








ORIGINAL ARTICLE

Long-term, prolonged-release tacrolimus-based immunosuppression in *de novo* kidney transplant recipients: 5-year prospective follow-up of the ADHERE study patients

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SUMMARY

The objectives of this study were to assess long-term graft survival, patient survival, renal function, and acute rejections in *de novo* kidney transplant recipients, treated with once-daily prolonged-release tacrolimus-based therapy. The study was a 5-year non-interventional prospective follow-up of patients from the ADHERE study, a Phase IV 12-month open-label assessment of patients randomized to receive prolonged-release tacrolimus in combination with mycophenolate mofetil (MMF) (Arm 1) or sirolimus (Arm 2). From 838 patients in the randomized study, 587 were included in the long-term follow-up, of whom 510 completed the study at year 5. At 1 year post-transplant, graft and patient survival rates were 93.0% and 97.8%, respectively, and at 5 years were 84.0% and 90.8%, respectively. Cox proportional hazards analysis showed no association between graft loss, initial randomized treatment arm, donor age, donor type, or sex. The 5-year acute rejection-free survival rate was 77.4%, and biopsy-confirmed acute rejection-free survival rate was 86.0%. Renal function remained stable over the follow-up period: mean \pm SD eGFR 4-variable modification diet in renal disease formula (MDRD4) was 52.3 ± 21.6 ml/min/1.73 m² at 6 months and 52.5 ± 23.0 ml/min/1.73 m² at 5 years post-transplant. These findings support the role of long-term once-daily prolonged-release tacrolimus-based immunosuppression, in combination with sirolimus or MMF, for renal transplant recipients in routine clinical practice.

Transplant International 2020; 33: 161–173

Key words

immunosuppression, kidney, kidney transplant, observational, prolonged-release tacrolimus, transplant

Received: 6 March 2019; Revision requested: 10 April 2019; Accepted: 17 September 2019; Published online: 24 October 2019

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Introduction

Maintaining long-term graft survival remains a challenge in kidney transplantation [1]. Overall post-transplantation graft survival rates of 91% have been reported at 1 year following deceased-donor transplantation in Europe; however, these rates fall to 77% at 5 years and 56% at 10 years [1]. Lifelong immunosuppressive [1] therapy following kidney transplantation generally comprises a calcineurin inhibitor (CNI), antiproliferative agent [mycophenolate mofetil (MMF) or mycophenolic acid (MPA)], and corticosteroids [2,3]. Tacrolimus is the most frequently used CNI and the mainstay of the immunosuppressive regimen, currently administered to over 80% of kidney transplant recipients [4]. Oral tacrolimus has been traditionally prescribed as a twice-daily immediate-release formulation; however, in 2007, a once-daily prolonged-release formulation was approved in many countries worldwide for the prevention of organ rejection in kidney transplant recipients [5].

The efficacy and safety of both formulations of tacrolimus are well-established [5–9], but prolonged-release delivery may offer several clinical advantages over the traditional formulation. Prolonged-release tacrolimus may improve long-term outcomes by reducing intra-patient variability in tacrolimus exposure, due to both the intrinsic pharmacokinetic properties of prolonged-release tacrolimus, and improved adherence to the simplified once-daily regimen [10–13]. Additional prospective studies of large patient cohorts are required to better characterize the long-term outcomes of prolonged-release tacrolimus in kidney transplant recipients, beyond the confined parameters of randomized controlled studies.

The ADHERE study was a 12-month, randomized, open-label, Phase IV study that compared renal function in *de novo* kidney transplant recipients receiving prolonged-release tacrolimus in combination with either MMF (Arm 1) or sirolimus (Arm 2) [14]. Renal function was found to be comparable between the two arms at 12 months post-transplant, with an acceptable tolerability profile. Here, we report a 5-year, prospective, non-interventional follow-up study of the ADHERE patient cohort. The primary objective of this follow-up study was to evaluate long-term graft survival in kidney transplant recipients, treated with once-daily prolonged-release tacrolimus-based therapy. Secondary objectives included evaluation of the long-term impact of prolonged-release tacrolimus on patient survival, renal function, and acute rejections (ARs).

Patients and methods

ADHERE (ClinicalTrials.gov identifier: NCT01363752) was a multicenter, randomized, open-label, parallel-group, 52-week, Phase IV study that evaluated renal function in *de novo* adult kidney transplant patients treated with once-daily prolonged-release tacrolimus (Advagraf®; Astellas Pharma Europe BV, Leiden, the Netherlands) plus MMF or sirolimus [14]. Eligible patients were aged 18 years or over, with end-stage kidney disease, suitable for primary renal transplantation or re-transplantation (unless the graft was lost from rejection within 6 months) and who received a kidney transplant from a deceased or living donor. Full study design details and inclusion/exclusion criteria of ADHERE were described previously [14].

The present study is a 5-year, non-interventional, prospective follow-up of patients from the primary ADHERE study. Patients were eligible for this study if they had received a kidney transplant and had been assigned to prolonged-release tacrolimus as participants of the ADHERE study. Centers that had participated in ADHERE were contacted and requested to invite patients to enroll into the long-term follow-up study at their next scheduled review appointment. Patients did not have to be currently receiving prolonged-release tacrolimus or to have completed ADHERE because the primary objective was to evaluate long-term graft survival in patients currently or previously treated with prolonged-release tacrolimus.

The long-term follow-up period comprised six study visits. Visit 1 was at 6 months post-transplant. Visit 2 was at 1 year post-transplant; for patients who completed the primary ADHERE study, this corresponded to the end-of-study (EOS) visit. Subsequent visits were scheduled annually at successive years post-transplant (± 4 months), so that Visit 3 was at 2 years post-transplant, and so on, with Visit 6 (the end of follow-up study visit) scheduled at 5 years post-transplant. For most patients, Visits 1 and 2 were held during the ADHERE initial study period, and Visits 3–6 during the follow-up study.

The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, International Conference on Harmonisation guidelines, and applicable national laws and regulations. An independent ethics committee at each site granted approval of the study before initiation. Written or verbal informed consent was obtained from all participants. Patients could withdraw from the study for any reason, at any time, without giving a reason for doing so and without

penalty or prejudice. Patients were discontinued from the study if they died, were lost to follow-up, or withdrew consent.

Treatment

In the primary ADHERE study, patients received once-daily prolonged-release tacrolimus at a starting dose of 0.2 mg/kg/day, corticosteroids, and MMF from Days 0–27. On Day 28, patients were randomized to receive prolonged-release tacrolimus plus MMF or plus sirolimus (Arm 1: prolonged-release tacrolimus, MMF, and steroids; Arm 2: prolonged-release tacrolimus, MMF replaced with sirolimus at Day 28 in combination with lower tacrolimus exposure at Day 42, and steroids). Prerandomization, tacrolimus target trough levels were 10–15 ng/ml until Day 14, then 8–12 ng/ml on Days 15–27 in both arms.

Post randomization, Arm 1 tacrolimus target trough levels were 8–12 ng/ml on Days 28–41, and 6–10 ng/ml from Day 42 to 1 year. In Arm 2, tacrolimus target trough levels were 8–12 ng/ml on Days 28–41; from Day 42 to 1 year, the tacrolimus dose was then decreased by $\geq 25\%$ to target tacrolimus trough levels of 4–5 ng/ml [14]. In the follow-up study, patients received prolonged-release or immediate-release tacrolimus.

A total of 5 years of follow-up post-transplant data are presented here: 1 year of the primary ADHERE study and a 4-year non-interventional follow-up period. The tacrolimus dose was administered by the investigator according to standard clinical practice. In this study, start of treatment was defined as the time when the first prolonged-release tacrolimus dose was administered during the ADHERE study period.

Endpoints

The primary endpoint was overall graft survival, namely the time from transplantation to graft loss, defined as re-transplantation, nephrectomy, death, or dialysis ongoing at EOS or at the time of patient discontinuation from the follow-up study.

Secondary endpoints included: overall patient survival; renal function, assessed by three methods—estimated glomerular filtration rate (eGFR) using the 4-variable modification diet in renal disease (MDRD4) formula [15], eGFR using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula [16], and estimated creatinine clearance (eCC) using the Cockcroft–Gault formula [17]; and biopsy-confirmed acute rejection (BCAR) episodes (severity assessed by Banff

2007 classification [18], both treated and untreated, and steroid-sensitive or steroid-resistant). Additional secondary endpoints included: concomitant immunosuppressant regimen, dose, and formulation (in addition to or instead of tacrolimus); tacrolimus dose, formulation and trough levels; medical conditions of interest (malignant diseases, diabetes mellitus, and cardiovascular events); and infections of interest [cytomegalovirus (CMV) and BK virus]. Safety data [adverse events (AEs) and adverse drug reactions (ADRs)] were also reported. Investigators provided a causality assessment for AEs potentially related to tacrolimus. ADRs were AEs considered to be possibly or probably tacrolimus-related.

Statistical analysis

The enrolled patient set (EPS) comprised all patients who enrolled in the primary ADHERE study and received a kidney transplant. The treatment arm (TA)-EPS comprised patients in the EPS who received randomized treatment with prolonged-release tacrolimus. The follow-up patient set (FPS) comprised patients in the EPS who consented to the follow-up study or who died before they could give consent. Patients in the FPS who received randomized treatment with prolonged-release tacrolimus were included in the TA-FPS. Patients who died before starting the follow-up study had only their date of death and ADHERE data included in these analyses, provided the date of death was available in the public domain.

The main analyses of the primary endpoint (graft survival) and all the secondary time-to-event endpoints (overall patient survival, AR and BCAR) were based on the EPS and the TA-EPS. This was done to ensure that all events that occurred during ADHERE were taken into account in time-to-event analyses in order to reduce bias in the analysis. Thus, the analysis included ADHERE data from patients who died or experienced graft loss before entering the follow-up study. Likewise, patients who were not approached for the follow-up study, who were approached but did not provide informed consent, or who were lost to follow-up between the end of the ADHERE study and the start of the follow-up study, had their ADHERE data included in the analyses up to the date of their discontinuation from the primary ADHERE study. The Kaplan–Meier method was used for the analyses of these endpoints. Patients without graft loss were censored at the EOS date or last evaluation date. A *post hoc* analysis of death-censored graft survival (graft survival censored for death with a functioning graft) was also performed using the Kaplan–Meier method. A Cox proportional

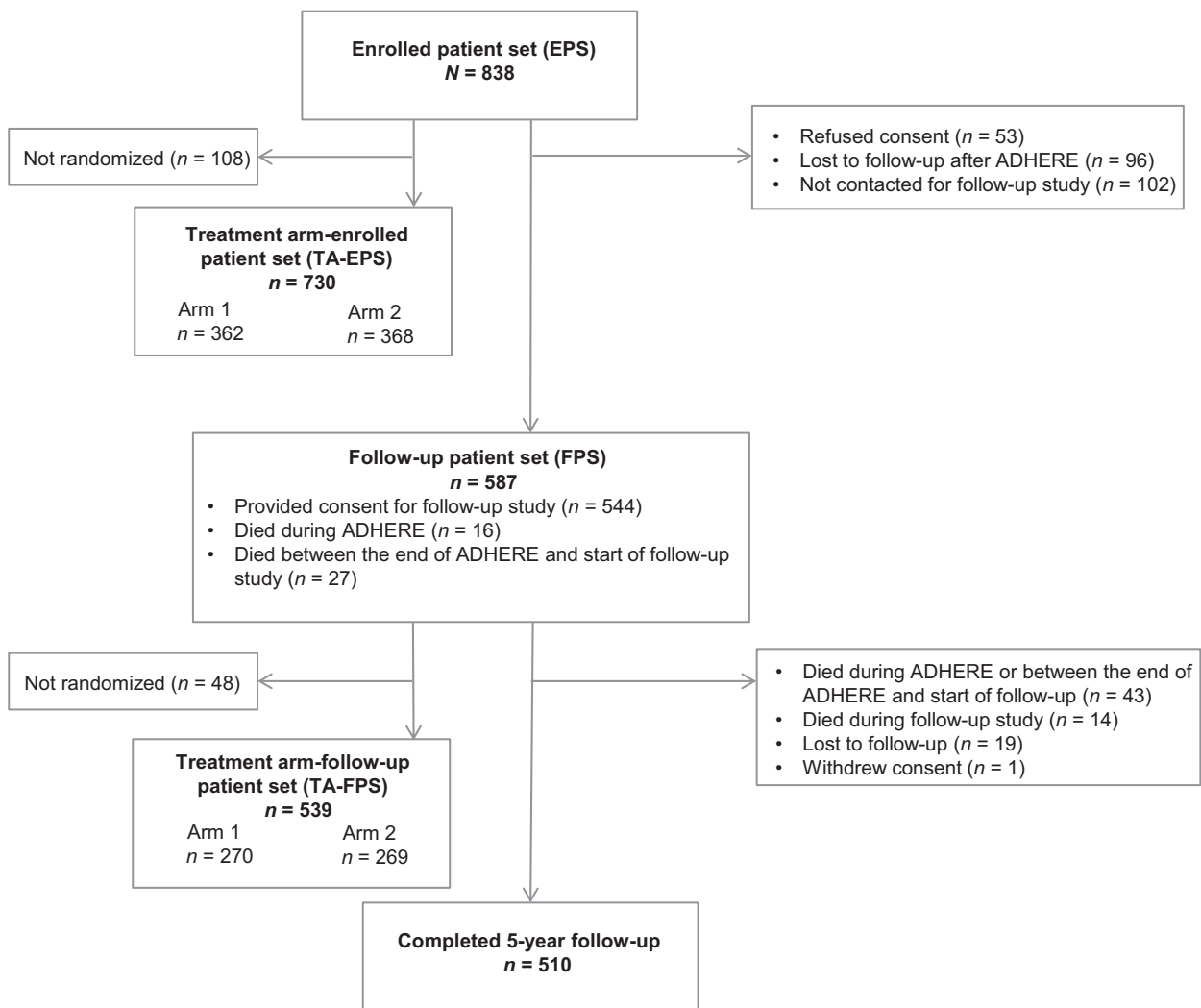


Figure 1 Patient population flow. EPS, enrolled patient set; FPS, follow-up patient set; TA-EPS, treatment arm-enrolled patient set; TA-FPS, treatment arm-follow-up patient set.

hazards model was used for the analyses of graft and overall patient survival adjusted for baseline characteristics, randomized treatment arm, donor age, donor type, and sex. Descriptive statistics were obtained for all the baseline characteristics and other secondary endpoints and the analyses based on the TA-FPS and the FPS.

Results

Patient characteristics

A total of 838 patients enrolled in the primary ADHERE study, received a kidney transplant, and were included in the EPS. Of the patients in the EPS, 108 were not randomized to treatment with prolonged-release tacrolimus, so that 730 patients were included in the TA-EPS. In total, 587 patients from the EPS

consented to the follow-up study (or died before they could give consent) and were included in the FPS, of whom 48 were not randomized to receive prolonged-release tacrolimus, so that 539 patients were included in the TA-FPS (Fig. 1).

Baseline demographics and clinical characteristics for the TA-FPS were similar between Arm 1 ($n = 270$) and Arm 2 ($n = 269$) and are presented in Table 1. Mean \pm SD age at baseline was 50.0 ± 13.4 years and 65.4% of study participants were male.

Tacrolimus dosing and exposure

In the FPS, the mean \pm SD tacrolimus total daily dose was 0.069 ± 0.048 mg/kg at 1 year post-transplant and 0.052 ± 0.035 mg/kg at 5 years post-transplant. In the TA-FPS, the mean \pm SD total daily dose in Arm 1 and

Table 1. Baseline demographics and clinical characteristics

Parameter	TA-FPS		
	Arm 1 (N = 270)	Arm 2 (N = 269)	FPS* (N = 587)
Age (years), mean ± SD	49.3 ± 13.29	49.5 ± 13.36	50.0 ± 13.40
Male sex, n (%)	167 (61.9)	182 (67.7)	384 (65.4)
Race			
White	238 (88.1)	238 (88.5)	515 (87.7)
Asian	19 (7.0)	16 (5.9)	39 (6.6)
Black or African American	1 (0.4)	4 (1.5)	8 (1.4)
Other	12 (4.4)	11 (4.1)	25 (4.3)
eGFR by MDRD4 (ml/min/1.73 m ²), mean ± SD	55.93 ± 18.36	55.32 ± 20.86	54.48 ± 21.63
eGFR by CKD-EPI (ml/min/1.73 m ²), mean ± SD	57.16 ± 19.76	56.10 ± 21.49	55.16 ± 21.74
eCC by Cockcroft–Gault (ml/min), mean ± SD	64.25 ± 21.84	63.85 ± 23.42	62.52 ± 24.01
Last measured tacrolimus trough levels (ng/ml), mean ± SD	7.32 ± 2.38	5.66 ± 2.42	6.86 ± 3.29
Patients with graft loss prior to start of follow-up study, n (%)	5 (1.9)	2 (0.7)	25 (4.3)
Recipient viral status, n (%)			
CMV positive	179 (66.3)	194 (72.1)	404 (68.8)
HBV negative	267 (98.9)	267 (99.3)	580 (98.8)
HCV negative	257 (95.2)	263 (97.8)	568 (96.8)
HIV negative	265 (98.1)	265 (98.5)	578 (98.5)
Donor age (years), mean ± SD	48.59 (15.41)	50.13 (14.82)	50.06 (15.17)
Organ donor type, n (%)			
Deceased	223 (82.6)	222 (82.5)	490 (83.5)
Living nonrelated	13 (4.8)	16 (5.9)	30 (5.1)
Living related	34 (12.6)	31 (11.5)	67 (11.4)

CKD-EPI, chronic kidney disease epidemiology collaboration; CMV, cytomegalovirus; eCC, estimated creatinine clearance; eGFR, estimated glomerular filtration rate; FPS, follow-up patient set; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MDRD4, 4-variable modification of diet in renal disease; SD, standard deviation; TA-FPS, treatment arm-follow-up patient set.

*The FPS includes 48 patients that were enrolled in the primary ADHERE study but not randomized to treatment with prolonged-release tacrolimus.

Arm 2, respectively, was 0.078 ± 0.049 mg/kg and 0.061 ± 0.043 mg/kg at 1 year post-transplant, and 0.056 ± 0.036 mg/kg and 0.052 ± 0.035 mg/kg at 5 years post-transplant (Table 2). At the end of the study, 441/539 (81.8%) patients in the TA-FPS had a recorded dose of tacrolimus.

Overall mean tacrolimus trough levels were 6.4 ± 2.4 ng/ml at 1 year and 5.5 ± 1.8 ng/ml at 5 years post-transplant. In the TA-FPS, mean ± SD tacrolimus trough levels in Arm 1 and Arm 2, respectively, were 7.2 ± 2.3 and 5.4 ± 1.9 ng/ml at 1 year, and 5.9 ± 1.9 and 5.1 ± 1.7 ng/ml at 5 years post-transplant (Table 2).

Concomitant immunosuppressants

In the TA-FPS, 92.0% of patients took at least one concomitant immunosuppressive medication during the follow-up period, including 54.0% and 10.0% who received MMF or MPA, respectively. A total of 35.1% (206/587) took sirolimus, most of whom [192/206 (93.2%)] were in

Arm 2. A summary of the sirolimus dosing during the follow-up period is shown in Table S1. Systemic corticosteroids were taken by 65.9% of patients. The only other immunosuppressive medication taken by more than 5% of patients was azathioprine (6.6%). MMF (Arm 1, 83.7%; Arm 2, 24.2%) and sirolimus (Arm 1, 3.7%; Arm 2, 71.4%) use were substantially different in each treatment arm (Table 3). Overall, 19 (3.2%) patients switched from tacrolimus to ciclosporin during the follow-up period. The number of patients taking ciclosporin at Years 1, 3, and 5 post-transplantation was 10 (1.7%), 10 (1.7%) and 5 (0.9%), respectively.

Primary efficacy endpoint: overall graft survival

In the EPS, at 1 year post-transplant, overall graft survival was 93.0% (95% confidence interval [CI]: 91.3, 94.8), falling to 84.0% (95% CI: 81.2, 86.9) at 5 years post-transplant. In the TA-EPS, graft survival rates for treatment Arms 1 and 2 were similar at 5 years: 89.3% (95% CI: 85.6,

Table 2. Tacrolimus (prolonged- and immediate-release formulations) total daily dose and whole blood trough levels over time

Time period post-transplantation	TA-FPS		FPS* (N = 587)
	Arm 1 (N = 270)	Arm 2 (N = 269)	
Total daily dose, mg/kg, mean ± SD			
6 months	0.090 ± 0.055	0.071 ± 0.047	0.084 ± 0.056
n (%)	270 (100)	269 (100)	587 (100)
1 year	0.078 ± 0.049	0.061 ± 0.043	0.069 ± 0.048
n (%)	264 (97.8)	264 (98.1)	550 (93.7)
2 years	0.066 ± 0.043	0.054 ± 0.040	0.060 ± 0.043
n (%)	242 (89.6)	242 (90.0)	506 (86.2)
3 years	0.059 ± 0.040	0.048 ± 0.035	0.055 ± 0.038
n (%)	239 (88.5)	238 (88.5)	500 (85.2)
4 years	0.057 ± 0.036	0.047 ± 0.032	0.053 ± 0.036
n (%)	238 (88.1)	228 (84.8)	490 (83.5)
5 years	0.056 ± 0.036	0.047 ± 0.032	0.052 ± 0.035
n (%)	223 (82.6)	218 (81.0)	462 (78.7)
Trough levels, ng/ml, mean ± SD			
6 months	8.1 ± 2.6	6.0 ± 2.7	7.4 ± 3.5
n (%)	270 (100)	269 (100)	581 (100)
1 year	7.2 ± 2.3	5.4 ± 1.9	6.4 ± 2.4
n (%)	257 (95.2)	252 (93.7)	528 (89.9)
2 years	6.5 ± 1.9	5.5 ± 1.8	6.0 ± 1.9
n (%)	234 (86.7)	233 (86.6)	486 (82.8)
3 years	6.3 ± 2.1	5.2 ± 1.8	5.8 ± 2.0
n (%)	232 (85.9)	223 (82.9)	476 (81.1)
4 years	6.0 ± 2.0	5.2 ± 2.5	5.7 ± 2.3
n (%)	228 (84.4)	210 (78.1)	458 (78.0)
5 years	5.9 ± 1.9	5.1 ± 1.7	5.5 ± 1.8
n (%)	211 (78.1)	201 (74.7)	432 (73.6)

FPS, follow-up patient set; SD, standard deviation; TA-FPS, treatment arm-follow-up patient set.

*The FPS includes 48 patients who were enrolled in the primary ADHERE study but not randomized to treatment with prolonged-release tacrolimus.

92.9) and 87.6% (95% CI: 83.7, 91.5), respectively (Fig. 2). A summary of the Kaplan–Meier analysis of overall graft survival for the EPS and TA-EPS is shown in Table S2. The *post hoc* analysis of overall death-censored graft survival (EPS) showed similar results to the primary analysis: 93.7% and 84.7% at 1 and 5 years post-transplant, respectively (Table S3). Cox proportional hazards model analysis showed no evidence of an association between hazard of graft loss and randomized treatment arm, donor age (<50 vs. ≥50 years), donor type (deceased vs. living), or sex (Table S4).

Secondary efficacy endpoints

Overall patient survival

In the EPS at 1 year post-transplant, overall patient survival was 97.8% (95% CI: 96.7, 98.8), falling to

90.8% (95% CI: 88.4, 93.1) at 5 years post-transplant. In the TA-EPS, the 5-year survival was similar across treatment Arms 1 and 2: 91.9% (95% CI: 88.5, 95.2) and 93.3% (95% CI: 90.3, 96.3), respectively (Fig. 3). A summary of the Kaplan–Meier analysis of overall patient survival for the EPS and TA-EPS is shown in Table S5. Cox proportional hazards model analysis showed no differences in hazard of death for the randomized arms, donor age (<50 vs. ≥50 years), donor type, or sex (Table S6).

Renal function

In the FPS, the mean ± SD eGFR (MDRD4) at the end of the initial ADHERE study period was 52.29 ± 21.64 ml/min/1.73 m² (Fig. 4; Table S7). This value was similar at 5 years post-transplant (52.5 ± 23.0 ml/min/1.73 m²). In the TA-FPS, mean eGFR was stable over time

Table 3. Concomitant immunosuppressant medications taken by $\geq 5\%$ of patients in the TA-FPS

n (%)	Arm 1 (N = 270)	Arm 2 (N = 269)
Patients with ≥ 1 concomitant immunosuppressant medication	249 (92.2)	247 (91.8)
Mycophenolate mofetil	226 (83.7)	65 (24.2)
Mycophenolic acid	27 (10.0)	27 (10.0)
Sirolimus*	10 (3.7)	192 (71.4)
Corticosteroid/glucocorticoid	196 (72.6)	195 (72.5)
Azathioprine	19 (7.0)	16 (5.9)

The Table shows medications taken during the follow-up study, which includes medications that were initiated before the start of the follow-up study and had an end date on or after the first day of the follow-up study.

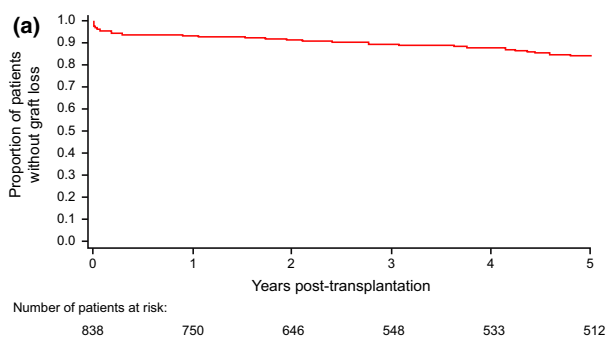
TA-FPS, treatment arm-follow-up patient set.

*In the primary ADHERE study, sirolimus was introduced at Day 28 post-transplantation for Arm 2 in combination with reduced-dose prolonged-release tacrolimus.

(6 months: 55.07 ± 21.64 ml/min/1.73 m²; 5 years: 52.01 ± 22.98 ml/min/1.73 m²); and the mean eGFRs of the two arms were not significantly different at any time-point. Similar results were observed after assessment of renal function by eGFR (CKD-EPI) and eCC (Cockcroft–Gault method) (Fig. 4; Table S7).

Acute rejections and biopsy-confirmed acute rejections

Most ARs and BCARs were reported in the first 6 months post-transplant. In the EPS, overall 5-year AR-free survival was 77.4% (Fig. 5) and overall BCAR-free survival rate was 86.0% (Fig. 6). Five-year estimates of AR- and BCAR-free survival were similarly high in both treatment arms. The number of patients with ARs and BCARs over time is shown in Table S8 and S9, respectively.



Safety and tolerability

One or more AEs were experienced by 211/587 patients (35.9%) in the FPS. AEs experienced by $\geq 1\%$ of patients in either treatment arm are summarized in Table 4. The most common system organ class was infections and infestations, reported in 92 patients (15.7%). In the TA-FPS, infections and infestations had a slightly higher rate in Arm 1 ($n = 48$, 17.8%) vs. Arm 2 ($n = 37$, 13.8%). The most frequently reported AEs included urinary tract infection (4.4%), diarrhea (2.7%), nasopharyngitis (2.6%), and peripheral edema (2.2%). The frequency of CMV ($n = 3$; 0.5%), and BK virus ($n = 1$; 0.2%) infections in the FPS were low. There was one AE leading to discontinuation of tacrolimus (moderate kidney transplant rejection), which was considered unrelated to tacrolimus.

In total, there were 50 patients (8.5%) with at least one ADR, of whom 11 (1.9%) had at least one severe ADR. Sixteen patients (2.7%) experienced ADRs that were considered by investigators to be probably related to tacrolimus, including infections and infestations (1.0%), neoplasms (benign, malignant, and unspecified; 0.9%) and renal and urinary disorders (0.5%). These are presented in Table 5. Three patients were reported to have ADRs leading to death, one originally randomized to Arm 1 (metastatic adenocarcinoma), and two originally randomized to Arm 2 (squamous cell carcinoma of the head and neck, and carcinoma of the small intestine). The 14 deaths reported during the follow-up study are listed in Table S10.

Discussion

In this long-term, 5-year follow-up of the patient cohort from the 12-month primary ADHERE study, follow-up data were obtained for 587 (70%) of the 838 patients

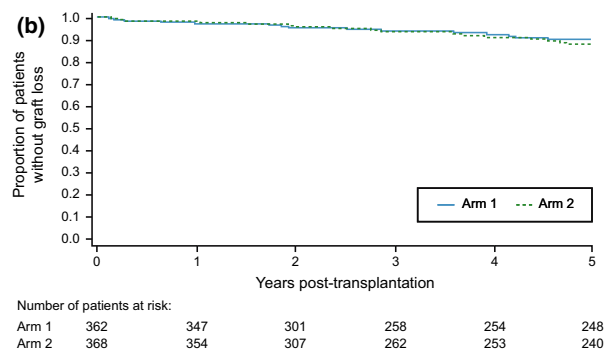


Figure 2 Kaplan–Meier plot of graft survival, overall in the EPS (a) and in Arms 1 and 2 of the TA-EPS (b). EPS, enrolled patient set; TA-EPS, treatment arm-enrolled patient set.

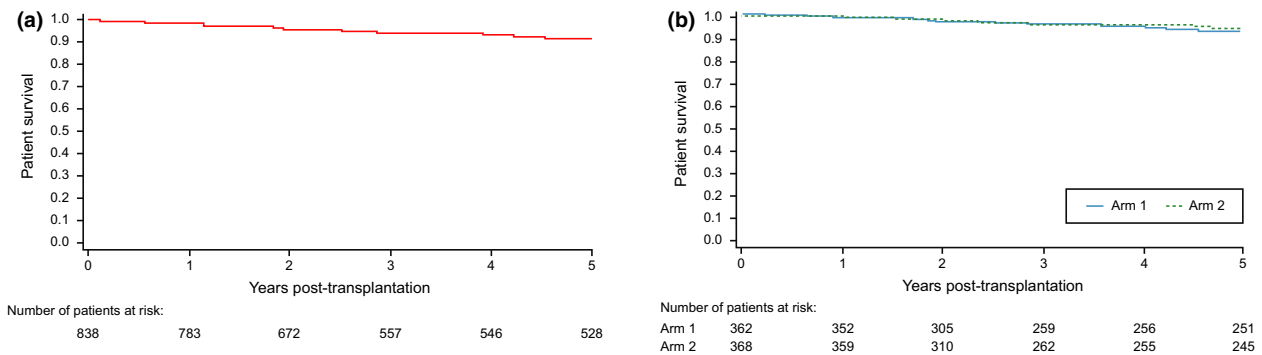


Figure 3 Kaplan–Meier plot of patient survival, overall in the EPS (a) and in Arms 1 and 2 of the TA-EPS (b). EPS, enrolled patient set; TA-EPS, treatment arm-enrolled patient set.

originally enrolled. Overall graft survival was high at 5 years post-transplant (84.0%), as was patient survival (90.8%). Renal function remained stable over 5 years post-transplant. Kaplan–Meier estimates of AR and BCAR events were low, with most occurring within the first 6 months post-transplant. The mean overall prolonged-release tacrolimus dose and trough levels decreased from 1 to 5 years post-transplant. Overall, 16 patients (2.7%) had ADRs that were considered probably tacrolimus-related during follow-up. These safety findings are consistent with those from the primary ADHERE study [14] and no new safety signals beyond the established safety profile for tacrolimus [19] were detected in this kidney transplant patient population.

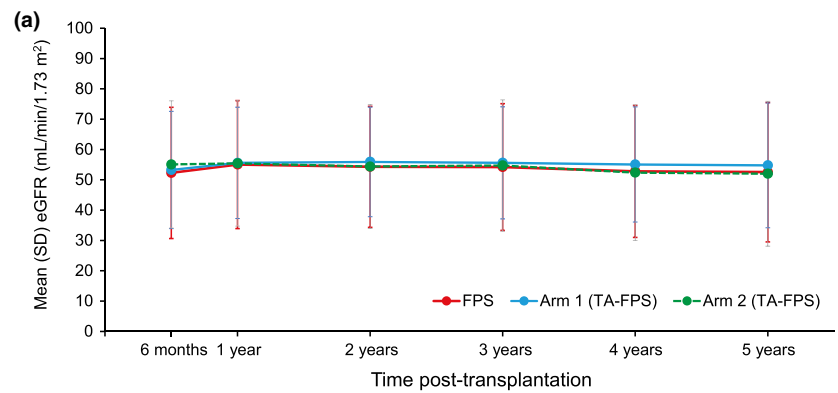
Our findings are broadly consistent with those reported by Silva *et al.* [20] in a 4-year follow-up of prolonged-release tacrolimus/MMF, immediate-release tacrolimus/MMF, and ciclosporin/MMF among 638 *de novo* kidney transplant recipients. Four-year Kaplan–Meier estimates of patient survival were 93.2%, 91.2%, and 91.7%, respectively. Estimates of graft survival were also high at 4 years: 84.7%, 82.7%, and 83.9%, respectively. Comparable safety profiles were reported between prolonged- and immediate-release tacrolimus, consistent with other reports of extended use of immunosuppressive regimens in transplant recipients [20].

In 2011, van Hooff *et al.* [21] presented 4-year, long-term follow-up data from four Phase II trials of prolonged-release tacrolimus, prescribed either *de novo* (in kidney and liver transplant recipients) or following conversion from immediate- to prolonged-release formulations (in kidney and heart transplant recipients) ($N = 240$). They reported that efficacy and safety of prolonged-release tacrolimus was maintained over 4 years in kidney, liver, and heart transplant recipients. The Kaplan–Meier estimate of graft survival at 4 years

was 100% in the *de novo* kidney study ($N = 47$), and 92.2% in the conversion kidney study ($N = 67$). Similarly, patient survival at 4 years was 100% in patients from the *de novo* kidney study and 93.6% in patients from the conversion kidney study. While 90.9% were BCAR-free at 4 years after the *de novo* kidney study, there were no reported BCAR episodes after the conversion study [21]. Overall, the 4-year survival rates reported by van Hooff *et al.* [21] were somewhat higher than the 5-year rates observed in the current study; however, it should be noted that the number of *de novo* kidney transplant patients in the van Hooff study was relatively small compared with that in our study.

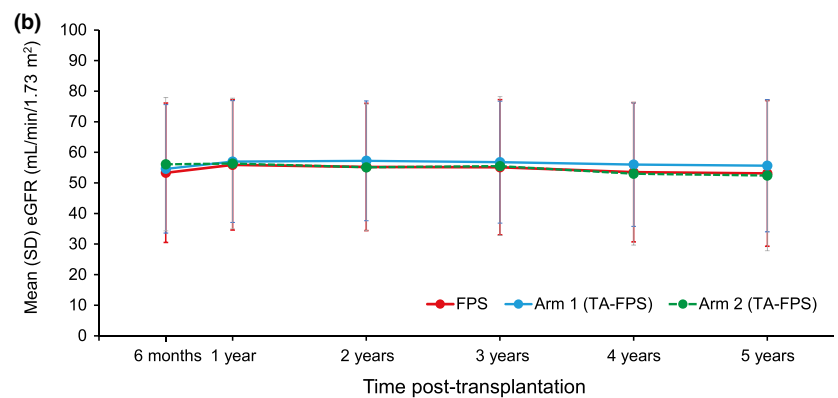
The objective of the primary ADHERE study was to assess whether sirolimus plus reduced-dose prolonged-release tacrolimus improved renal function compared with MMF plus prolonged-release tacrolimus. In accordance with the original randomization, most of the patients who took sirolimus during the follow-up period were in Arm 2 (93.2%). Renal function remained stable and there was no significant difference in renal function between treatment arms at any timepoint. These findings are broadly consistent with those of previous long-term studies of graft function in kidney transplant recipients [22,23].

Graft survival and patient survival were similar between the treatment arms. Kaplan–Meier estimates of AR and BCAR were also similar between treatment arms. Regarding mean tacrolimus trough levels at 1 year, higher values were seen in Arm 1 than in Arm 2 (5.46 ng/ml vs. 4.42 ng/ml, respectively), which reflects the design of the randomized study; however, this difference narrowed at 5 years (5.9 and 5.1 ng/ml, respectively). In accordance with the higher tacrolimus trough levels in Arm 1, the rate of infections and infestations was slightly higher in Arm 1 (17.8%) than Arm 2



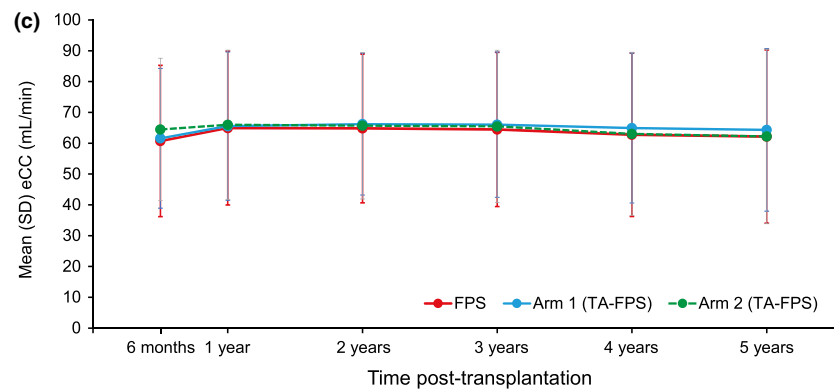
Number of patients with eGFR measurement:

FPS	587	566	533	530	520	493
Arm 1	270	267	246	248	248	237
Arm 2	269	265	254	251	241	239



Number of patients with eGFR measurement:

FPS	587	566	533	530	520	493
Arm 1	270	267	246	248	248	237
Arm 2	269	265	254	251	241	239



Number of patients with eCC measurement:

FPS	587	566	533	530	520	493
Arm 1	270	267	246	248	248	237
Arm 2	269	265	254	251	241	239

Figure 4 Renal function over time: eGFR by MDRD4 (a) and CKD-EPI (b) formulae, and eCC by Cockcroft–Gault method (c) in the FPS and in Arms 1 and 2 of the TA-FPS. CKD-EPI, chronic kidney disease epidemiology collaboration; eCC, estimated creatinine clearance; eGFR, estimated glomerular filtration rate; FPS, follow-up patient set; MDRD4, 4-variable modification of diet in renal disease; SD, standard deviation; TA-FPS, treatment arm-follow-up patient set. The FPS includes 48 patients who were enrolled in the primary ADHERE study but not randomized to treatment with prolonged-release tacrolimus. For patients with graft loss, renal function values were set to zero from the time of graft loss.

(13.8%), but a formal statistical comparison between arms was not performed. Both tacrolimus-based regimens appear to have a role in long-term immunosuppression following kidney transplant.

The recently published TRANSFORM study [24] also evaluated efficacy and safety outcomes of a CNI (cyclosporin A or tacrolimus) with everolimus or MPA as concomitant medication. Similar to the ADHERE

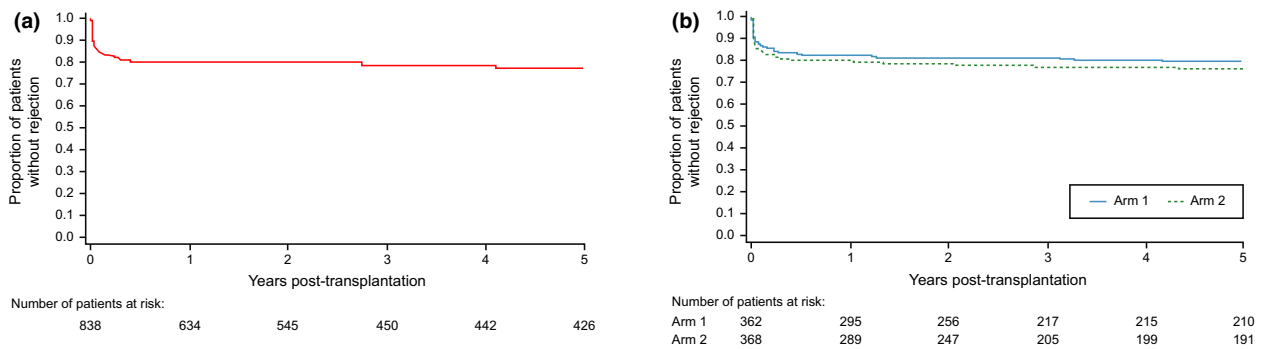


Figure 5 Kaplan–Meier plot of time to first episode of acute rejection, overall in the EPS (a) and in Arms 1 and 2 of the TA-EPS (b). EPS, enrolled patient set; TA-EPS, treatment arm-enrolled patient set.

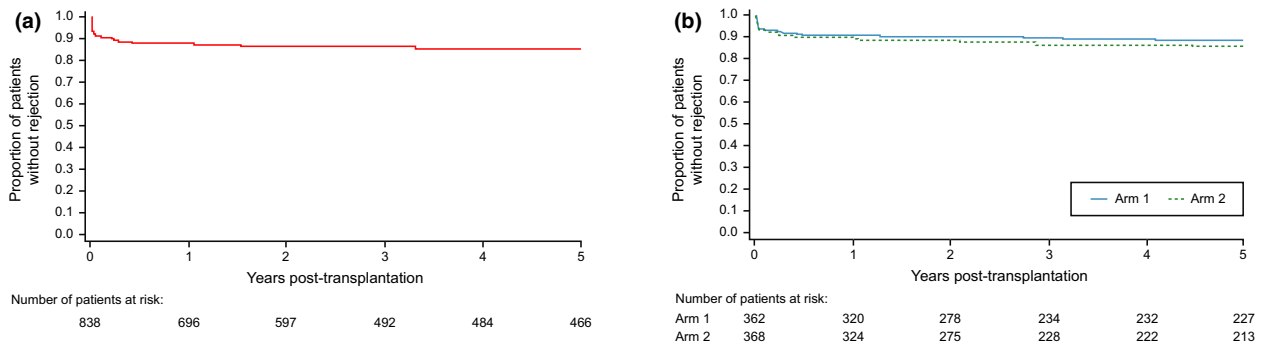


Figure 6 Kaplan–Meier plot of time to first episode of BCAR, overall in the EPS (a) and in Arms 1 and 2 of the TA-EPS (b). BCAR, biopsy-confirmed acute rejection; EPS, enrolled patient set; TA-EPS, treatment arm-enrolled patient set.

Table 4. Adverse events (AEs) experienced by $\geq 1\%$ of patients in the TA-FPS and in patients in the FPS who were not randomized to treatment with prolonged-release tacrolimus in the primary ADHERE study

Adverse event (SOC/PT), n (%)	Arm 1 (N = 270)	Arm 2 (N = 269)	Not randomized (N = 48)*
Patients with ≥ 1 AE	100 (37.0)	99 (36.8)	12 (25.0)
Infections and infestations			
Urinary tract infection [†]	14 (5.2)	11 (4.1)	1 (2.1)
Nasopharyngitis	4 (1.5)	9 (3.3)	2 (4.2)
Bronchitis	4 (1.5)	5 (1.9)	0 (0.0)
Pneumonia	3 (1.1)	4 (1.5)	0 (0.0)
Gastrointestinal disorders			
Diarrhea	7 (2.6)	9 (3.3)	0 (0.0)
Renal and urinary disorders			
Renal impairment	3 (1.1)	4 (1.5)	0 (0.0)
Neoplasms (benign, malignant and unspecified, including cysts and polyps)			
Basal cell carcinoma	3 (1.1)	3 (1.1)	0 (0.0)
General disorders and administration site conditions			
Peripheral edema	3 (1.1)	9 (3.3)	1 (2.1)

Only adverse events that started on or after the date of informed consent for this follow-up study are included.

AE, adverse event; FPS, follow-up patient set; PT, preferred term; SOC, system organ class; TA-FPS, treatment arm-follow-up patient set.

*The FPS includes 48 patients that were enrolled in the primary ADHERE study but not randomized to treatment with prolonged-release tacrolimus.

[†]A composite of the preferred terms “Escherichia urinary tract infection”, “urinary tract infection”, and “urinary tract infection bacterial”.

Table 5. Adverse drug reactions (ADRs) probably related to tacrolimus (FPS)

Adverse event (SOC/PT), n (%)	Frequency, n (%) (N = 587)
Patients with ≥ 1 probably tacrolimus-related ADR	16 (2.7)
Infections and infestations	6 (1.0)
Urinary tract infection	2 (0.3)
Herpes zoster	3 (0.5)
Cytomegalovirus infection	1 (0.2)
Oral herpes	1 (0.2)
Neoplasms benign, malignant and unspecified (inc. cysts and polyps)	5 (0.9)
Basal cell carcinoma	1 (0.2)
Squamous cell carcinoma of skin	3 (0.5)
Squamous cell carcinoma of head and neck	1 (0.2)
Bowen's disease	1 (0.2)
Skin cancer	1 (0.2)
Skin papilloma	1 (0.2)
Renal and urinary disorders	3 (0.5)
Renal impairment	3 (0.5)
Nervous system disorders	1 (0.2)
Tremor	1 (0.2)
Investigations	1 (0.2)
Blood creatinine increased	1 (0.2)
Metabolism and nutrition disorders	1 (0.2)
Diabetes mellitus	1 (0.2)

ADR, adverse drug reaction; FPS, follow-up patient set; PT, preferred term; SOC, system organ class.

study design, a reduced dose of CNI was prescribed with everolimus. However, in TRANSFORM the trough concentration of everolimus was specified, unlike in ADHERE where a fixed dose was set for sirolimus. The primary endpoint was a composite of treated BCAR or eGFR < 50 ml/min/1.73 m². Graft loss was 3.2% at 12 months post-transplant in the TRANSFORM study, comparable to the rates reported in ADHERE at 12 months (2.9% and 2.2% in Arms 1 and 2, respectively) [14]. However, the Kaplan–Meier estimate of AR was 13.8% and BCAR was 12% at 12 months in TRANSFORM [24], higher than those reported in ADHERE in the same time frame (7.3% and 8.3% AR, and 4.3% and 3.6% BCAR, in ADHERE Arms 1 and 2, respectively) [14]. The results of the TRANSFORM study suggested that everolimus was noninferior to MPA for immunosuppressive efficacy and preservation of graft function in *de novo* kidney transplant recipients at 12 months post-transplant.

The present study has several limitations. Owing to the limited amount of data available for analysis in real-world practice, data on some endpoints were not collected. For example, neither adherence nor variability in exposure was assessed despite being potentially key factors associated with long-term graft survival. Although the patients who entered the follow-up study were representative of the original ADHERE patient population, these results should be interpreted with caution given that not all the patients in the EPS ($n = 838$) from the initial 1-year ADHERE study were included in the TA-FPS ($n = 539$) and had 5-year follow-up data. It is important to note that safety data in postmarketing observational studies are subject to underreporting bias [25,26]. Therefore, the safety findings reported in the current study should be interpreted with caution.

In summary, long-term follow-up of the ADHERE study revealed high rates of both graft survival and patient survival at 5 years post-transplant. Renal function remained stable over 5 years, and rates of AR and BCAR were low, with most cases being reported during the first 6 months post-transplant. The safety findings reported here are consistent with those from the primary ADHERE study, and no new safety signals were reported. These findings support the role of long-term administration of once-daily prolonged-release tacrolimus-based immunosuppression in combination with sirolimus or MMF for renal transplant recipients in the clinical setting.

Authorship

All authors were involved in data interpretation and critical review of the article at each stage of development. OR, MC, NK, AD, CM, FC, ZM, MHLC, DRJK, JK, and FL were also involved in data acquisition. GK, NU, and SA were involved with the study design and data analysis while MH was involved with the study design.

Funding

This study was sponsored by Astellas Pharma Europe Ltd. Medical writing and editorial support were funded by Astellas Pharma, Inc.

Conflict of interest

All authors report nonfinancial support from Astellas Pharma, during the conduct of the study. NK reports personal fees from Chiesi, Novartis, Neovii, Sanofi,

Merck Sharp & Dohme, Abbvie, Shire, Amgen, and Gilead during the conduct of the study. AD is married to an employee of Astellas. SA, MH, and NU are employed by Astellas; GK is a consultant statistician working on behalf of Astellas, and he has also received support for travel from Astellas.

Acknowledgements

The authors would like to thank all centers participating in this study. This study was sponsored by Astellas Pharma Europe, Ltd. Paola Accalai and Fernando Gibson, from Cello Health MedErgy, assisted in drafting the manuscript under the direction of the authors. Editorial support was provided by Cello Health MedErgy throughout the development process. Editorial support was funded by Astellas Pharma, Inc.

Data statement

Access to anonymized individual participant-level data will not be provided for this trial as it meets one or more of the exceptions described on www.clinicalstudydatarequest.com under “Sponsor Specific Details for Astellas.”

SUPPORTING INFORMATION

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

Table S1. Summary of sirolimus dosing throughout the follow-up period (FPS).

Table S2. Kaplan–Meier summary of graft survival (EPS and TA-EPS).

Table S3. Kaplan–Meier summary of death-censored graft survival (EPS).

Table S4. Cox proportional hazards model analysis of overall graft survival (TA-EPS).

Table S5. Kaplan–Meier summary of patient survival (EPS and TA-EPS).

Table S6. Cox proportional hazards model analysis of patient survival (TA-EPS).

Table S7. Renal function over time (FPS and TA-FPS).

Table S8. Kaplan–Meier summary of AR-free survival (EPS and TA-EPS).

Table S9. Kaplan–Meier summary of BCAR-free survival (EPS and TA-EPS).

Table S10. Patient deaths during the follow-up study (FPS).

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