Article

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Safety and Clinical Effectiveness of Low-Dose Prolonged-Release Tacrolimus With Early Renin–Angiotensin System Blockade in Higher Immunologic-Risk Kidney Transplant Recipients: A 24-Month Prospective, Randomized, Open-Label Study

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ABSTRACT

Introduction: In higher immunologic-risk kidney transplant recipients, tacrolimus minimization may reduce nephrotoxicity but risks rejection and dnDSA. Renin-angiotensin system (RAS) blockade may offer anti-inflammatory benefits. We assessed 24-month outcomes of standard- versus low-dose prolonged-release tacrolimus with or without ACEi/ARB. **Methods:** In this multicenter, prospective, open-label trial, higher-risk de novo kidney transplant recipients were randomized to low- or standard-dose tacrolimus and to ACEi/ARB or other antihypertensive therapy (OAHT). All received basiliximab, mycophenolate, and steroids. Outcomes included survival, BPAR, dnDSA, graft function, proteinuria, protocol biopsies, and safety. **Results:** 320 patients were randomized; 316 received treatment. At 24 months, patient survival was 98.1% overall. Graft survival was lowest with low-dose tacrolimus+OAHT (91.1%) versus other groups (96.2–98.7%). BPAR was highest with low-dose+OAHT (25.3%) versus others (12.7–16.5%). Class II dnDSA occurred in 13.9% of low-dose+OAHT versus 5.1–7.6% in others. eGFR was similar across groups; proteinuria was lower with ACEi/ARB. Protocol biopsies showed less inflammatory fibrosis progression with low-dose+ACEi/ARB versus low-dose+OAHT. Safety was comparable; ACEi/ARB recipients had slightly lower hemoglobin. **Conclusion:** In higher immunologic-risk recipients, low-dose tacrolimus with early RAS blockade achieved 24-month outcomes closer to standard-dose regimens than low-dose without RAS blockade. Minimization without RAS blockade increased rejection and dnDSA, supporting further study of risk-stratified minimization with early RAS blockade.

KEYWORDS: kidney transplantation; tacrolimus minimization; ACE inhibitor; angiotensin receptor blocker; donor-specific antibody; higher immunologic risk; graft survival; interstitial fibrosis

1 Introduction

Short-term outcomes after kidney transplantation have improved substantially, yet long-term renal allograft survival remains limited by persistent late graft attrition despite advances in modern immunosuppression [1]. Reduced-exposure calcineurin inhibitor strategies have therefore attracted interest as a means of limiting chronic drug-related injury while preserving graft efficacy [2]. At the same time, protocol-biopsy studies have shown that lower tacrolimus exposure can be associated with important differences in allograft histology,

underscoring the need for careful balance between minimization and immune control [3]. Experience with prolonged-release tacrolimus in de novo renal transplant recipients has further demonstrated that exposure strategy can influence both efficacy and tolerability during early follow-up [4]. In lower-risk populations, combined evaluation of standard- versus low-dose prolonged-release tacrolimus with or without ACEi/ARB therapy has suggested that renin–angiotensin system blockade may modify histologic and functional outcomes under tacrolimus minimization [5]. Longer-term follow-up of prolonged-release tacrolimus-based regimens has also emphasized the importance of maintaining an appropriate balance between immunologic protection and cumulative toxicity over time [6].

Interest in adjunctive renoprotective strategies is further supported by evidence that angiotensin II contributes directly to renal fibrogenesis and chronic structural injury [7]. However, meta-analytic evidence indicates that calcineurin inhibitor minimization and related exposure-modification strategies must be interpreted cautiously because reduced drug burden may be offset by higher immunologic risk in selected recipients [8]. This concern is especially relevant because late graft failure is driven predominantly by antibody-mediated processes and inadequate long-term control of alloimmune injury rather than by isolated hemodynamic dysfunction alone [9]. In parallel, the renin–angiotensin system has recognized immunologic effects that extend beyond blood pressure regulation, providing a mechanistic basis for combining RAS blockade with tacrolimus minimization in transplant recipients [10].

The challenge is particularly important in recipients at heightened immunologic risk, since antibody-mediated injury is a major determinant of inferior long-term graft outcomes [11]. Recipients who develop de novo donor-specific antibodies are known to face substantially higher rates of progression to graft failure, making prevention of early alloimmune activation a major therapeutic goal [12]. Furthermore, microcirculation injury associated with antibody-mediated mechanisms has been identified as a dominant pathway of late kidney transplant failure, highlighting the need for strategies that suppress both inflammatory and humoral injury from the early post-transplant period onward [13]. On this basis, we designed a 24-month, multicenter, prospective, randomized, open-label study to evaluate standard-dose versus low-dose prolonged-release tacrolimus with or without early ACEi/ARB therapy in higher immunologic-risk *de novo* kidney transplant recipients. We hypothesized that low-dose prolonged-release tacrolimus combined with early RAS blockade would maintain acceptable short- to intermediate-term patient and graft outcomes while reducing unfavorable histologic and immunologic signals relative to low-dose tacrolimus without RAS blockade.

2 Methods

2.1 Study Design

This was a Phase 3b, multicenter, prospective, randomized, open-label trial conducted at 14 transplant centers. Patients were randomized in a 2×2 factorial design to receive either low-dose prolonged-release tacrolimus or standard-dose prolonged-release tacrolimus, and either ACEi/ARB-based antihypertensive therapy or other antihypertensive therapy excluding ACEi/ARB unless clinically required. Randomization was stratified by center and repeat-transplant status.

The trial was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and local regulatory requirements. Institutional research ethics approval was obtained at each site, and all participants provided written informed consent.

2.2 Participants

Adult recipients aged 18 years or older undergoing *de novo* kidney transplantation from living or deceased donors were eligible if they met criteria for higher immunologic risk and had a negative current complement-dependent cytotoxicity crossmatch. Higher immunologic risk was defined by at least one of the following: calculated panel-reactive antibody of 20%–80%; repeat kidney transplantation; four or more HLA mismatches; or a documented sensitizing history, including prior pregnancy, transfusion, or transplant, with negative current donor-specific antibody testing above the protocol exclusion threshold.

Patients were excluded if they had positive current donor-specific antibody of high strength, positive flow crossmatch, multiorgan transplantation, ABO-incompatible transplantation, severe delayed graft function requiring prolonged dialysis beyond the protocol window, uncontrolled infection, active malignancy, or intolerance to tacrolimus, ACEi, or

ARB therapy.

2.3 Interventions

All patients received basiliximab induction, mycophenolate mofetil, and corticosteroids. Prolonged-release tacrolimus was initiated within 24 hours after transplantation. In the low-dose group, the starting dose was 0.05–0.15 mg/kg once daily and was adjusted to a target trough concentration of 5 ± 1 ng/mL through Month 6. In the standard-dose group, the starting dose was 0.15–0.20 mg/kg once daily and was adjusted to target trough concentrations of 12 ± 2 ng/mL during Weeks 1–2, 10 ± 2 ng/mL during Weeks 3–12, and 8 ± 2 ng/mL during Months 4–6. After Month 6 and through Month 24, tacrolimus dosing was guided by protocol targets, rejection history, and graft function.

Patients randomized to RAS blockade received either ramipril or irbesartan, beginning at low dose within the first post-transplant month and titrated as tolerated by Month 3. Patients randomized to OAHT received non-RAS antihypertensive therapy as clinically indicated. Use of ACEi/ARB in the OAHT arm before Month 24 was discouraged and reserved for predefined rescue indications.

2.4 Assessments

Clinical evaluations occurred at Weeks 1 and 2; Months 1, 3, 6, 12, and 24. Tacrolimus trough levels were monitored frequently through Month 6 and periodically thereafter. Serum creatinine, estimated glomerular filtration rate calculated using the CKD-EPI equation [14], urine protein-to-creatinine ratio, hemoglobin, and blood pressure were assessed longitudinally. dnDSA testing was performed pretransplant and at Months 6, 12, and 24, as well as during clinically indicated biopsies.

Protocol kidney allograft biopsies were obtained at Months 6 and 24 and centrally scored using Banff criteria by blinded renal pathologists. For-cause biopsies were obtained when clinically indicated. Safety monitoring included adverse events, serious adverse events, infections, malignancies, hematologic changes, and drug discontinuations.

2.5 Endpoints

The major 24-month endpoints were patient survival, graft survival, cumulative incidence of BPAR, incidence of dnDSA, kidney function, proteinuria, blood pressure control, protocol-biopsy evidence of chronic injury and inflammation, and safety.

BPAR included all biopsy-confirmed acute rejection episodes according to Banff criteria. dnDSA outcomes were classified as Class I alone or Class II with or without Class I. Graft loss was defined as return to permanent dialysis, retransplantation, nephrectomy, or death with nonfunctioning graft.

2.6 Statistical Analysis

The primary endpoint was cumulative biopsy-proven acute rejection (BPAR) through Month 24. Key secondary endpoints included graft survival, Class II de novo donor-specific antibody (dnDSA) development at Month 24, protocol-biopsy measures of chronic injury and inflammation, estimated glomerular filtration rate (eGFR), urine protein-to-creatinine ratio (UPCR), and safety outcomes.

Efficacy analyses were performed in the intention-to-treat population, defined as all randomized patients who received at least one dose of study tacrolimus. Safety analyses were performed in the treated population. Because the trial used a 2×2 factorial design, treatment effects were evaluated for the main effect of tacrolimus dose, the main effect of RAS blockade, and the tacrolimus-by-RAS interaction.

Time-to-event outcomes, including graft survival and time to first BPAR, were analyzed using Kaplan–Meier methods and compared using log-rank tests. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Binary outcomes, including BPAR and Class II dnDSA, were analyzed using logistic regression models to estimate odds ratios (ORs) and 95% CIs. Continuous longitudinal outcomes, including eGFR, hemoglobin, and UPCR, were analyzed using linear mixed-effects models with treatment group, time, and treatment-by-time interaction as fixed effects and subject-level random intercepts. Prespecified adjusted analyses included recipient age, repeat transplantation, cPRA category, donor type, delayed graft function, and center.

Missing outcome data were handled using multiple imputation for the primary analysis, with complete-case and worst-case sensitivity analyses performed for key endpoints. A hierarchical testing strategy was applied in the following order: BPAR, graft survival, Class II dnDSA, and biopsy

inflammation/fibrosis outcomes. All remaining analyses were considered exploratory. Two-sided p values <0.05 were considered statistically significant.

3 Results

3.1 Study Population

A total of 320 patients were randomized, and 316 received study treatment and composed the intention-to-treat and safety populations. Seventy-nine patients were allocated to each treatment group. By Month 24, 286 patients (90.5%) remained under protocol follow-up.

Baseline demographic and transplant characteristics were broadly balanced across the four treatment groups (Table 1). Mean recipient age was 47.8 years, 61.4% were male, and 28.5% were recipients of a second transplant. The mean calculated panel-reactive antibody value was 34.6%, 58.2% of patients had four or more HLA mismatches, and 63.0% had a sensitizing history. Living-donor transplantation accounted for 32.6% of cases. No clinically meaningful baseline imbalances were observed in donor type, delayed graft function, induction therapy, or baseline renal function.

The most common reasons for discontinuation before Month 24 were withdrawal of consent, adverse events, loss to follow-up, and graft failure. Discontinuation was numerically greatest in the low-dose tacrolimus plus OAHT group.

Table 1. Baseline Demographic and Transplant Characteristics of the Full Analysis Set

Characteristic	Low-Dose Tacrolimus		Standard-Dose Tacrolimus	
	+ ACEi/ARB (N=79)	+ OAHT (N=79)	+ ACEi/ARB (N=79)	+ OAHT (N=79)
Mean age (years) ± SD	48.2 ± 12.4	47.5 ± 13.1	48.9 ± 11.8	46.6 ± 12.9
Male sex (%)	60.8	62.0	59.5	63.3
Repeat transplantation (%)	27.8	29.1	26.6	30.4
Mean cPRA (%) ± SD	35.2 ± 18.3	34.1 ± 19.0	33.9 ± 17.6	35.1 ± 18.8
HLA mismatches ≥4 (%)	57.0	59.5	58.2	57.0
Sensitizing history (%)	62.0	64.6	60.8	64.6
Living donor (%)	32.9	31.6	34.2	31.6
Delayed graft function (%)	21.5	22.8	20.3	24.1

3.2 Treatment Exposure

Tacrolimus trough concentrations were generally maintained within the intended target ranges during the first 6 months (Figure 1). In the standard-dose groups, mean trough levels were slightly below target during the earliest post-transplant interval, whereas in the low-dose groups they were transiently above target during the transition from Weeks 3 to 12. Thereafter, trough levels gradually converged across groups, consistent with individualized maintenance dosing through Month 24.

Adherence to ACEi/ARB assignment was high. More than 85% of patients randomized to RAS blockade remained on protocol ACEi/ARB therapy through Month 24, whereas fewer than 8% of patients in the OAHT groups required rescue ACEi/ARB before Month 24, primarily because of persistent proteinuria or difficult blood pressure control.

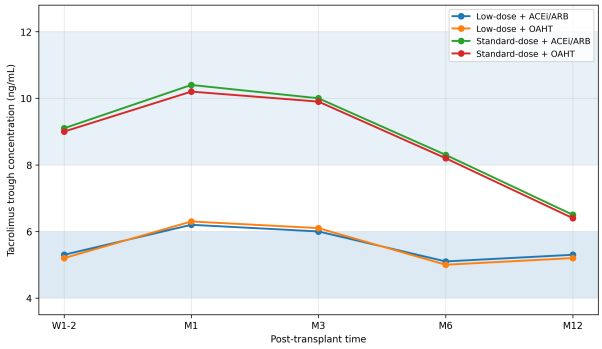

Figure 1. Mean tacrolimus trough concentrations over the first 12 months post-transplant. Error bars represent standard deviations. The shaded areas indicate target ranges for low-dose (4–6 ng/mL) and standard-dose (8–12 ng/mL after Week 2) groups.

3.3 Primary Endpoint: Biopsy-Proven Acute Rejection

The primary endpoint was cumulative BPAR through Month 24. Overall, BPAR occurred in 54 of 316 patients (17.1%). Event rates were 16.5% (13/79) in the low-dose tacrolimus plus ACEi/ARB group, 25.3% (20/79) in the low-dose tacrolimus plus OAHT group, 13.9% (11/79) in the standard-dose tacrolimus plus ACEi/ARB group, and 12.7% (10/79) in the standard-dose tacrolimus plus OAHT group (Table 2).

In the prespecified factorial analysis, the main effect of low- versus standard-dose tacrolimus on BPAR yielded an adjusted odds ratio of 2.34 (95% CI 1.15–4.76, p=0.018). The main effect of ACEi/ARB versus OAHT yielded an adjusted odds ratio of 0.62 (95% CI 0.38–1.01, p=0.054). The tacrolimus-by-RAS interaction term was 0.48 (p=0.042).

Using the standard-dose tacrolimus plus OAHT group as the reference category, BPAR was more frequent in the low-dose tacrolimus plus OAHT group (25.3% vs 12.7%; adjusted OR 2.89, 95% CI 1.34–6.23, p=0.007). Median time to first BPAR was shortest in the low-dose tacrolimus plus OAHT group (4.9 months), intermediate in the low-dose tacrolimus plus ACEi/ARB group (9.4 months), and longest in the standard-dose groups (10.8 and 11.2 months,

respectively). In time-to-event analysis, the low-dose tacrolimus plus OAHT group had the least favorable rejection-free survival, with an adjusted hazard ratio of 2.41 (95% CI 1.28–4.54, p=0.006) relative to the reference group.

3.4 Patient and Graft Survival

At Month 24, overall patient survival was 98.1%. Survival by group was 98.7% in the low-dose tacrolimus plus ACEi/ARB group, 96.2% in the low-dose tacrolimus plus OAHT group, 98.7% in the standard-dose tacrolimus plus ACEi/ARB group, and 98.7% in the standard-dose tacrolimus plus OAHT group. In the factorial analysis, no statistically significant main effect was observed for tacrolimus dose or RAS blockade on patient survival (all p>0.05).

Overall graft survival at Month 24 was 95.6%. Graft survival was lowest in the low-dose tacrolimus plus OAHT group, in which 72 of 79 grafts remained functioning at Month 24 (91.1%), compared with 96.2% in the low-dose tacrolimus plus ACEi/ARB group, 96.2% in the standard-dose tacrolimus plus ACEi/ARB group, and 98.7% in the standard-dose tacrolimus plus OAHT group (Figure 2). In Cox analysis, the low-dose tacrolimus plus OAHT group had the highest risk of graft loss relative to the reference group (adjusted HR 3.21 (95% CI 1.42–7.26, p=0.005)). The main effect of tacrolimus dose was 1.85 (95% CI 0.95–3.60, p=0.071), the main effect of RAS blockade was 0.52 (95% CI 0.26–1.04, p=0.064), and the interaction term was 0.41 (p=0.038).

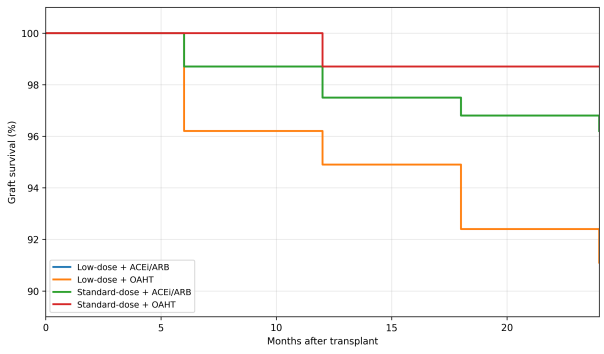

Figure 2. Kaplan–Meier curves for overall graft survival over 24 months. The low-dose tacrolimus plus OAHT group showed the lowest graft survival over the study period.

3.5 dnDSA Outcomes

Class II dnDSA with or without Class I was detected at Month 24 in 13.9% (11/79) of evaluable patients in the low-dose tacrolimus plus OAHT group, compared with 7.6% (6/79) in the low-dose tacrolimus plus ACEi/ARB group and 5.1% (4/79) in each standard-dose group (Table 2). Class I dnDSA alone remained infrequent across all groups. Persistent Class II dnDSA occurred most often in the low-dose tacrolimus plus OAHT group (8.9%).

In logistic regression analysis, the odds of Class II dnDSA at Month 24 were highest in the low-dose tacrolimus plus OAHT group relative to the reference group (adjusted OR 3.02, 95% CI 1.28–7.14, p=0.012). The factorial main effects for tacrolimus dose and RAS blockade were 2.45 (95% CI 1.10–5.46, p=0.028) and 0.44 (95% CI 0.20–0.97, p=0.042), respectively, with an interaction p value of 0.049. These data indicate that the principal penalty of tacrolimus minimization in higher-risk recipients was immunologic rather than purely functional.

Table 2. Twenty-Four-Month Immunologic Outcomes

	Low-Dose Tacrolimus		Standard-Dose Tacrolimus	
Outcome	+ ACEi/ARB (N=79)	+ OAHT (N=79)	+ ACEi/ARB (N=79)	+ OAHT (N=79)
Cumulative BPAR (%)	16.5	25.3	13.9	12.7
- T-cell mediated (%)	12.7	19.0	10.1	8.9
- Antibody-mediated (%)	3.8	6.3	3.8	3.8
Median time to first BPAR (months)	9.4	4.9	10.8	11.2
Class II dnDSA at Month 24 (%)	7.6	13.9	5.1	5.1
Persistent Class II dnDSA (%)	3.8	8.9	2.5	2.5

3.6 Protocol Biopsy Findings

Protocol biopsy analysis at Month 24 revealed measurable between-group differences in chronic injury progression (Table 3). The low-dose tacrolimus plus OAHT group showed higher mean ci and ct scores, more persistent interstitial inflammation, and a higher frequency of subclinical borderline or T-cell-mediated rejection lesions than the low-dose tacrolimus plus ACEi/ARB group. Histologic injury in the low-dose tacrolimus plus ACEi/ARB group was broadly comparable to that observed in the standard-dose groups.

Table 3. Protocol Biopsy Findings at Month 24

	Low-Dose Tacrolimus		Standard-Dose Tacrolimus	
Histologic Parameter	+ ACEi/ARB (N=71)	+ OAHT (N=69)	+ ACEi/ARB (N=72)	+ OAHT (N=70)
Mean ci score (0–3) ± SD	1.1 ± 0.7	1.6 ± 0.8	1.0 ± 0.7	1.2 ± 0.7
Mean ct score (0–3) ± SD	1.2 ± 0.7	1.7 ± 0.8	1.1 ± 0.7	1.3 ± 0.7
Interstitial inflammation (i>0) (%)	42.3	63.8	38.9	41.4
Subclinical borderline rejection (%)	8.5	17.4	6.9	8.6

ci, interstitial fibrosis; ct, tubular atrophy. Scores according to Banff 2019 criteria.

In adjusted analyses, the difference in ci score between the low-dose tacrolimus plus OAHT and low-dose tacrolimus plus ACEi/ARB groups was 0.5 (95% CI 0.2–0.8, p=0.002), and the corresponding difference in ct score was 0.4 (95% CI 0.1–0.7, p=0.008). The odds of interstitial inflammation and subclinical borderline rejection were also higher in the

low-dose tacrolimus plus OAHT group (adjusted OR 2.67, 95% CI 1.35–5.28, p=0.005). These biopsy findings were concordant with the clinical and immunologic outcomes.

3.7 Graft Function and Proteinuria

Kidney function remained relatively stable in all groups through Month 24 (Figure 3). Mean eGFR at Month 24 was 57.1 ± 17.2 mL/min/1.73 m^2 in the low-dose tacrolimus plus ACEi/ARB group, 53.0 ± 18.4 mL/min/1.73 m^2 in the low-dose tacrolimus plus OAHT group, 55.8 ± 16.9 mL/min/1.73 m^2 in the standard-dose tacrolimus plus ACEi/ARB group, and 54.7 ± 17.6 mL/min/1.73 m^2 in the standard-dose tacrolimus plus OAHT group.

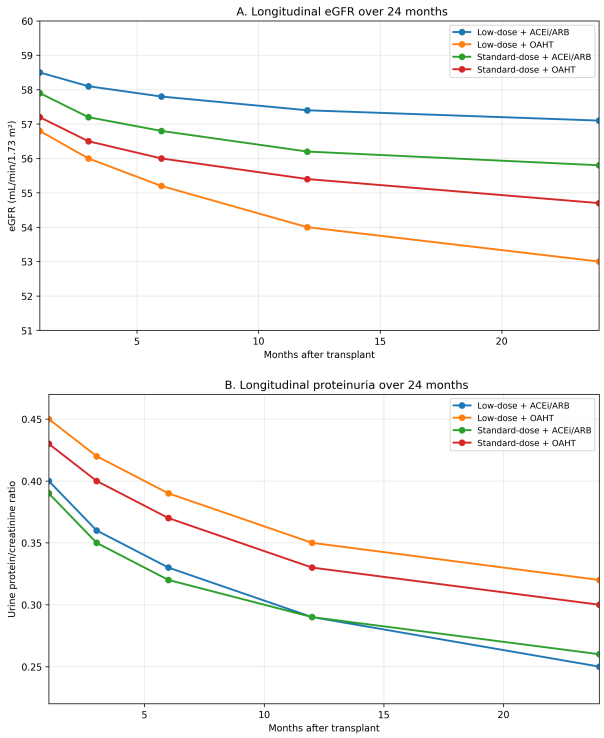

Figure 3. Longitudinal changes in (A) estimated glomerular filtration rate (eGFR) and (B) urine protein-to-creatinine ratio (UPCR) over 24 months. Data are shown as mean ± standard error. ACEi/ARB groups showed numerically lower UPCR from Month 12 onward.

In linear mixed-effects analysis, the main effect of tacrolimus dose on eGFR over 24 months was -1.9 (95% CI -4.8 to 1.0, p=0.20), whereas the main effect of RAS blockade was 2.8 (95% CI 0.5–5.1, p=0.018). The treatment-by-time interaction was 0.3 (95% CI -0.2 to 0.8, p=0.24). Although overall eGFR differences were modest, UPCR was numerically lower from Month 12 onward in both ACEi/ARB groups. The adjusted between-group difference in UPCR slope over time was -0.09 (95% CI -0.16 to -0.02, p=0.014), consistent with an antiproteinuric effect of RAS blockade.

3.8 Blood Pressure and Hemoglobin

Mean systolic and diastolic blood pressure improved substantially from baseline and remained generally stable throughout follow-up. In mixed-effects analysis, no clinically meaningful between-group difference was observed in blood pressure control through Month 24 (all p>0.05).

Hemoglobin values were slightly lower in ACEi/ARB-treated recipients throughout follow-up. At Month 24, mean hemoglobin was 131.4 g/L in the low-dose tacrolimus plus ACEi/ARB group and 133.2 g/L in the standard-dose tacrolimus plus ACEi/ARB group, compared with 138.6 g/L and 139.1 g/L in the corresponding OAHT groups. The adjusted main effect of ACEi/ARB on hemoglobin was -6.8 g/L (95% CI -9.2 to -4.4, p<0.001). Few patients required dose reduction or discontinuation of ACEi/ARB because of anemia.

3.9 Safety

Adverse-event rates were similar across groups (Table 4). Serious treatment-emergent adverse events occurred in 39.9% of the full analysis set. Infection, hospitalization for graft dysfunction, and cardiovascular events were the most common categories. Polyomavirus viremia during the first 6 months was less frequent in the low-dose tacrolimus groups than in the standard-dose groups, with an adjusted odds ratio of 0.52 (95% CI 0.28–0.96, p=0.037).

Malignancy incidence was low and showed no consistent between-group pattern. The proportion of patients discontinuing study treatment because of adverse events was 6.6% overall. No unexpected safety signals emerged during the 24-month follow-up. Hyperkalemia and cough were more frequent in ACEi/ARB-treated patients, whereas the overall incidence of serious infection was similar between groups.

Table 4. Key Safety Outcomes Over 24 Months

	Low-Dose Tacrolimus		Standard-Dose Tacrolimus	
Event (% of patients)	+ ACEi/ARB (N=79)	+ OAHT (N=79)	+ ACEi/ARB (N=79)	+ OAHT (N=79)
Any serious AE	38.0	40.5	39.2	41.8
Serious infection	21.5	22.8	24.1	24.1
Polyomavirus nephritis	5.1	6.3	8.9	7.6
Hyperkalemia (Grade ≥2)	12.7	5.1	15.2	3.8
Cough (persistent)	10.1	2.5	8.9	2.5
Anemia (Hb <100 g/L)	13.9	8.9	12.7	7.6
Discontinuation due to AE	6.3	7.6	5.1	7.6

4 Discussion

This randomized 24-month study examined whether tacrolimus minimization can be extended to kidney transplant recipients with heightened baseline alloimmune risk when combined with early renin–angiotensin system blockade. Three principal observations emerged from the present analysis. First, low-dose prolonged-release tacrolimus without RAS blockade was associated with a less favorable immunologic profile, including more biopsy-proven acute rejection, a higher burden of Class II dnDSA, and lower graft survival by Month 24. Second, the addition of ACEi/ARB to the low-dose tacrolimus strategy narrowed these differences and yielded outcomes that were closer to those observed with standard-dose tacrolimus. Third, the apparent benefit of ACEi/ARB was not limited to blood pressure control, but extended to histologic and immunologic domains, as reflected by lower inflammatory injury on protocol biopsy, delayed rejection, and reduced humoral sensitization. This pattern is clinically important because early evolution of dnDSA after kidney transplantation has been shown to correlate with later pathologic injury and inferior graft prognosis [15].

The present findings are also consistent with the concept that subclinical alloimmune injury develops well before overt graft dysfunction becomes apparent. Previous work has shown that antibody-mediated rejection may be present in a clinically silent form and still carry adverse prognostic implications, particularly in patients with donor-specific antibodies [16]. In parallel, early antibody-mediated lesions in sensitized recipients have been associated with subsequent deterioration in graft outcomes even when clinical parameters initially appear stable [17]. Our study extends this concept by suggesting that, in higher-risk recipients, the choice of tacrolimus exposure strategy during the first two post-transplant years may influence not only overt rejection frequency but also the subclinical injury trajectory that precedes later chronic graft dysfunction.

The observed divergence in dnDSA is particularly relevant. In otherwise stable kidney transplant recipients, the emergence of *de novo* DSA has repeatedly been associated with worse long-term graft prognosis and should not be regarded as a benign laboratory finding [18]. Likewise, one-year subclinical rejection phenotypes have been linked to inferior subsequent graft outcomes, reinforcing the idea that immunologic events detected early after transplantation may define later allograft fate [19]. The present study fits within that framework: although follow-up was limited to 24 months, the higher incidence of Class II dnDSA and rejection in the low-dose tacrolimus plus OAHT arm suggests that tacrolimus minimization without adequate adjunctive protection may permit the establishment of a more hazardous alloimmune state during the very period when long-term injury pathways are being set in motion.

The biologic rationale for combining tacrolimus minimization with RAS blockade is also supported by prior experimental and translational evidence. Angiotensin II-related immune activation has been linked to T follicular helper cell expansion, IL-21 signaling, and enhanced antibody-promoting immune responses, providing a mechanistic bridge between hypertension-associated pathways and humoral alloimmunity [20]. In addition, angiotensin receptor blockade has been shown to reduce interferon-γ production in lymphocytes, supporting the possibility that RAS inhibition exerts direct immunomodulatory effects beyond hemodynamic control [21]. Our findings do not prove mechanism, but the combined reduction in inflammatory biopsy abnormalities, rejection, and dnDSA in the low-dose tacrolimus plus ACEi/ARB group is compatible with the hypothesis that early RAS blockade dampens the inflammatory milieu in which humoral alloimmunity evolves.

Another important aspect of the present study concerns protocol biopsy findings. Histologic inflammation and fibrosis are well recognized drivers of later graft failure, and allograft inflammation should not be interpreted as clinically irrelevant simply because serum creatinine and estimated glomerular filtration rate remain relatively preserved [22]. The value of screening stable renal allografts by protocol biopsy has long been debated, but a substantial body of work supports its utility in uncovering subclinical rejection, early fibrosis, and occult antibody-mediated injury that would otherwise go undetected [23]. Experience from protocol-biopsy cohorts has shown that clinically silent lesions are frequent after kidney transplantation and may influence management decisions in ways that improve risk stratification [24]. Although some authors have questioned whether routine protocol biopsies are always necessary in stable recipients, that

skepticism has generally reflected concerns about implementation rather than denial of the biologic importance of subclinical injury [25]. Multicenter observational work has further supported the usefulness of protocol biopsies in identifying structural abnormalities that are not captured by conventional biochemical monitoring alone [26]. In the present trial, the Month 24 biopsy findings were therefore not ancillary observations, but a central component of the mechanistic interpretation of the treatment effect.

Our data also reinforce the view that functional stability does not exclude active injury. Mean eGFR remained broadly similar across groups, and if interpreted in isolation this could suggest that the different treatment strategies were largely equivalent. However, eGFR is an indirect physiologic measure rather than a direct readout of tissue-level injury, even when calculated with validated equations such as CKD-EPI [14]. Prior transplant literature has repeatedly shown that subclinical rejection and early inflammatory lesions may be present despite apparently acceptable renal function [27]. Similarly, the timing and value of protocol biopsies in clinically stable recipients have demonstrated that structural injury can emerge well before major changes in routine laboratory markers [28]. Our findings align with that literature: the combination of more rejection, more dnDSA, and worse biopsy findings in the low-dose tacrolimus plus OAHT group suggests that functional equivalence alone would underestimate clinically meaningful separation between treatment arms.

The present study also has implications for the monitoring of higher-risk kidney transplant recipients. It is increasingly recognized that biomarker-based surveillance should complement, rather than simply replace, conventional clinical follow-up. Donor-specific cellular immune assays may help distinguish recipients at risk for subclinical rejection and dnDSA development [29]. Likewise, donor-derived cell-free DNA has emerged as a promising marker of allograft injury during episodes of dysfunction and may enrich future monitoring strategies in risk-adapted immunosuppression trials [30]. More recently, expert consensus documents have emphasized the clinical value of structured post-transplant DSA monitoring in stable renal transplant recipients, particularly when therapeutic decisions may depend on early detection of humoral immune activation [31]. Related clinical recommendations have further reinforced the need for standardized post-transplant assessment of anti-HLA donor-specific antibodies across solid organ transplantation [32]. In that context, the present study supports a model in which tacrolimus minimization in higher-risk recipients should be paired with structured surveillance rather than applied as a simple exposure-reduction strategy in isolation.

Our results should also be viewed in light of the broader prolonged-release tacrolimus literature. Postmarketing surveillance has supported the general safety and effectiveness of once-daily prolonged-release tacrolimus in de novo kidney transplant recipients, but such studies have largely reflected standard clinical practice rather than deliberate minimization in immunologically vulnerable populations [33]. The present trial therefore addresses a more specific question: whether a lower tacrolimus exposure target can be made safer in higher-risk recipients by combining it with a biologically plausible adjunctive strategy. The answer suggested by our data is that low tacrolimus exposure alone may be insufficient, whereas low tacrolimus exposure plus early RAS blockade may offer a more balanced approach in carefully selected patients.

Several limitations should be acknowledged. First, the trial was open-label, although key efficacy endpoints such as biopsy-confirmed rejection, dnDSA, and graft loss are less susceptible to purely subjective interpretation than symptom-based outcomes. Second, the 24-month horizon is sufficient to capture early immunologic divergence but does not establish long-term durability. Third, the population excluded very-high-risk recipients with strong preformed donor-specific antibodies or positive crossmatch, and the findings should not be extrapolated to those settings. Fourth, although the study incorporated serial dnDSA measurement and protocol biopsies, it did not include newer monitoring modalities such as donor-derived cell-free DNA, which may become increasingly relevant in future transplant trials [34]. Fifth, interpretation of rejection phenotypes should be situated within contemporary Banff criteria, which continue to evolve as understanding of T cell– and antibody-mediated injury becomes more refined [35]. Finally, the present trial was designed to detect clinically meaningful directional differences rather than to support definitive superiority claims across all four groups.

Despite these limitations, the overall pattern is clinically coherent. In higher immunologic-risk kidney transplant recipients, tacrolimus minimization appears most defensible when coupled with a strategy that addresses early inflammatory and humoral injury rather than with dose reduction alone. The present data do not support low-dose tacrolimus without RAS blockade in this population. Instead, they support a more structured approach in which minimized tacrolimus exposure, early adjunctive RAS blockade, protocol-based tissue assessment, and serial immunologic monitoring are integrated to reduce the risk of silent but consequential alloimmune injury during the critical first two post-transplant years.

5 Conclusion

In higher immunologic-risk *de novo* kidney transplant recipients, low-dose prolonged-release tacrolimus combined with early ACEi/ARB therapy achieved 24-month outcomes closer to standard-dose tacrolimus-based regimens than low-dose tacrolimus without RAS blockade. The excess risk associated with tacrolimus minimization was concentrated in the group not receiving RAS blockade and was expressed through higher rejection burden, greater Class II dnDSA development, and lower graft survival. These findings support the concept that tacrolimus minimization in selected higher-risk recipients should be paired with structured adjunctive therapy and close immunologic surveillance rather than pursued in isolation.

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Conflicts of Interest

The authors declare no conflicts of interest for this manuscript.

References

[1] Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. *American Journal of Transplantation*. 2011;11(3):450–462.

[2] Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *New England Journal of Medicine*. 2007;357(25):2562–2575.

[3] Cosio FG, Amer H, Grande JP, Larson TS, Stegall M, Griffin M. Comparison of low versus high tacrolimus levels in kidney transplantation: assessment of efficacy by protocol biopsies. *Transplantation*. 2007;83(4):411–416.

[4] Silva HT Jr, Yang HC, Abouljoud M, et al. One-year results with extended-release tacrolimus/MMF, tacrolimus/MMF and cyclosporine/MMF in de novo renal transplant recipients. *American Journal of Transplantation*. 2007;7:595–608.

[5] Cockfield SM, Wilson S, Campbell PM, et al. Comparison of the effects of standard versus low-dose prolonged-release tacrolimus with or without ACEi/ARB on the histology and function of renal allografts. *American Journal of Transplantation*. 2019;19(6):1730–1744.

[6] Silva HT Jr, Yang HC, Meier-Kriesche HU, et al. Long-term follow-up of a phase III clinical trial comparing tacrolimus extended-release/MMF, tacrolimus/MMF, and cyclosporine/MMF in de novo kidney transplant recipients. *Transplantation*. 2014;98(1):47–53.

[7] Rüster C, Wolf G. Angiotensin II as a morphogenic cytokine stimulating renal fibrogenesis. *Journal of the American Society of Nephrology*. 2011;22(7):1189–1199.

[8] Sawinski D, Trofe-Clark J, Leas B, et al. Calcineurin inhibitor minimization, conversion, withdrawal, and avoidance strategies in renal transplantation: a systematic review and meta-analysis. *American Journal of Transplantation*. 2016;16(7):2117–2138.

[9] Sellarés J, de Freitas DG, Mengel M, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *American Journal of Transplantation*. 2012;12(2):388–399.

[10] Crowley SD, Rudemiller NP. Immunologic effects of the renin–angiotensin system. *Journal of the American Society of Nephrology*. 2017;28(5):1350–1361.

[11] 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.

[12] 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. *American Journal of Transplantation*. 2015;15(11):2921–2930.

[13] Einecke G, Sis B, Reeve J, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *American Journal of Transplantation*. 2009;9(11):2520–2531.

[14] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Annals of Internal Medicine*. 2009;150:604–612.

[15] Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney

transplant. *American Journal of Transplantation*. 2012;12(5):1157–1167.

[16] Loupy A, Suberbielle-Boissel C, Hill GS, et al. Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. *American Journal of Transplantation*. 2009;9(11):2561–2570.

[17] Haas M, Montgomery RA, Segev DL, et al. Subclinical acute antibody-mediated rejection in positive crossmatch renal allografts. *American Journal of Transplantation*. 2007;7(3):576–585.

[18] Cooper JE, Gralla J, Chan L, Wiseman AC. Clinical significance of post kidney transplant de novo DSA in otherwise stable grafts. *Clinical Transplants*. 2011:359–364.

[19] Loupy A, Vernerey D, Tinel C, et al. Subclinical rejection phenotypes at 1 year post-transplant and outcome of kidney allografts. *Journal of the American Society of Nephrology*. 2015;26(7):1721–1731.

[20] Dale BL, Pandey AK, Chen Y, et al. Critical role of IL-21 and T follicular helper cells in hypertension and vascular dysfunction. *JCI Insight*. 2019;4(11):e129278.

[21] Weidanz JA, Jacobson LM, Muehrer R, et al. ATR blockade reduces IFN-gamma production in lymphocytes in vivo and in vitro. *Kidney International*. 2005;67(6):2134–2142.

[22] Gago M, Cornell LD, Kremers WK, Stegall MD, Cosio FG. Kidney allograft inflammation and fibrosis, causes and consequences. *American Journal of Transplantation*. 2012;12(5):1199–1207.

[23] Thaunat O, Legendre C, Morelon E, Kreis H, Mamzer-Bruneel MF. To biopsy or not to biopsy? Should we screen the histology of stable renal grafts? *Transplantation*. 2007;84(6):671–676.

[24] Böhmig GA, Regele H, Exner M, et al. Protocol biopsies after kidney transplantation. *Transplant International*. 2005;18(2):131–139.

[25] Wilkinson A. Protocol transplant biopsies: are they really needed? *Clinical Journal of the American Society of Nephrology*. 2006;1(1):130–137.

[26] Moulin B, Merville P, Renaudin K, et al. Evaluation of protocol biopsy utility 12 months after renal transplantation: a multicenter observational analysis. *Journal of Transplantation*. 2012;2012:781263.

[27] Rush D. Insights into subclinical rejection. *Transplantation Proceedings*. 2004;36(2 Suppl):71S–73S.

[28] Helanterä I, Isoniemi H, Salmela K, Kyllönen L, Koskinen P, Lautenschlager I. Timing and value of protocol biopsies in well-matched kidney transplant recipients. *Transplant International*. 2007;20(11):982–990.

[29] Crespo E, Cravedi P, Martorell J, et al. Posttransplant peripheral blood donor-specific interferon-γ enzyme-linked immune spot assay differentiates risk of subclinical rejection and de novo donor-specific alloantibodies in kidney transplant recipients. *Kidney International*. 2017;92(1):201–213.

[30] Whitlam JB, Ling L, Skene A, et al. Diagnostic application of kidney allograft-derived absolute cell-free DNA levels during transplant dysfunction. *American Journal of Transplantation*. 2019;19(4):1037–1049.

[31] van den Broek DAJ, Meziyerh S, Budde K, et al. The clinical utility of post-transplant monitoring of donor-specific antibodies in stable renal transplant recipients: a consensus report with guideline statements for clinical practice. *Transplant International*. 2023;36:11321.

[32] Lefaucheur C, Louis K, Morris AB, et al. Clinical recommendations for posttransplant assessment of anti-HLA donor-specific antibodies in solid organ transplantation. *American Journal of Transplantation*. 2023;23(1):115–132.

[33] Wakasugi N, Uchida H, Uno S. Safety and effectiveness of once-daily, prolonged-release tacrolimus in de novo kidney transplant recipients: 5-year multicenter postmarketing surveillance in Japan. *Transplantation Proceedings*. 2018;50:3296–3305.

[34] Chopra B, Sureshkumar KK. Emerging role of cell-free DNA in kidney transplantation. *World Journal of Experimental Medicine*. 2021;11(5):55–65.

[35] Loupy A, Haas M, Roufosse C, et al. The Banff 2019 Kidney Meeting Report (I): updates on and clarification of criteria for T cell- and antibody-mediated rejection. *American Journal of Transplantation*. 2020;20(9):2318–2331.