hours

before and 4 days after implantation), steroids (200-500 mg intravenous methylprednisolone preoperatively followed by either methylprednisolone intravenously or prednisone orally, starting at 1 mg/kg and tapering to ≥ 5 mg daily by month 5), and mycophenolate mofetil (MMF; 1 g twice daily from day 1 posttransplant, with adjustment as clinically indicated).

Prolonged-release tacrolimus was initiated in the STD intervention group as a single dose of 0.15-0.20 mg/kg, with dose adjustments as needed to achieve the target trough concentrations of 12 ± 2 ng/mL for weeks 1 and 2, 10 ± 2 ng/mL for week 3 through month 3, and 8 ± 2 ng/mL for month 4 through month 6. For patients randomized to the LOW group, the initial dose was 0.05-0.15 mg/kg, adjusted thereafter to achieve a target trough concentration of 5 ± 1 ng/mL through month 6. Tacrolimus trough targets and dosing after month 6 were at the Investigator's discretion for all patients.

Patients randomized to the ACEi/ARB intervention group received ramipril (initially 5 mg/day, increasing to 10 mg/day by month 3 posttransplant), or irbesartan (150 mg/day, increasing to 300 mg/day) by month 1 posttransplant, continuing to month 24. For patients randomized to the OAHT intervention group, non–ACEi/ARB-based antihypertensive therapy was initiated if the patients became hypertensive.

2.4 | Procedures

Renal biopsies were performed per protocol at baseline, month 6, and month 24 to assess the coprimary efficacy endpoints (ie, presence of IF/TA ≥ 2 at month 6 [comparing the LOW vs STD tacrolimus intervention groups] and at month 24 [comparing the ACEi/ARB vs OAHT intervention groups]).

Sera were tested for DSA at implant, month 6, and then yearly from month 12. Serum screening for polyomavirus occurred at months 3, 6, 9, and 12. Renal function and blood pressure were evaluated at months 1, 3, and 6 and then yearly, starting at month 12.

Mononuclear cell interstitial inflammation was assessed prospectively at months 6 and 24 at a central pathology laboratory by using the current Banff semiquantitative criteria ("i") for renal allograft inflammation of the unscarred (non-IF/TA) parenchyma. Furthermore, the extent of inflammation of the entire cortical area present (including the subcapsular cortex, perivascular cortex, and areas of IF/TA) was reported on a semiquantitative scale ("ti") based on the Banff 2007 classification.[37]

2.5 | Statistical analysis

The sample size of 240 evaluable patients was based on a statistical power of 80% to detect a 15% difference in IF/TA prevalence between 2 groups, using a .05 significance level and 2-tailed test.

Tacrolimus trough concentrations were estimated using 4 piecewise, mixed-effects models corresponding to the 4 sets of dosing guidelines. Each model used log tacrolimus concentration as the response with fixed effects of time, dosing group, and interaction (between time and dosing group) and a random effect for within-patient assessments.

ACEi/ARB use, steroid dose, and MMF dose were assessed for each nominal time period in the full analysis set (FAS).

For the coprimary endpoints, logistic regression was used to assess differences in IF/TA prevalence between intervention groups while adjusting for fixed effects, including donor status, delayed graft function (DGF), donor age, recipient sex, and baseline ci + ct. Modified FAS (FAS patients with evaluable biopsies at implant and month 6 [mFAS6] or month 24 [mFAS24]) populations were used for this analysis. IF/TA progression was assessed in the mFAS6/24 population (mFAS with evaluable biopsies at months 6 and 24). mFAS included all patients of the FAS who had evaluable biopsies (marginal or adequate specimen), per central pathology assessment, with consideration of missing protocol biopsy replaced with for-cause biopsy, at the following time points: mFAS6, patients have evaluable biopsies at implant and month 6; mFAS24, patients have evaluable biopsies at implant and month 24; and mFAS6/24, patients have evaluable biopsies at months 6 and 24.

3 | RESULTS

3.1 | Patients

The intent-to-treat (ITT) population included 281 adult de novo RTRs at 13 Canadian study centers. Of these patients, 235

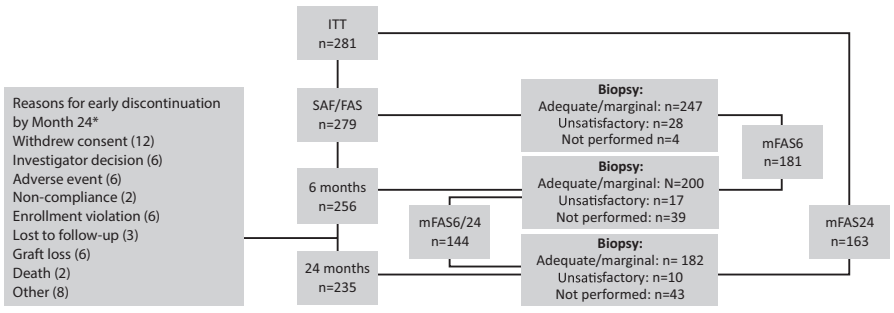


FIGURE 2 Patient disposition in FKC-014. *Multiple reasons could be given for early discontinuation. mFAS6, patients have evaluable biopsies at implant and month 6; mFAS24, patients have evaluable biopsies at implant and month 24. ITT, intent-to-treat set; SAF, safety set; (m)FAS, (modified) full analysis set

remained in the study by month 24. Biopsy material was suitable for histologic analysis for 247, 200, and 182 of the patients at baseline, month 6 and month 24, respectively (Figure 2; Table S1). For-cause biopsy rates in each treatment group are shown in Table S2. Mean patient age was 50.3 years, and 68% were male. Donor age was >50 years in 41.6%; the donor was deceased in 60.9% and identified as an extended criteria donor (ECD) in 21.4% of cases. Baseline characteristics, including stratification factors (recipient sex, donor age and status, and DGF) and other parameters, were generally well distributed among the 4 treatment groups. However, diabetic nephropathy was more common in patients randomized to the STD + OAHT treatment group than in the general ITT population (27.1% vs 18.1%). In addition, the LOW + OAHT treatment group had a lower rate of DGF than the ITT population (18.8% vs 24.2%). Other baseline differences included imbalances in the proportion of patients with ECDs (more common in the ACEi/ARB intervention group) and with donors who died from cerebrovascular accident (more common in the STD intervention group) (Table 1 and data not shown).

3.2 | Dosing of immunosuppressive therapies and AHTs

Prolonged-release tacrolimus doses administered in each treatment group throughout the study period are summarized in Table S3. A difference in tacrolimus trough concentration was observed, per protocol, over the first 6 months following transplantation. For both the LOW and STD intervention groups, mean trough tacrolimus concentrations were within the target range by week 3 and remained so through month 6 (Figure 3). Tacrolimus trough concentrations converged thereafter, with 95% confidence intervals (CIs) overlapping by day 576.

TABLE 1 Baseline characteristics of renal transplant recipients

	LOW Tac + ACEi/ARB	LOW Tac + OAHT	STD Tac + ACEi/ARB	STD Tac + OAHT	Total
	n = 71	n = 69	n = 71	n = 70	N = 281
Age, y (mean [SD])	50.5 (11.73)	48.0 (12.67)	50.4 (12.04)	52.4 (11.22)	50.3 (11.96)
Male, n[a]	47 (66.2%)	48 (69.6%)	47 (66.2%)	49 (70.0%)	191 (68.0%)
White, n	56 (78.9%)	52 (75.4%)	58 (81.7%)	57 (81.4%)	223 (79.4%)
Body mass index, kg/m^2 (mean [SD])	27.6 (5.89)	27.3 (5.54)	28.3 (5.65)	27.0 (4.49)	27.6 (5.41)
Epstein-Barr virus positive, n	65 (91.5%)	63 (91.3%)	64 (90.1%)	61 (87.1%)	253 (90.0%)
Donor age >50 y, n[a]	29 (40.8%)	28 (40.6%)	31 (43.6%)	29 (41.4%)	117 (41.6%)
Donor deceased, n[a]	43 (60.6%)	42 (60.9%)	43 (60.6%)	43 (61.4%)	171 (60.9%)
Delayed graft function, n[a]	18 (25.4%)	13 (18.8%)	19 (26.8%)	18 (25.7%)	68 (24.2%)
Extended criteria donor, n[b]	17 (23.9%)	14 (20.3%)	18 (25.4%)	11 (15.7%)	60 (21.4%)
Primary reason for transplant[c]					
Diabetic nephropathy	10 (14.1%)	8 (11.6%)	14 (19.7%)	19 (27.1%)	51 (18.1%)
Polycystic kidney disease	10 (14.1%)	13 (18.8%)	10 (14.1%)	15 (21.4%)	48 (17.1%)
Glomerulonephritis	11 (15.5%)	10 (14.5%)	11 (15.5%)	5 (7.1%)	37 (13.2%)
IgA nephropathy	5 (7.0%)	10 (14.5%)	9 (12.7%)	9 (12.9%)	33 (11.7%)
Hypertension	3 (4.2%)	5 (7.2%)	5 (7.0%)	4 (5.7%)	17 (6.0%)
Glomerulosclerosis	5 (7.0%)	4 (5.8%)	2 (2.8%)	3 (4.3%)	14 (5.0%)
PRA (mean [SD])	11.2 (19.43)	9.7 (24.91)	12.1 (21.08)	23.0 (31.56)	13.9 (24.65)
HLA-A > 1 mismatch, n	28 (39.4%)	26 (37.7%)	26 (36.6%)	25 (36.2%)	105 (37.5%)
HLA-B > 1 mismatch, n	36 (50.7%)	33 (47.8%)	38 (53.5%)	34 (49.3%)	141 (50.4%)
HLA-C > 1 mismatch, n	21 (29.6%)	24 (34.8%)	27 (38.0%)	28 (40.6%)	100 (35.7%)
HLA-DRB1 > 1 mismatch, n	21 (29.6%)	19 (27.5%)	18 (25.4%)	20 (29.0%)	78 (27.9%)
HLA-DQB1 > 1 mismatch, n	12 (16.9%)	13 (18.8%)	14 (19.7%)	13 (18.8%)	52 (18.6%)

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor 1 blocker; EBV, Epstein-Barr virus; IgA, immunoglobulin A; LOW, low dose; OAHT, other antihypertensive treatment; PRA, panel-reactive antibody; SD, standard deviation; STD, standard dose; Tac, prolonged-release tacrolimus.

[a]Modeling covariate property, used for stratification of patients at randomization.

[b]Extended criteria donor defined as age ≥60 or age 50-59 years with ≥2 of the following 3 risk factors: (1) history of hypertension, (2) terminal creatinine is ≥132.6 μmol/L, or (3) death due to cerebrovascular accident.

[c]Primary reasons for transplantation shown here were those cited by ≥5.0% of patients; for 19.2% of patients, the primary reason cited was "Other."

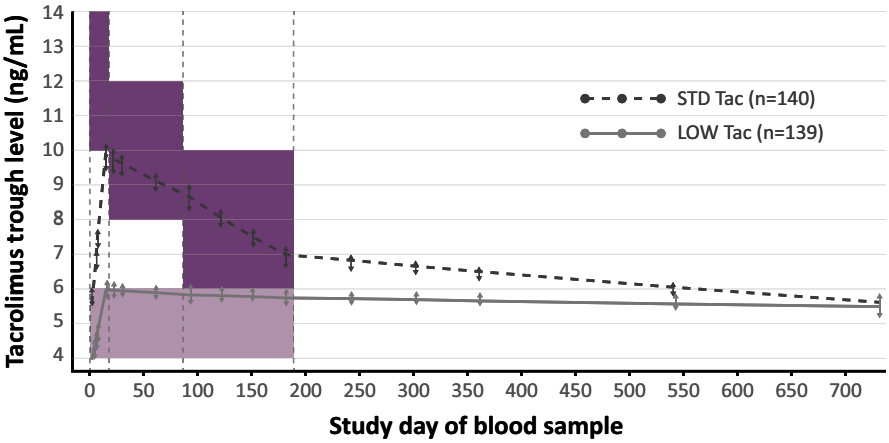


FIGURE 3 Least-squares mean (±standard error) tacrolimus trough concentrations by time for patients randomized to standard-dose (STD) vs low-dose (LOW) tacrolimus (Tac). Light and dark purple shading indicate protocol-specified target trough concentrations for patients randomized to LOW Tac and STD Tac. After month 6, no target was specified. Trough concentrations were estimated from a 4-period mixed model

All patients received steroid treatment during the study. Mean oral prednisone dose declined from ~30 mg daily in the first 2 weeks posttransplant to <10 mg daily from month 3 to month 24; however, a higher mean dose of prednisone between months 13 and 24 was used in the LOW + OAHT treatment group, compared with the other 3 groups (Table S4). MMF dosing was therapeutically similar across treatment groups from transplant to month 24 (Table S5).

Use of ACEi/ARBs was likewise generally per protocol. In the ACEi/ARB (n = 142) and OAHT (n = 137) intervention groups, ACEi/ARBs were used in >83% and <16% of patients, respectively, at all times up to month 24. Antihypertensive compliance in each treatment group throughout the study is summarized in Table S6. Median time taking ACEi/ARBs was 22.2 months in the ACEi/ARB group vs 0.0 months for the OAHT group.

3.3 | IF/TA prevalence and progression

Prevalence of IF/TA (defined as ci + ct ≥ 2) did not differ significantly between the LOW and STD intervention groups (36.8% vs 39.5%; $P = .80$) or between the ACEi/ARB and OAHT groups (33.7% vs 42.7%; $P = .09$) at month 6. Prevalence of IF/TA remained similar between the ACEi/ARB and OAHT groups at month 24 (54.8% vs 58.2%; $P = .33$); however, the STD intervention group had increased IF/TA prevalence compared with the LOW intervention group (71.6% vs 43.8%; $P < .001$). IF/TA prevalence was also significantly higher in the STD + ACEi vs LOW + ACEi treatment group (73.7% vs 39.1%; $P = .02$), and the STD + OAHT vs LOW + OAHT treatment group (69.4% vs 48.8%; $P = .007$), at month 24 (Figure 4A). The IF/TA grade at 24 months was considerably less in the LOW + ACEi treatment group compared with the other groups (Table S7).

In an analysis of patients with biopsies available at months 6 and 24, mean [SD] change in IF/TA score differed significantly between the LOW and STD intervention groups (+0.42 [1.477] vs +1.10 [1.577]; $P = .0039$) but not between the ACEi/ARB and OAHT intervention groups (+0.56 [1.431] vs +0.91 [1.675]; $P = .15$). A trend toward an interaction between the interventions was apparent when IF/TA progression was analyzed by treatment group. Thus, patients in the LOW + ACEi/ARB group experienced numerically less IF/TA progression, relative to all other treatment groups. This difference reached statistical significance in comparison with patients in the STD + ACEi/ARB group (+0.19 [1.144] vs + 1.05 [1.627]; $P = .03$) (Figure 4B).

Similarly, in comparisons with baseline IF/TA, mean [SD] change, the LOW + ACEi/ARB showed a significantly smaller increase in IF/TA score from month 0 to month 6 and month 24, relative to either the LOW + OAHT or the STD + ACEi/ARB groups (Figure 5).

3.4 | IF/TA+i

IF/TA+i was also examined in a post hoc analysis of all intervention and treatment groups (Figure 6). IF/TA+i accounted for less than half of the overall IF/TA prevalence (22% vs 56% of patients in the mFAS24 population at month 24; data not shown). Comparison between Figures 4 and 6 shows that much of the observed progression of IF/TA from months 6 to 24 occurred in the absence of inflammation, particularly in patients in the STD intervention group.

The ACEi/ARB intervention group experienced lower prevalence of IF/TA+i, relative to OAHT-treated patients, at months 6 and 24. Analysis by treatment group showed that prevalence of IF/TA+i declined between month 6 and month 24 in the LOW + ACEi/ARB treatment group. Similarly, the prevalence of IF/TA+i was significantly lower in the LOW + ACEi/ARB group than in the LOW + OAHT treatment group at month 24 (8.7% vs 37.2%; $P = .0022$). Conversely, in the STD tacrolimus intervention group, the addition of ACEi/ARB treatment had little effect on IF/TA+i by month 24 (Figure 6).

More detailed histological Banff acute and chronic scores at 6 and 24 months for the 4 treatment groups are shown in Table S8. Notably, these show a reduced tubulointerstitial and peritubular capillary inflammation in the LOW ACEi/ARB group compared with all other groups, and a similar degree of arteriolar hyalinosis for all groups at 24 months.

3.5 | Immunologic events

Rejection events were observed in protocol and for-cause biopsies over 24 months posttransplant. Time to first TCMR of Banff grade

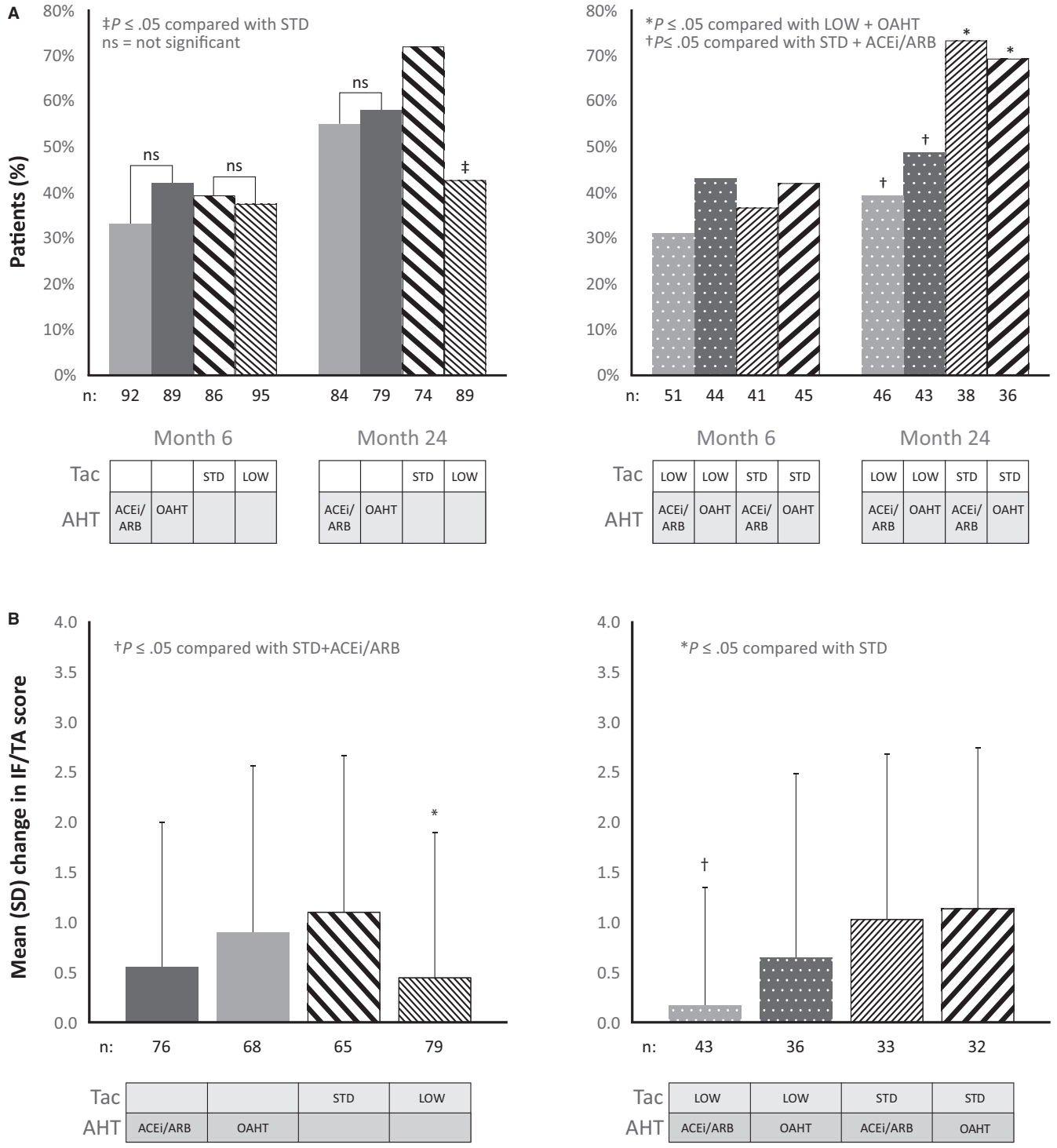


FIGURE 4 Prevalence of IF/TA at month 6 and month 24 (A) and progression of IF/TA from month 6 to month 24 by intervention and treatment group (B). Brackets indicate comparisons specified as coprimary or as key secondary efficacy endpoints. For treatment group comparisons, statistical significance was tested relative to the LOW + OAHT group and the STD + ACEi/ARB group. No test was performed comparing LOW + ACEi/ARB with STD + OAHT. Data in B show mean change in IF/TA from month 6 to month 24. ns, nonsignificant ($P \geq .05$); ACEi, angiotensin-converting enzyme inhibitor; AHT, antihypertensive treatment; ARB, angiotensin II receptor 1 blocker; FAS, full analysis set; IF/TA, interstitial fibrosis/tubular atrophy; LOW, low dose; OAHT, other antihypertensive treatment; SD, standard deviation; STD, standard dose; Tac, prolonged-release tacrolimus

1A or higher (Figure 7) was shortest, corresponding to the highest risk of rejection, in the LOW + OAHT treatment group, relative to the STD + OAHT treatment group, with a hazard ratio (90% CI) for TCMR of 2.48 (1.13-5.43; $P = .023$). Likewise, the hazard ratio (90% CI) for the LOW + OAHT vs the LOW + ACEi/ARB group was 2.69 (1.22-5.92; $P = .014$).

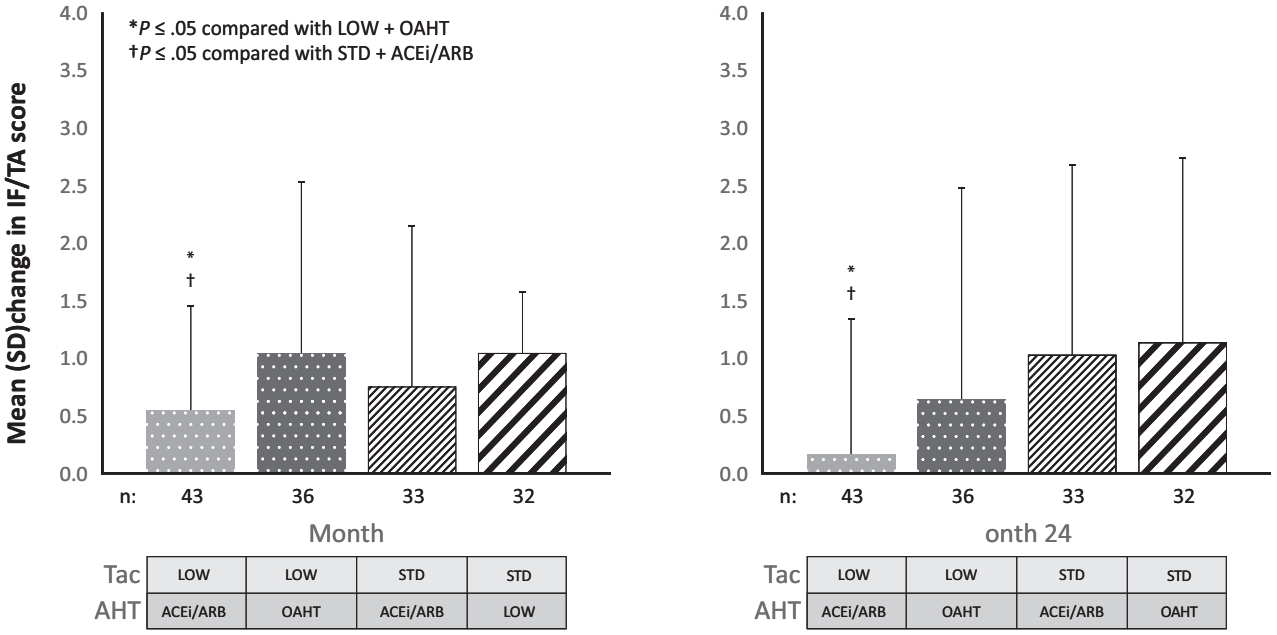


FIGURE 5 Change from baseline in IF/TA score by treatment group by month 6 and month 24. Statistical significance was tested relative to the LOW + OAHT group and the STD + ACEi/ARB group. No test was performed comparing LOW + ACEi/ARB with STD + OAHT. Data displayed show mean change in IF/TA score from month 0 to month 6 and month 24. ACEi, angiotensin-converting enzyme inhibitor; AHT, antihypertensive treatment; ARB, angiotensin II receptor 1 blocker; IF/TA, interstitial fibrosis/tubular atrophy; LOW, low dose; OAHT, other antihypertensive treatment; SD, standard deviation; STD, standard dose

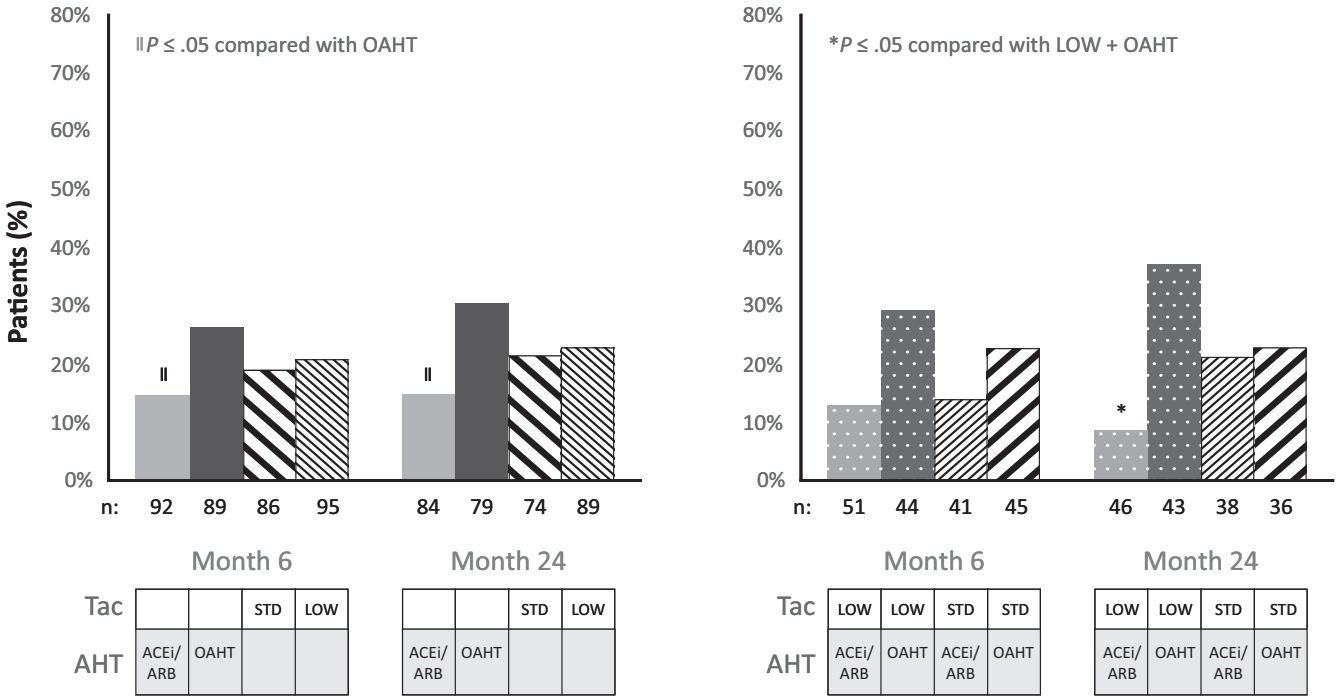


FIGURE 6 Prevalence of interstitial fibrosis/tubular atrophy with inflammation at month 6 and month 24

For TCMR including borderline changes (TCMR/B), the results were qualitatively similar, with median event-free survival of 9 months in the LOW + OAHT group vs 25 months for the LOW + ACEi/ARB group. Median time to TCMR/B could not be estimated for the STD intervention groups (data not shown). At both month 6 and month 24, prevalence of TCMR/B was greater in the LOW + OAHT treatment group, relative to any of the other treatment groups; some, but not all, of these comparisons reached statistical significance (Figure 8).

De novo DSA formation was identified in a small number of patients in all treatment groups at months 6 and 24 (Table 2). In

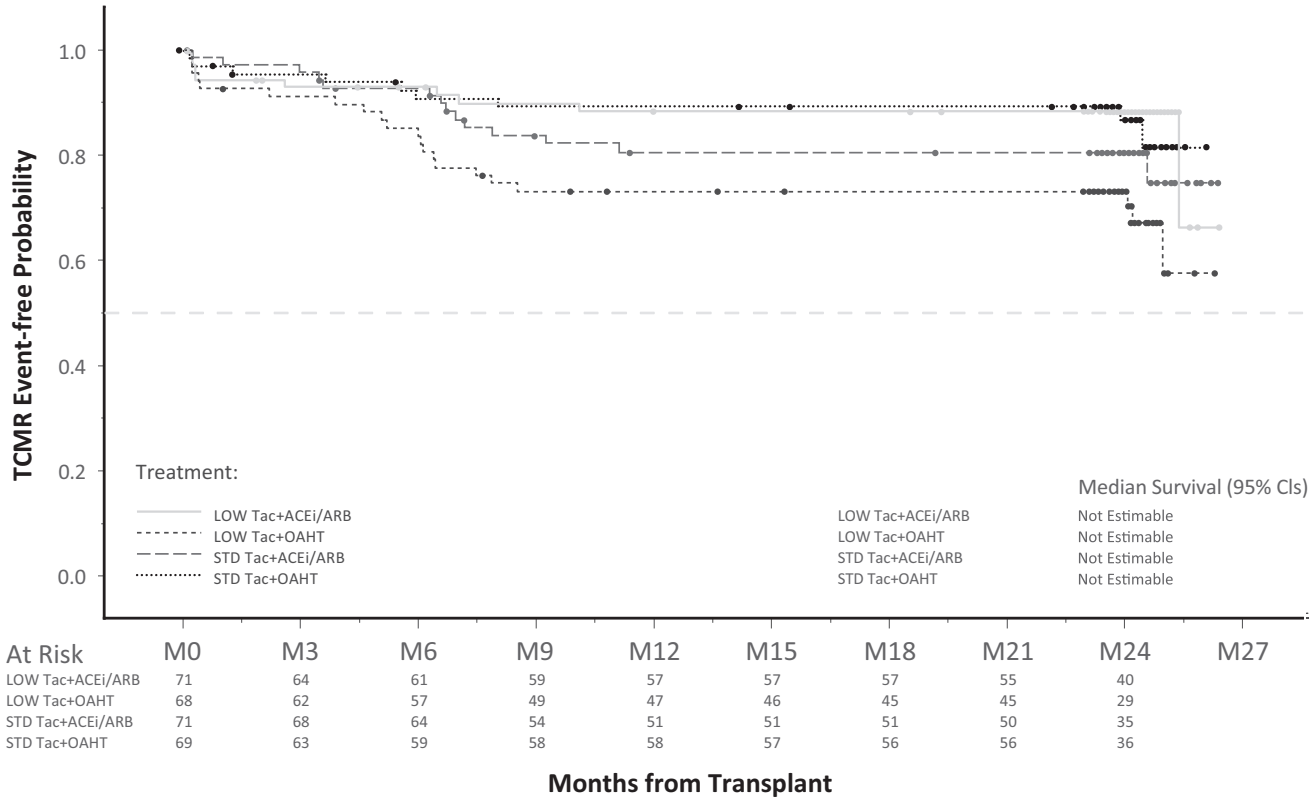


FIGURE 7 Time to first TCMR of Banff grade 1A or higher (Kaplan-Meier estimation). Data derive from protocol and for-cause biopsies, with protocol biopsies mandated at months 6 and 24

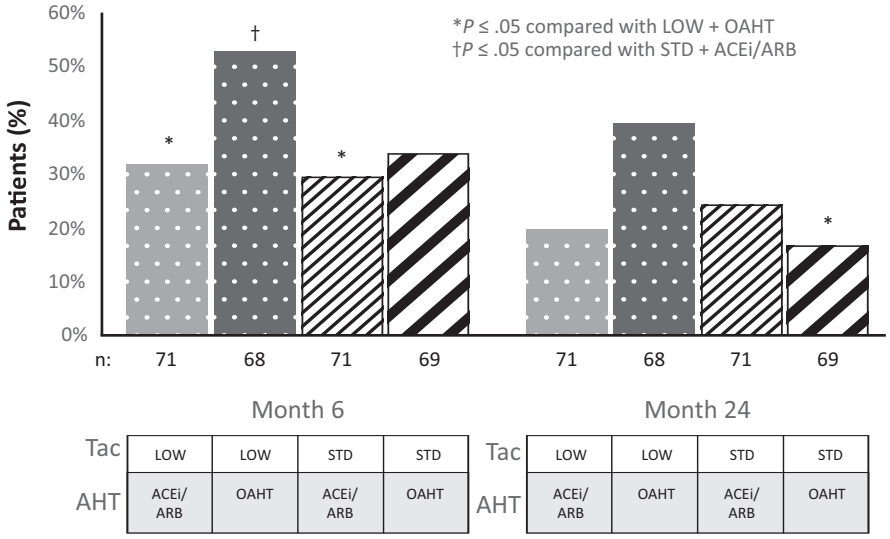


FIGURE 8 Prevalence of TCMR/B at months 6 and 24. Statistical significance was tested relative to the LOW + OAHT group and the STD + ACEi/ARB group. No test was performed comparing LOW + ACEi/ARB with STD + OAHT. ACEi, angiotensin-converting enzyme inhibitor; AHT, antihypertensive treatment; ARB, angiotensin II receptor 1 blocker; LOW, low dose; OAHT, other antihypertensive treatment; STD, standard dose; TCMR/B, T cell–mediated rejection including borderline changes

addition, AMR occurred in 6 of 279 patients (2.5%). Antibody-mediated rejection events were observed only after month 6 and were reported for all 4 treatment groups (data not shown).

3.6 | Polyomavirus activation

Polyomavirus viremia was detected in all treatment groups from month 3 through month 12. By month 6, the prevalence of viremia was significantly reduced in the LOW vs STD intervention group (6.4% vs 16.3%; $P = .028$), whereas use of an ACEi/ARB had no effect on prevalence of viremia. After month 6, viremia remained detectable in 4.8%-9.1% of patients across treatment groups. Viral load decreased over time, with 27 (93%) of 29 viremic patients at month 3, but only 7 (50%) of 14 at month 12, carrying >2000 copies/mL of the viral genome (data not shown).

3.7 | Clinical outcomes and patient safety

Renal function, as assessed by eGFR using the Chronic Kidney Disease Epidemiology Collaboration formula, was stable over time from month 1 to month 24 in all treatment groups (Figure 9). Mean diastolic and systolic blood pressures were likewise stable from month 1 to 24 and did not differ across treatment groups (Figure 10). Treatment-emergent adverse events (TEAEs) were observed in nearly all patients in all treatment groups, consistent with expectations for an RTR patient population and the medications mandated in this trial. Serious TEAEs occurred in 64% of patients (Table 3). TEAEs with a prevalence of ≥10% during the study are presented in Table S9. Over the 24 months, there were 11 instances of graft loss and 3 deaths (embolic stroke, infective endocarditis, and unknown cause). Overall, 90% of patients randomized to ACEi/ARB remained on this treatment for the duration of the study.

4 | DISCUSSION

The prevalence of IF/TA ≥2 in FKC-014 was similar for the LOW and STD tacrolimus intervention groups at month 6 and for the ACEi/ARB and OAHT intervention groups at month 24, findings that represent the 2 coprimary objectives of this study. Of particular interest, however, was the observation that ACEi/ARB use reduced IF/TA progression in the context of reduced exposure to prolonged-release tacrolimus and that the IF/TA grade was lower in this group compared with all others. RAS blockade also abrogated the heightened rejection risk otherwise observed with LOW tacrolimus. These findings suggest a potentially important interaction between the 2 interventions tested in this study.

IF/TA observed during the first year after transplant has been associated with late graft loss or other adverse outcomes,[38,39] particularly when other markers of allograft injury are evident as well.[26,40] Recently, several studies have highlighted the prognostic significance of an inflammatory infiltrate, either alone (Banff i score)[18,41] or in the context of fibrosis (IF/TA+i)[17,20,39] as a potentially more powerful prognostic factor. In the current study, IF/TA progression from baseline, IF/TA+I, and tubulointerstitial inflammation and peritubular capillaritis at 24 months were reduced in the low ACEi/ARB intervention group, suggesting that the addition of an ACEi/ARB exerts an anti-inflammatory and antifibrotic effect independent of conventional immunosuppression.

IF/TA represents a common endpoint of several chronic pathologic processes, including TCMR, AMR, and polyomavirus activation,[9,13,42] as well as normal renal aging[43]; CNI toxicity may also contribute to the development of IF/TA according to some authors,[10,44] although this is not universally accepted.[12,45] In the current study, no significant association emerged between IF/TA and tacrolimus dosing up to month 6, although progression of bland IF/TA was significantly greater with STD vs the LOW tacrolimus. The progression of IF/TA in the STD group from month 6 to 24 may be explained in part by the higher prevalence of polyomavirus activation,[9,46] which was significantly more common in STD than in LOW patients at month 6.

TABLE 2 Prevalence of the development of de novo donor-specific antibodies across treatment groups

	LOW Tac + ACEi/ARB	LOW Tac + OAHT	STD Tac + ACEi/ARB	STD Tac + OAHT
	n = 71	n = 68	n = 71	n = 69
DSA developed by month 6, n	2 (3.0%)	1 (1.5%)	1 (1.5%)	1 (1.6%)
DSA developed by month 24, n	4 (5.9%)	6 (8.8%)	3 (4.5%)	2 (3.1%)

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor 1 blocker; DSA, donor-specific antibody; FAS, full analysis set; LOW, low dose; OAHT, other antihypertensive treatment; STD, standard dose; Tac, prolonged-release tacrolimus.
Percentages are based on the number of patients in each group with recorded DSA status.

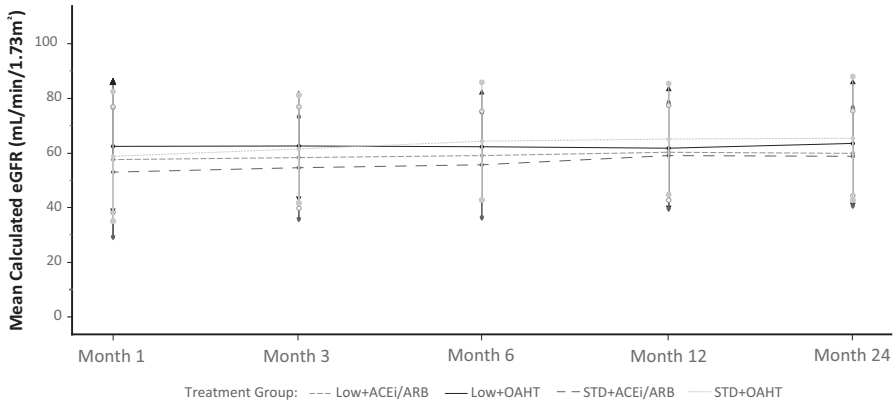
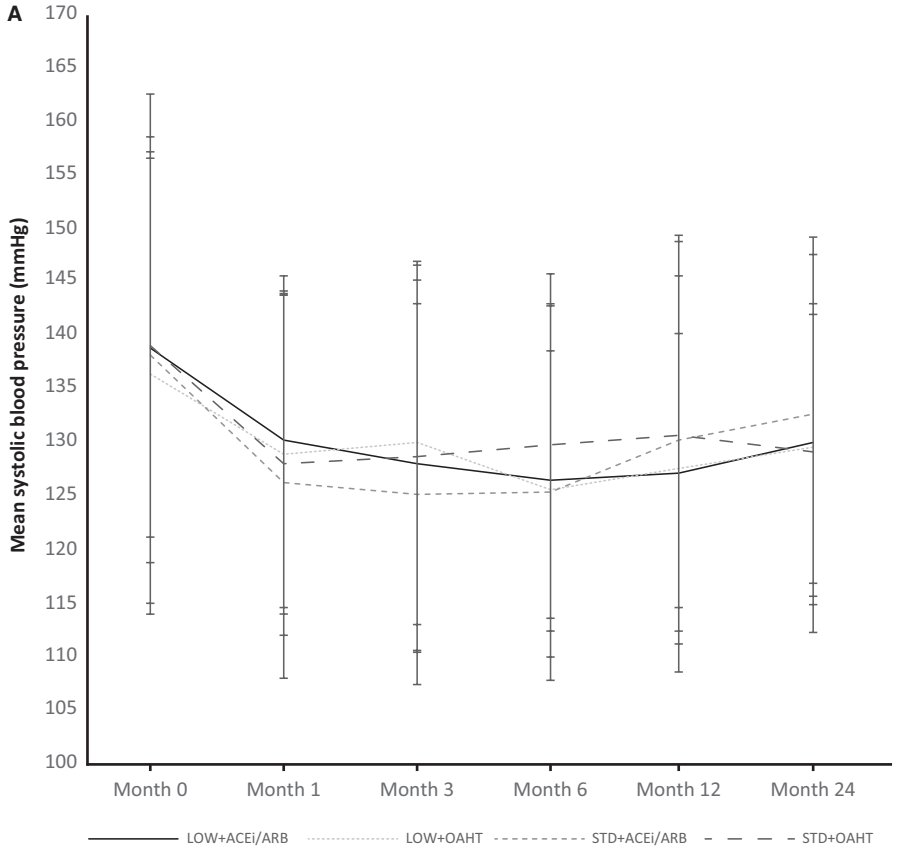


FIGURE 9 Renal function by estimated glomerular filtration rate (eGFR; Chronic Kidney Disease Epidemiology Collaboration) over time by treatment group



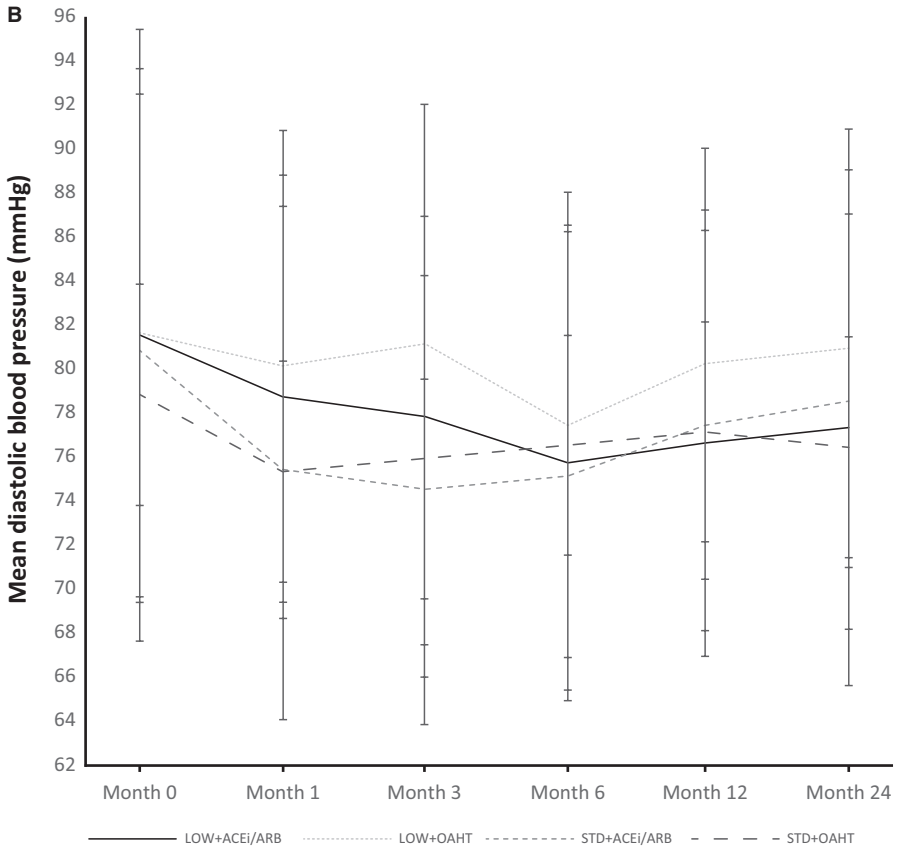


FIGURE 10 Mean systolic blood pressure (A) and diastolic blood pressure (B) over time by treatment group. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor 1 blocker; LOW, low dose; OAHT, other antihypertensive treatment; STD, standard dose

TABLE 3 Overview of safety data over 24 months

	LOW Tac + ACEi/ARB		LOW Tac + OAHT		STD Tac + ACEi/ARB		STD Tac + OAHT	
	Patients	Events	Patients	Events	Patients	Events	Patients	Events
TEAEs, n	70	2113	68	2435	71	2407	69	2162
Serious TEAEs, n	42 (59.4%)	108 (5.1%)	48 (70.6%)	148 (6.1%)	46 (64.8%)	161 (6.7)	42 (60.9%)	117 (5.4%)
Graft loss, n	2 (2.9%)	—	4 (5.9%)	—	3 (4.2%)	—	2 (2.9%)	—
Death, n	0 (0.0%)	—	2 (2.9%)	—	0 (0.0%)	—	1 (1.4%)	—

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor 1 blocker; FAS, full analysis set; LOW, low dose; OAHT, other antihypertensive treatment; STD, standard dose; Tac, prolonged-release tacrolimus; TEAE, treatment-emergent adverse event.

Optimizing CNI dosing has been proposed to delay onset of subclinical and clinical rejection and ultimately to improve graft and patient survival. Historically, at a time when immunosuppressant dosing was typically higher than in the current era, lowering tacrolimus exposure significantly reduced the prevalence of polyomavirus-associated nephropathy and IF/TA, while preserving allograft function more completely than higher-dose tacrolimus.[6] Conversely, negative effects of relaxing immunosuppression by reduction or elimination of CNIs include the increased risk of rejection, even in patients deemed low risk.[14,47-49]

Despite these concerns, clinical evidence suggests that CNI-sparing regimens can be used with acceptable results. In SYMPHONY, patients receiving a low-dose tacrolimus regimen maintained better allograft function and survival, relative to patients on other immunosuppressive treatments, such as standard-dose cyclosporine.[7,8] Unfortunately, SYMPHONY lacked a comparator dose for tacrolimus, and patients' actual tacrolimus exposure was higher than intended.[50] Indeed, these limitations provided part of the impetus for conducting the current study and for treating with prolonged-release tacrolimus formulation,[36] which is associated with more precise control of drug exposure, relative to immediate-release tacrolimus.[50]

In the current study, LOW tacrolimus dosing, combined with ACEi/ARB use, reduced progression of IF/TA from baseline compared with either of these interventions alone. Another striking interaction was seen in the risk of TCMR over the course of 24 months, which was >2-fold higher in the LOW vs STD intervention groups, reinforcing the notion that reduced tacrolimus exposure may not be without immunological risk. Combining LOW tacrolimus exposure with ACEi/ARB use attenuated development of TCMR and TCMR/B, again suggesting a beneficial effect of RAS blockade in this setting.

The apparent impact of RAS-blocking AHTs on rejection in the context of suboptimal immunosuppression is consistent with a large body of evidence that the RAS acts in multiple cell types that drive inflammation and immune responses in various organs and allografts. These include T cells, macrophages, and dendritic cells, all of which express RAS components and respond to RAS stimulation or inhibition.[51-54] Moreover, in animal models as well as in humans, ACEi/ARB treatment reduces expression of proinflammatory and profibrotic mediators, such as monocyte chemoattractant protein-1, tumor necrosis factor α, transforming growth factor β, and interferon γ.[25,55-58] Finally, allograft-specific T cells may be directly inhibited by RAS blockade.

This final possibility was suggested first by Nataraj et al,[59] who showed that autocrine signaling through the angiotensin II receptor $ATIIR_1$ activated the phosphatase calcineurin in murine T cells, leading to transactivation of genes related to T cell proliferation and activation. Conversely, blockade of $ATIIR_1$ mimicked the effect of CNIs, leading to the suppression of T cell responses.[59] It is tempting to speculate that a convergence of inhibitory signals on calcineurin via tacrolimus/FK-binding protein and ATII/cyclophilin blockade via ACEi/ARB occurred in the present study, potentially accounting for the decreased incidence of rejection and of IF/TA+i observed in the LOW + ACEi/ARB, compared with the LOW + OAHT treatment group.

This study has several notable strengths and limitations. Strengths include the fact that the 2 interventions were carried out per protocol, such that the CIs for tacrolimus trough concentrations in the LOW vs STD groups separated and, after the first 2 weeks posttransplant, remained within the designated target ranges. This contrasts with other studies, such as SYMPHONY, where trough values of tacrolimus and other study drugs showed substantial variance and were commonly outside the target range.[50] ACEi/ARB use was likewise largely per protocol.

Limitations include the fact that histopathologic comparisons were statistically underpowered, due to a higher-than-expected number of allograft biopsies being unavailable or inadequate. However, the co-primary endpoints of this study (IF/TA comparisons at month 6 and at month 24) are unlikely to have been substantially affected by this loss of statistical power, given the relative differences between groups. Another limitation is the nonuniform distribution of risk-associated baseline characteristics across treatment groups, including DGF and use of ECD organs. In addition, the current analysis was restricted to surrogate markers; clinical outcomes such as allograft and patient survival will be reported upon study completion at Year 5. A meaningful analysis of de novo DSA formation was precluded by the low number of patients that developed DSA by 24 months posttransplant.

It is unclear whether the interaction observed in this trial between tacrolimus dose and RAS-blocking AHTs can be generalized to other RTR patient populations or other immunosuppressive protocols. However, it is reassuring to compare the present findings with those in a recent European study examining the effects of CNI dose-minimization.[48] As in the current study, Gatault and coworkers

used basiliximab (Simulect®, Novartis Pharmaceuticals Canada Inc., Dorval, QC, Canada) induction and prolonged-release tacrolimus for maintenance immunosuppression. In contrast to the current study, these authors aimed for steroid-free maintenance for most of their RTRs. Because AHT use was not reported in their study, findings can only be compared with FKC-014 data in the OAHT intervention group. With this restriction, some striking parallels emerge between the 2 studies, particularly related to the elevated risk of rejection and higher rates of IF/TA+i in patients receiving lower-dose tacrolimus.[48] Increased risk of rejection has also been reported in patients using immediate-release tacrolimus whose tacrolimus exposure over 6 months was similar to that of the LOW group in the present study.[60]

5 | CONCLUSIONS

Whereas prevalence of IF/TA was not significantly affected by tacrolimus dose at month 6 or by use of RAS-blocking AHTs at month 24, IF/TA and histologic markers of rejection (TCMR/B) or inflammation (IF/TA+i) showed strong evidence of interaction between these 2 interventions. Among patients treated with LOW tacrolimus, IF/TA+i, rejection, and progression of IF/TA, were substantially suppressed among patients using RAS-blocking AHTs. As clinical outcomes emerge at the end of this 5-year study, it will be of great interest to learn whether these early results are correlated with long-term patient and allograft outcomes. These findings may inform the design of future studies and help optimize the monitoring and immunosuppressive treatment of RTRs.

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DISCLOSURE

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REFERENCES

1. Lamb KE, Lodhi S, Meier-Kriesche H-U. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant*. 2011;11(3):450-462.
2. Lodhi SA, Meier-Kriesche H-U. Kidney allograft survival: the long and short of it. *J Lab Clin Med Nephrol Dial Transplant*. 2011;26(1):15-17.
3. Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2015 annual data report: kidney. *Am J Transplant*. 2017;17(suppl 1):21-116.
4. Knoll G. Trends in kidney transplantation over the past decade. *Drugs*. 2008;68(suppl 1):3-10.
5. Rush D, Arlen D, Boucher A, et al. Lack of benefit of early protocol biopsies in renal transplant patients receiving TAC and MMF: a randomized study. *Am J Transplant*. 2007;7(11):2538-2545.
6. Cosio FG, Amer H, Grande JP, Larson TS, Stegall MD, Griffin MD. Comparison of low versus high tacrolimus levels in kidney transplantation: assessment of efficacy by protocol biopsies. *Transplantation*. 2007;83(4):411-416.
7. Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med*. 2007;357(25):2562-2575.
8. Ekberg H, Bernasconi C, Tedesco-Silva H, et al. Calcineurin inhibitor minimization in the SYMPHONY study: observational results 3 years after transplantation. *Am J Transplant*. 2009;9(8):1876-1885.
9. Bohl DL, Brennan DC. BK virus nephropathy and kidney transplantation. *Clin J Am Soc Nephrol*. 2007;2(suppl 1):S36-S46.
10. Nankivell BJ, Ng CHP, O'Connell PJ, Chapman JR. Calcineurin inhibitor nephrotoxicity through the lens of longitudinal histology: comparison of cyclosporine and tacrolimus eras. *Transplantation*. 2016;100(8):1723-1731.
11. Nicholson ML, McCulloch TA, Harper SJ, et al. Early measurement of interstitial fibrosis predicts long-term renal function and graft survival in renal transplantation. *Br J Surg*. 1996;83(8):1082-1085.
12. El-Zoghby ZM, Stegall MD, Lager DJ, et al. Identifying specific causes of kidney allograft loss. *Am J Transplant*. 2009;9(3):527-535.
13. Moreso F, Carrera M, Goma M, et al. Early subclinical rejection as a risk factor for late chronic humoral rejection. *Transplantation*. 2012;93(1):41-46.
14. Gaston RS, Cecka JM, Kasiske BL, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplantation*. 2010;90(1):68-74.
15. Gourishankar S, Leduc R, Connett J, et al. Pathological and clinical characterization of the "troubled transplant": data from the DeKAF study. *Am J Transplant*. 2010;10(2):324-330.
16. Naesens M, Lerut E. Calcineurin inhibitor nephrotoxicity in the era of antibody-mediated rejection. *Transplantation*. 2016;100(8):1599-1600.
17. Gago M, Cornell LD, Kremers WK, Stegall MD, Cosio FG. Kidney allograft inflammation and fibrosis, causes and consequences. *Am J Transplant*. 2012;12(5):1199-1207.
18. García-Carro C, Dörje C, Åsberg A, et al. Inflammation in early kidney allograft surveillance biopsies with and without associated tubulointerstitial chronic damage as a predictor of fibrosis

progression & development of de novo donor specific antibodies. *Transplantation*. 2017;101(6):1410-1415.
19. Li X, Zhuang S. Recent advances in renal interstitial fibrosis and tubular atrophy after kidney transplantation. *Fibrogenes Tissue Repair*. 2014;7:15.
20. Park WD, Griffin MD, Cornell LD, Cosio FG, Stegall MD. Fibrosis with inflammation at one year predicts transplant functional decline. *J Am Soc Nephrol*. 2010;21(11):1987-1997.
21. Wiebe C, Rush DN, Nevins TE, et al. Class II eplet mismatch modulates tacrolimus trough levels required to prevent donor-specific antibody development. *J Am Soc Nephrol*. 2017;28:3353-3362.
22. Béland MA, Lapointe I, Noël R, et al. Higher calcineurin inhibitor levels predict better kidney graft survival in patients with de novo donor-specific anti-HLA antibodies: a cohort study. *Transpl Int*. 2017;30(5):502-509.
23. Nickerson P, Blydt-Hansen T, Rush D, et al. De novo donor-specific antibody (DSA) is associated with decreased kidney graft survival and subclinical antibody-mediated rejection. *Am J Transpl*. 2010;10(Suppl):124.
24. Crowley SD, Rudemiller NP. Immunologic effects of the renin-angiotensin system. *J Am Soc Nephrol*. 2017;28(5):1350-1361.
25. Koh KK, Ahn JY, Han SH, et al. Pleiotropic effects of angiotensin II receptor blocker in hypertensive patients. *J Am Coll Cardiol*. 2003;42(5):905-910.
26. Vanhove T, Goldschmeding R, Kuypers D. Kidney fibrosis: origins and interventions. *Transplantation*. 2017;101(4):713-726.
27. el-Agroudy AE, Hassan NA, Foda MA, et al. Effect of angiotensin II receptor blocker on plasma levels of TGF-beta 1 and interstitial fibrosis in hypertensive kidney transplant patients. *Am J Nephrol*. 2003;23(5):300-306.
28. Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int Suppl*. 2012;2:139-274.
29. Arumugam S, Sreedhar R, Thandavarayan RA, et al. Angiotensin receptor blockers: focus on cardiac and renal injury. *Trends Cardiovasc Med*. 2016;26(3):221-228.
30. Rush DN, Cockfield SM, Nickerson PW, et al. Factors associated with progression of interstitial fibrosis in renal transplant patients receiving tacrolimus and mycophenolate mofetil. *Transplantation*. 2009;88(7):897-903.
31. Knoll GA, Fergusson D, Chasse M, et al. Ramipril versus placebo in kidney transplant patients with proteinuria: a multicentre, double-blind, randomised controlled trial. *LANCET Diabetes Endocrinol*. 2016;4(4):308-326.
32. Noris M, Mister M, Pezzotta A, et al. ACE inhibition limits chronic injury of kidney transplant even with treatment started when lesions are established. *Kidney Int*. 2003;64(6):2253-2261.
33. Ibrahim HN, Jackson S, Connaire J, et al. Angiotensin II blockade in kidney transplant recipients. *J Am Soc Nephrol*. 2013;24(2):320-327.
34. Paoletti E, Bellino D, Marsano L, Cassottana P, Rolla D, Ratto E. Effects of ACE inhibitors on long-term outcome of renal transplant recipients: a randomized controlled trial. *Transplantation*. 2013;95(6):889-895.
35. Hiremath S, Fergusson DA, Fergusson N, Bennett A, Knoll GA. Renin-angiotensin system blockade and long-term clinical outcomes in kidney transplant recipients: a meta-analysis of randomized controlled trials. *Am J Kidney Dis*. 2017;69(1):78-86.
36. Astellas Pharma Canada I. Advagraf Product Monograph. https://www.astellas.ca/Uploads/pdf/2015-07-31%20Advagraf%20PM-Approved.pdf Accessed January 11, 2019.
37. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant*. 2008;8:753-760.
38. Cosio FG, Grande JP, Larson TS, et al. Kidney allograft fibrosis and atrophy early after living donor transplantation. *Am J Transplant*. 2005;5(5):1130-1136.
39. El Ters M, Grande JP, Keddis MT, et al. Kidney allograft survival after acute rejection, the value of follow-up biopsies. *Am J Transplant*. 2013;13(9):2334-2341.
40. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Rates and determinants of progression to graft failure in kidney allograft recipients with de novo donor-specific antibody. *Am J Transplant*. 2015;15(11):2921-2930.
41. Ortiz F, Gelpi R, Helanterä I, et al. Decreased kidney graft survival in low immunological risk patients showing inflammation in normal protocol biopsies. *PLoS ONE*. 2016;11(8):e0159717.
42. Mehta R, Sood P, Hariharan S. Subclinical rejection in renal transplantation: reappraised. *Transplantation*. 2016;100(8):1610-1618.
43. Yang HC, Fogo AB. Fibrosis and renal aging. *Kidney Int Suppl*. 2014;4(1):75-78.
44. Naesens M, Kuypers DRJ, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol*. 2009;4(2):481-508.
45. Matas AJ. Chronic progressive calcineurin nephrotoxicity: an overstated concept. *Am J Transplant*. 2011;11(4):687-692.
46. Lamarche C, Orio J, Collette S, et al. BK polyomavirus and the transplanted kidney: immunopathology and therapeutic approaches. *Transplantation*. 2016;100(11):2276-2287.
47. Dugast E, Soulillou J-PP, Foucher Y, et al. Failure of calcineurin inhibitor (tacrolimus) weaning randomized trial in long-term stable kidney transplant recipients. *Am J Transplant*. 2016;16(11):3255-3261.
48. Gatault P, Kamar N, Büchler M, et al. Reduction of extended-release tacrolimus dose in low-immunological-risk kidney transplant recipients increases risk of rejection and appearance of donor-specific antibodies: a randomized study. *Am J Transplant*. 2017;17(5):1370-1379.
49. Israni AK, Riad SM, Leduc R, et al. Tacrolimus trough levels after month 3 as a predictor of acute rejection following kidney transplantation: a lesson learned from DeKAF genomics. *Transpl Int*. 2013;26(10):982-989.
50. Ekberg H, Mamelok RD, Pearson TC, Vincenti F, Tedesco-Silva H, Daloze P. The challenge of achieving target drug concentrations in clinical trials: experience from the SYMPHONY study. *Transplantation*. 2009;87(9):1360-1366.
51. Ahn KO, Lim SW, Li C, et al. Influence of angiotensin II on expression of Toll-like receptor 2 and maturation of dendritic cells in chronic cyclosporine nephropathy. *Transplantation*. 2007;83(7):938-947.
52. Okwan-Doudu D, Datta V, Shen X, et al. Angiotensin converting enzyme (ACE) over-expression in mouse macrophages upregulates iNOS and markedly increases resistance to listeria and MRSA. *FASEB J*. 2011;25(suppl):614.
53. Hahn AW, Jonas U, Bühler FR, Resink TJ. Activation of human peripheral monocytes by angiotensin II. *FEBS Lett*. 1994;347:178-180.
54. Sonmez A, Kisa U, Uckaya G, et al. Effects of losartan treatment on T-cell activities and plasma leptin concentrations in primary hypertension. *JRAAS*. 2001;2:112-116.
55. Kwang KK, Quon MJ, Seung HH, et al. Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. *Circulation*. 2004;110(24):3687-3692.
56. Maeda A, Okazaki T, Inoue M, et al. Immunosuppressive effect of angiotensin receptor blocker on stimulation of mice CTLs by angiotensin II. *Int Immunopharmacol*. 2009;9(10):1183-1188.
57. Iñigo P, Campistol JM, Lario S, et al. Effects of losartan and amlodipine on intrarenal hemodynamics and TGF-beta(1) plasma levels in a crossover trial in renal transplant recipients. *J Am Soc Nephrol*. 2001;12(4):822-827.
58. Mas VR, Alvarellos T, Maluf DG, et al. Molecular and clinical response to angiotensin II receptor antagonist in kidney transplant patients with chronic allograft nephropathy. *Transpl Int*. 2004;17(9):540-544.

59. Nataraj C, Oliverio MI, Mannon RB, et al. Angiotensin II regulates cellular immune responses through a calcineurin-dependent pathway. *J Clin Invest*. 1999;104(12):1693-1701.
60. Davis S, Gralla J, Klem P, et al. Lower mean tacrolimus troughs increase risk of de novo donor-specific antibodies in the first year of kidney transplant. *Am J Transpl*. 2017;17(suppl):208.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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