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One-year Outcome of Everolimus With Standard-dose Tacrolimus Immunosuppression in De Novo ABO-incompatible Living Donor Kidney Transplantation: A Retrospective, Single-center, Propensity Score Matching Comparison With Mycophenolate in 42 Transplants

Hiroshi Noguchi, MD, PhD,¹ Akihiro Tsuchimoto, MD, PhD,² Kenji Ueki, MD,² Keizo Kaku, MD, PhD,¹ Yasuhiro Okabe, MD,¹ and Masafumi Nakamura, MD, PhD¹

Background. Despite improvement in immunosuppressive therapy, long-term kidney allograft survival remains a major challenge. The outcomes of therapy with everolimus (EVR) and standard-dose tacrolimus (Tac) have not been compared with those of mycophenolate mofetil (MMF) and standard-dose Tac in recipients of de novo ABO-incompatible (ABOi) living donor kidney transplantation (LDKT). **Methods.** This retrospective, observational, single-center, propensity score matching (PSM) study compared the outcomes of EVR and standard-dose Tac with those of MMF and standard-dose Tac following de novo ABOi LDKT. In total, 153 recipients of ABOi LDKT between January 2008 and March 2018 were screened for inclusion in the study. The variables considered for PSM were: recipient age/sex, duration of dialysis, cytomegalovirus mismatch (seronegative recipient and seropositive donor), cause of kidney disease, donor age/sex, and numbers of mismatches (HLA-A, HLA-B, and HLA-DR). After PSM, there were 21 patients in each group (n = 42 overall). **Results.** Four patients in the EVR group and 1 patient in the MMF group were withdrawn because of adverse effects. There were no significant differences between the 2 groups in 1-year outcomes regarding patient death, graft loss, delayed graft function, biopsy-proven acute rejection, infection requiring hospital admission, or estimated glomerular filtration rate. The 1-year protocol biopsy showed that the severity of interstitial fibrosis/tubular atrophy was significantly milder in the EVR group than in the MMF group. **Conclusions.** The findings suggest that the renal efficacy and safety of EVR and standard-dose Tac in recipients of de novo ABOi LDKT are comparable with those of MMF and standard-dose Tac.

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Kidney transplantation (KT) is the preferred option for improving life expectancy and quality of life in patients with end-stage renal disease. Despite improvement in immunosuppressive therapy, long-term kidney allograft survival remains a major challenge. A current major threat to graft

survival is premature death caused by cardiovascular events, malignancy, or infectious diseases, all of which are associated with the prolonged use of immunosuppressive agents.¹ The use of calcineurin inhibitors (CNIs) and corticosteroids can promote atherosclerosis. Many methods to avoid CNI toxicity have been proposed, although no established treatment

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¹ Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

² Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

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A.T. participated in the pathological assessment. K.U. participated in the pathological assessment. K.K. participated in generating the study population database.

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Correspondence: Yasuhiro Okabe, MD, Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. (y-okabe@surg1.med.kyushu-u.ac.jp).

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strategy has been adopted.² The use of immunosuppressive agents, including mycophenolate mofetil (MMF), can promote the onset of infectious diseases.

A severe shortage of deceased donors has forced the inclusion of a broader range of donor types for KT. ABO-incompatible (ABOi) living donor KT (LDKT) has been adopted in many centers worldwide,³ and modern immunosuppressive management has improved the outcome of ABOi LDKT.⁴ A recent meta-review reported that the risk of sepsis in ABOi KT is higher than that in ABO-compatible (ABOc) KT; moreover, patient survival in the first 5 years after ABOi KT is inferior to that after ABOc KT. This increased mortality presumably results from oversuppression of the immune system following desensitization, which permits the emergence of life-threatening bacterial and viral infections.⁵

Everolimus (EVR) is a newly introduced immunosuppressive drug that is classified as an inhibitor of mammalian target of rapamycin (mTOR).⁶ mTOR blocks growth-factor-mediated cell proliferation, suppresses T-cell activation, and exerts potent immunosuppression in transplant recipients.⁶ The mTOR signaling pathway regulates a variety of other cellular functions involved in metabolism, apoptosis, and growth.⁷ Compared with MMF, EVR exhibits antineoplastic, antiviral, antiatherosclerosis, and antiproliferative properties. KT recipients taking mTOR inhibitors are at risk of developing cytomegalovirus (CMV) infection.⁸ EVR has no obvious nephrotoxicity, and its use may offer an opportunity to reduce or withdraw MMF. Therefore, several studies have assessed a variety of EVR-based, CNI-sparing protocols to identify the optimal balance between preventing rejection and preserving graft function.^{9–13} The recent TRANSFORM study (Advancing renal TRANSplant efficacy and safety Outcomes with an everoliMus-based regimen) compared de novo EVR with reduced-exposure CNI, in the context of the current standard of care.¹⁴ Both treatments yielded a comparable incidence of adverse effects, although with a different pattern. However, no studies have compared the outcomes of standard therapy with MMF and tacrolimus (Tac) with those of EVR and Tac in recipients of de novo ABOi LDKT. EVR has been approved for use in recipients of KT in Japan since 2011. Based on existing research, we consider EVR-based immunosuppression to be the optimal treatment for KT. In our hospital, we introduced an EVR-based protocol for all patients with low immunological risk who were undergoing de novo KT, including ABOi KT, in 2016. Because the safety and efficacy of the EVR protocol for ABOi KT have not yet been established, we adopted a protocol in which EVR was combined with standard-dose Tac, before the adoption of combination therapy with low-dose Tac. The aim of the present study was to compare the outcomes of EVR and standard Tac immunosuppression with those of MMF and standard Tac immunosuppression in recipients of de novo ABOi LDKT.

MATERIALS AND METHODS

Patients

This retrospective study included patients who underwent KT between January 2008 and March 2018. Patient data were extracted from the medical records at the Kyushu University Hospital, Fukuoka, Japan. We introduced an EVR-based protocol for all patients with low immunological risk who were undergoing de novo KT, including ABOi KT, in September

2016; therefore, all patients in the MMF group underwent transplantation during the period from January 2008 to August 2016. In contrast, all patients in the EVR group underwent transplantation during the period from September 2016 to March 2018. To reduce bias based on the differing lengths of the study period for each group, we compared 1-year outcomes between the 2 groups. In addition, all patients were considered to have low immunological risk, based on the use of the following exclusion criteria: (1) recipients of ABOc KT; (2) recipients of deceased donor KT; (3) pediatric recipients aged <18 years; (4) patients with antiphospholipid syndrome; (5) recipients of living donor simultaneous pancreas and KT; (6) patients with preformed donor-specific anti-HLA antibody (DSA); and (7) retransplantation recipients. A single method was used to evaluate DSA in all patients in this study. We identified HLA classes I (A or B) and II (DR) IgG using the FACSCanto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and a commercially available kit (FlowPRA Single Antigen Beads; One Lambda Inc., Canoga Park, CA, USA), in accordance with the manufacturer's instructions. The study protocol was approved by the Ethics Committee of Kyushu University (IRB-No 24-54) and was registered in the University Hospital Medical Information Network Clinical Trials Registry System (UMIN000008475). All included patients received full verbal and written explanations of the nature and purpose of this study and provided written informed consent to participate.

Immunosuppression Protocol

All patients who underwent KT were administered 20 mg/d basiliximab at the time of the operation and on postoperative day 4. The orally administered immunosuppressive agents were a once-daily, prolonged-release formulation of tacrolimus (Tac-QD), MMF, EVR, and methylprednisolone. Recipients of ABOi LDKT received oral immunosuppression beginning on preoperative day 14 (described below) and low-dose rituximab (200 mg total) on preoperative day 7.¹⁵ They underwent 1–6 plasmapheresis treatments until the anti-A/-B titers were reduced to ≤ 32 , before transplantation.¹⁵ No splenectomy or postoperative plasmapheresis was performed. Methylprednisolone (250 mg) was administered intravenously on the day of surgery. In all patients, Tac-QD was initiated at 0.10 mg/kg/d; it was adjusted to maintain a trough concentration of 5–8 ng/mL in the whole blood for 1 or 2 months postoperatively, and a trough concentration of 4–8 ng/mL thereafter. We previously reported that the required doses and trough concentrations tended to decrease in patients receiving Tac-QD, compared with a twice-daily formulation.¹⁶ In our institution, the trough concentration has decreased in a manner observed in other institutions.^{4,17,18} In the EVR group, EVR was started at 2.0 mg/d (beginning on preoperative day 14) and adjusted to target a trough concentration of 3–8 ng/mL throughout the study.¹⁴ In the MMF group, MMF was started at 1.0 g/d (beginning on preoperative day 14) and was increased to 2.0 g/d on preoperative day 7. The dose was reduced to 1.5 g/d after 2 months and to 1 g/d after 3 months (Figure 1). CMV prophylaxis was low-dose valganciclovir (450 mg/d) for ≥ 3 months post KT for all cases in which the donor was CMV-seropositive and the recipient was CMV seronegative.¹⁹ For prophylaxis of pneumonia caused by *Pneumocystis jirovecii* (previously known as *Pneumocystis carinii*), all patients received sulfamethoxazole/trimethoprim for 1 week after transplantation.²⁰

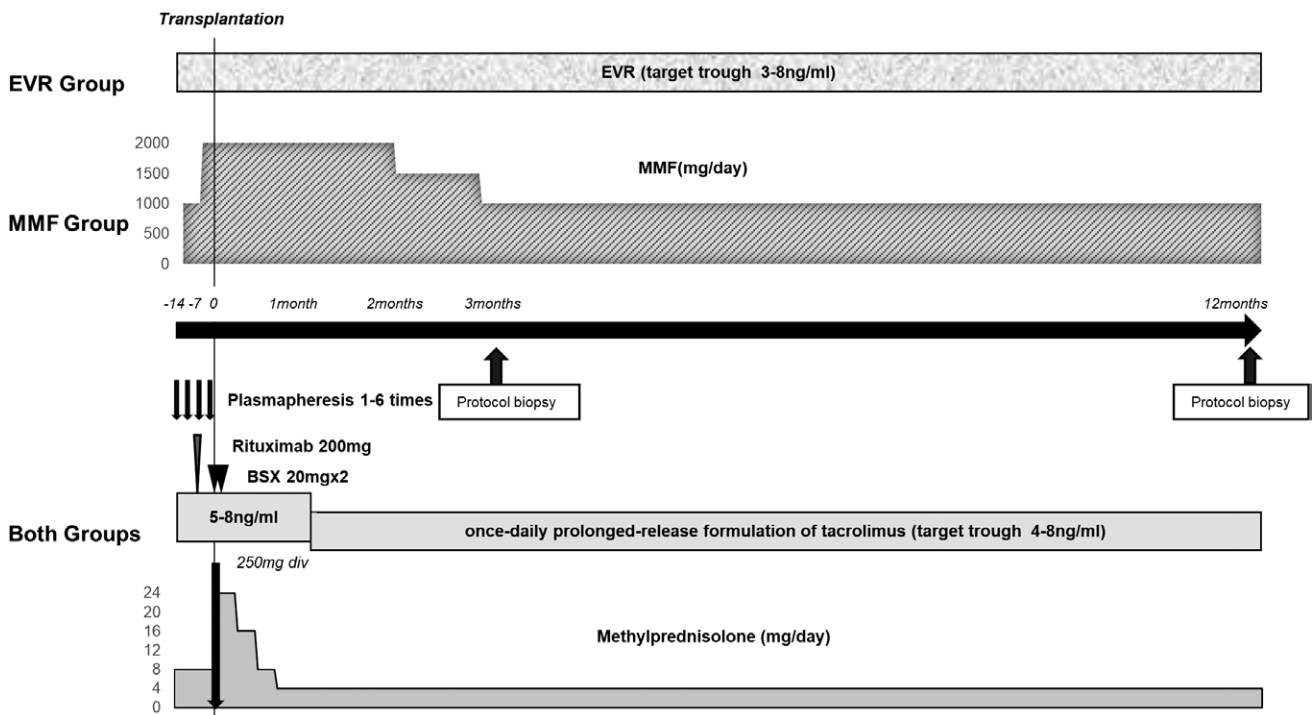


FIGURE 1. Flowchart of patient selection. EVR, everolimus; MMF, mycophenolate mofetil.

Study Variables and Definitions

The following demographic and clinical data were retrospectively collected from medical records: kidney function, urinary protein, patient death, graft loss, biopsy-proven acute rejection, BK-virus-associated nephropathy, surgical complications, infection including CMV antigenemia, delayed graft function, new-onset diabetes after transplantation, hyperlipidemia, leukopenia, and anemia. To evaluate baseline kidney function, the estimated glomerular filtration rate was calculated using the appropriate equation for Japanese chronic kidney disease patients.²¹ Urinary protein was assessed as spot urine protein/creatinine ratio at 6 and 12 months. Allograft diagnoses were performed using episode biopsies or 3- and 12-month protocol biopsies, in accordance with the Banff 2013 working classification.²² Patients with acute rejection were classified into borderline changes, acute T-cell-mediated rejection (Banff grade IA or higher), and/or acute antibody-mediated rejection. Subclinical acute rejection was defined as rejection diagnosed by protocol biopsy without an increase of >15% in the serum creatinine concentration from baseline (defined as mean serum creatinine concentration at 3 months before protocol biopsy) and no rejection episodes within the previous 1 month.²³ BK-virus-associated nephropathy and graft rejection were restricted to biopsy-proven diagnoses. All biopsy specimens were evaluated by 2 experienced nephrologists (A.T. and U.K.) who reached a consensus using a dual-light microscope. Postoperative complications were graded in accordance with the Clavien–Dindo classification,²⁴ with grade ≥ 3 indicating the presence of postoperative complications. Infection was defined as clinical symptoms and a need for hospitalization. CMV infection was defined as detection of CMV replication based on the presence of CMV p65 antigenemia (≥ 10 CMV p65-positive cells per 200 000 peripheral blood leukocytes). Delayed graft function was defined as a need for dialysis therapy within 1 week of transplantation

despite the diagnosis determined via biopsy. New-onset diabetes after transplantation was diagnosed according to the American Diabetes Association definition²⁵ (hemoglobin A1c > 6.5%; fasting plasma glucose ≥ 126 mg/dL; 2-hour plasma glucose level of ≥ 200 mg/dL during a 75-g oral glucose tolerance test; random plasma glucose level of ≥ 200 mg/dL on 2 occasions; or the requirement of medication for the management of hyperglycemia). Hyperlipidemia, leukopenia, and anemia were assessed by serum triglyceride, low-density lipoprotein, white blood cell, and hemoglobin levels at 6 and 12 months.

Statistical Analysis

Results are presented as mean \pm SD for normally distributed variables, as median (interquartile range) for variables that were not normally distributed, and as count and percentage for categorical variables. For normally distributed continuous variables, mean bivariate differences between 2 groups were assessed using Student's *t*-test; for continuous variables that were not normally distributed, median differences were compared using the Mann–Whitney U test. Grades of interstitial fibrosis (IF) and tubular atrophy (TA) without any specific etiology were compared using 2-sided Mann–Whitney U tests. Categorical variables were compared using the χ^2 test or Fisher's exact test. Time-dependent changes in the Tac trough concentration and estimated glomerular filtration rate were compared between the EVR and MMF groups, using repeated-measures ANOVA. Bonferroni correction was used to reduce type I error because of the multiple comparisons among multiple time points. To overcome bias from different distributions of covariables among patients in the 2 study groups, propensity score matching (PSM) was performed using logistic regression analysis to create propensity scores for both groups. The following variables were entered into the propensity model: recipient age/sex, duration of

dialysis, CMV mismatch (seronegative recipient and seropositive donor), ABO titer (\log_2), cause of end-stage renal disease, donor age/sex, and number of HLA mismatches. One-to-one matching between the 2 groups was performed using nearest neighbor matching with a caliper method. All statistical analyses were performed using JMP software (version 13, SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient Characteristics

From January 2008 to March 2018, a total of 762 consecutive KT were performed at Kyushu University Hospital. Of these, 153 patients met the criteria for inclusion in this study. Before PSM, only the duration of dialysis was significantly longer in the MMF group than in the EVR group ($P = 0.0276$). After PSM, 21 patients were chosen for each group (Figure 2). The baseline characteristics of each group (before and after PSM) are shown in Table 1. The mean recipient age/sex, duration of dialysis, ABO titer (\log_2), CMV mismatch, number of HLA mismatches, and donor age/sex of the groups were statistically similar after PSM. Four patients in the EVR group were withdrawn because of graft loss caused by acute T-cell-mediated rejection ($n = 1$), severe proteinuria ($n = 2$), or leg edema ($n = 1$). One patient in the MMF group was withdrawn because of elevated liver function and was converted to EVR treatment ($n = 1$).

Immunosuppression

In both groups, the mean Tac trough concentration was in the target range throughout. Repeated-measures ANOVA showed significant time-dependent interactions of Tac trough concentration ($P = 0.0058$) between the EVR and MMF groups. Post hoc multiple comparisons analysis revealed that the changes in Tac trough concentrations between 1

and 5 months, as well as between 1 and 10 months, were significantly different between the EVR and MMF groups ($P = 0.0074$ and $P = 0.0275$, respectively; Figure 3A). In the EVR group, the mean EVR trough concentration was in the target range (3–8 ng/dL) throughout the study (Figure 3B).

Perioperative Outcomes

Perioperative outcomes are summarized in Table 2. The 2 groups did not significantly differ regarding warm ischemia time, cold ischemia time, incidence of death, graft loss, biopsy-proven acute rejection, BK-virus-induced nephropathy, surgical complications (Clavien–Dindo grade ≥ 3), infection needing hospital admission, CMV viremia (≥ 10 p65-positive cells per 200 000 peripheral blood leukocytes), delayed graft function, or new-onset diabetes after transplantation. There were no episodes of thrombosis or pulmonary adverse events.

Graft Function

Repeated-measures ANOVA showed no significant time-dependent interactions of estimated glomerular filtration rate ($P = 0.7772$) between the EVR and MMF groups throughout the study (Figure 3C).

Proteinuria, Triglyceride, Low-density Lipoprotein, White Blood Cells, and Hemoglobin

There were no significant differences in spot urine protein/creatinine ratio, triglycerides, white blood cells, or hemoglobin at 6 and 12 months post KT. However, the mean low-density lipoprotein concentration at 6 and 12 months was significantly higher in the EVR group than in the MMF group (Table 3).

Pathological Outcomes

There were no significant differences in the rates of sub-clinical acute rejection and borderline changes in the 3-month and 1-year protocol biopsies (Figure 4A). Furthermore, there

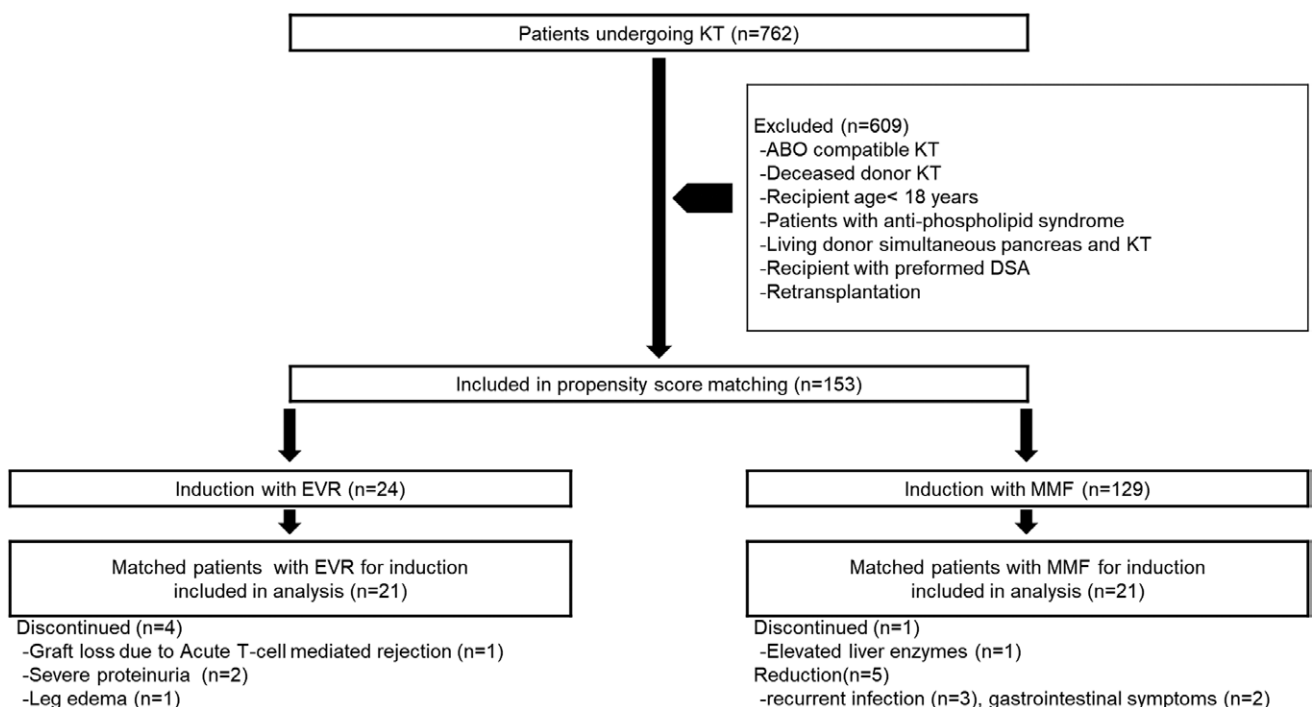


FIGURE 2. Immunosuppression protocols of both groups. DSA, donor-specific anti-HLA antibody; EVR, everolimus; KT, kidney transplantation; MMF, mycophenolate mofetil.

TABLE 1.
Patient characteristics

Characteristics	Overall (n = 153)			Matched (n = 42)		
	EVR group (n = 24)	MMF group (n = 129)	P	EVR group (n = 21)	MMF group (n = 21)	P
Recipient						
Age (y)	43.8 ± 13.0	49.2 ± 13.4	0.0666	44.2 ± 13.2	46.3 ± 15.2	0.6323
Sex (female: male)	8:16	55:74	0.3905	7:14	7:14	–
Duration of dialysis (mo) ^a	0 (0–29)	15 (0–73)	0.0276	0 (0–53)	0 (0–34)	0.9425
ABO titer (log ₂)	6.7 ± 2.3	7.1 ± 2.7	0.4400	6.7 ± 2.2	6.2 ± 2.6	0.5586
CMV mismatch (D+R–)	1 (4.2%)	19 (14.7%)	0.1138	1 (4.8%)	0 (0%)	0.2349
HLA mismatch	3.4 ± 1.6	3.3 ± 1.6	0.7471	3.4 ± 1.5	3.3 ± 1.6	0.9577
Primary disease (CAKUT: DM: GN: PCKD: other: nephrosclerosis: unknown)	2:2:12:2:2:3:1	6:40:59:8:6:4:6	0.1716	2:2:11:2:2:1:1	0:3:10:1:2:2:3	0.5766
Donor						
Age (y)	56.8 ± 11.0	55.9 ± 11.0	0.8311	55.8 ± 11.2	54.5 ± 11.8	0.7915
Sex (female: male)	15:9	82:47	0.9208	13:8	14:7	0.7474

^aMedian (interquartile range).

CAKUT, congenital anomalies of the kidney and urinary tract; CMV, cytomegalovirus; DM, diabetes mellitus; EVR, everolimus; GN, glomerulonephritis; MMF, mycophenolate mofetil; PCKD, polycystic kidney disease.

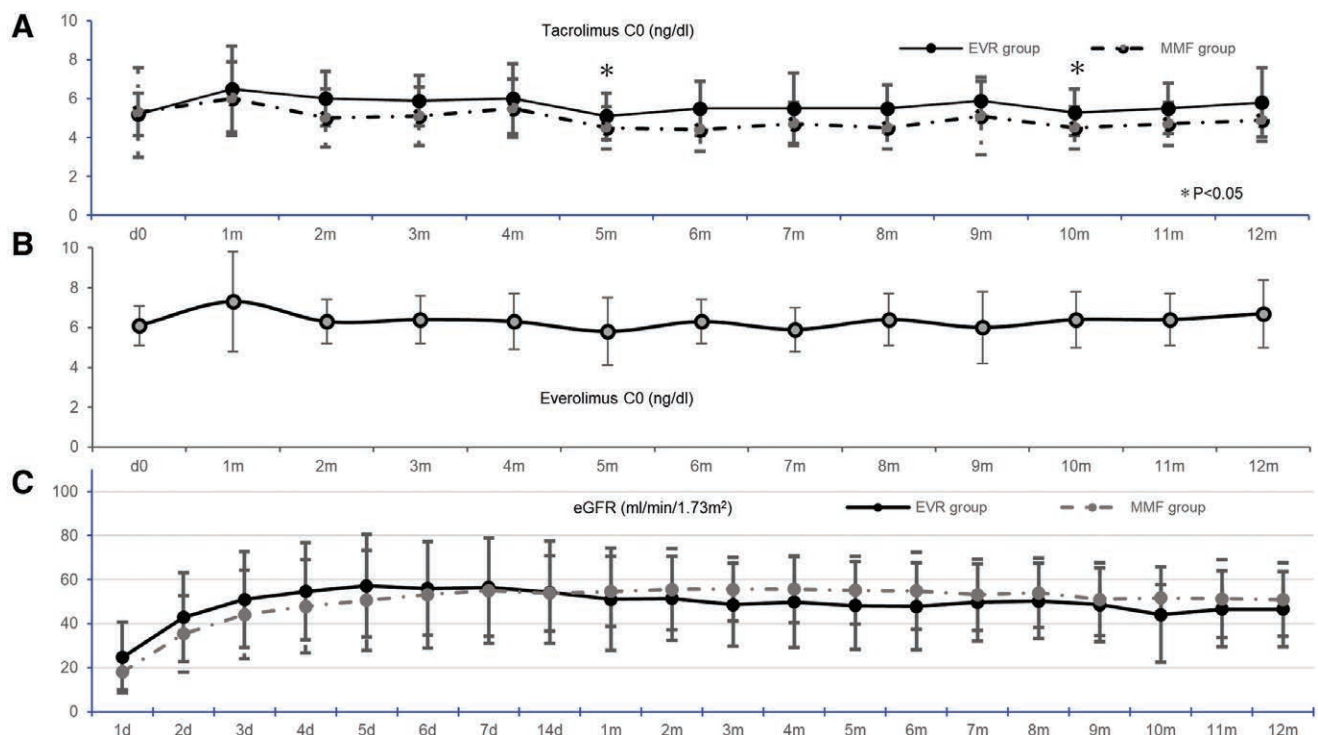


FIGURE 3. A, Repeated-measures ANOVA showed significant time-dependent interactions of Tac trough concentration ($P = 0.0058$) between the EVR and MMF groups. Post hoc multiple comparison analysis revealed that the changes in Tac trough concentrations between 1 and 5 mo, as well as between 1 and 10 mo, were significantly different between the EVR and MMF groups ($P = 0.0074$ and $P = 0.0275$, respectively). B, Mean EVR trough concentrations and SDs of the EVR group. C, Mean estimated glomerular filtration rates and SDs of both groups. Repeated-measures ANOVA showed no significant time-dependent interactions of estimated glomerular filtration rate ($P = 0.7772$) between the EVR and MMF groups. EVR, everolimus; MMF, mycophenolate mofetil; SD, standard deviation; Tac, tacrolimus.

was no significant difference in the severity of IF/TA in the 3-month protocol biopsy. However, the severity of IF/TA in the 1-year biopsy was significantly milder in the EVR group than in the MMF group (Figure 4B).

DISCUSSION

In our retrospective PSM study, we assessed the 1-year safety and efficacy of EVR with standard-dose Tac in recipients of de novo ABOi LDKT. Our study suggested that EVR

with standard-dose Tac achieved short-term beneficial outcomes for recipients of de novo ABOi LDKT, compared with the standard MMF-based regimen.

Several reports have shown that EVR-based regimens with low-dose CNI reduce the incidence of infection, particularly CMV antigenemia.^{8,26} However, our study did not show any significant differences between the EVR- and MMF-based regimens in terms of the incidence of infection. One possible explanation is that, unlike previous investigators, we used standard-dose Tac. Furthermore, Tac trough concentrations in

TABLE 2.**Postoperative outcome at 1 y**

Parameter	EVR (n = 21)	MMF (n = 21)	P
WIT (min)	3.8 ± 1.3	4.1 ± 1.8	0.9485
TIT (min)	152 ± 72	131 ± 43	0.6286
Death	0	0	–
Graft loss	1 (4.8%)	0 (0%)	0.2349
BPAR	4 (19.1%)	5 (23.8%)	0.7066
BK nephropathy	0 (0%)	1 (4.8%)	0.2349
Surgical complication	2 (9.5%)	1 (4.8%)	0.5455
Infection	2 (9.5%)	6 (28.6%)	0.1093
CMV antigenemia (≥10)	1 (4.8%)	3 (14.3%)	0.2832
DGF	0	0	–
NODAT	2 (9.5%)	1 (4.8%)	0.5455

BPAR, biopsy-proven acute rejection; CMV, cytomegalovirus; DGF, delayed graft function; EVR, everolimus; MMF, mycophenolate mofetil; NODAT, new-onset diabetes after transplantation; TIT, total ischemia time; WIT, warm ischemia time.

TABLE 3.**UPCR, triglyceride, LDL, WBC, and hemoglobin at 6 and 12 mo**

	Time	EVR (n = 21)			MMF (n = 21)			P
		n	Mean	SD	n	Mean	SD	
UPCR (g/g Cr)	Month 6	18	0.26	0.44	19	0.12	0.12	0.3690
	Month 12	17	0.25	0.32	17	0.15	0.15	0.2848
Triglyceride (mg/dL)	Before KT	21	110	59	21	172	140	0.1908
	Month 6	19	178	101	18	173	113	0.7382
	Month 12	17	142	77	16	134	76	0.7187
LDL (mg/dL)	Before KT	21	108	42	20	103	38	0.6955
	Month 6	19	128	33	18	110	27	0.0404
	Month 12	17	122	25	16	102	29	0.0376
WBC (/ μ L)	Before KT	21	6272	1767	21	6276	2072	0.5212
	Month 6	19	6145	5057	19	5527	2210	0.3891
	Month 12	17	6651	1986	17	6236	2282	0.4908
Hemoglobin (g/dL)	Before KT	21	11.2	1.5	21	11.6	1.8	0.3716
	Month 6	19	11.7	1.4	19	12.3	1.6	0.0959
	Month 12	17	12.2	1.4	17	12.5	2.1	0.1840

Cr, creatinine; EVR, everolimus; KT, kidney transplantation; LDL, low-density lipoprotein; MMF, mycophenolate mofetil; SD, standard deviation; UPCR, spot urine protein/creatinine ratio; WBC, white blood cell.

the EVR group sometimes seemed to be higher than those in the MMF group, which might have resulted in a comparable incidence of infection. Therefore, in patients with infection, CNI dose may be more important than the decision to administer EVR or MMF. It has also been reported that Tac concentration is associated with CMV replication and the development of CMV-specific cell-mediated responses.²⁷

Tac trough concentrations seemed to be higher in the EVR group than in the MMF group, and the severity of IF/TA at 12 months was significantly milder in the EVR group than in the MMF group. The prevention of interstitial renal fibrosis remains an unresolved problem after organ transplantation. CNI minimization is associated with a modest increase in renal function, but persistent damage is present on biopsies for the duration of CNI administration.²⁸ In contrast, the effect of serum Tac concentration on short-term IF/TA in allografts is controversial.^{29,30} A recent report suggested that low-concentration Tac exposure was independently associated with greater increase in chronicity score (ie, the sum of 4 basic “chronic” Banff qualifiers: chronic glomerular damage, IF, TA, and vascular intimal thickening).²⁹ However, another report suggested that higher concentrations of Tac might influence

the development of IF/TA.³⁰ In this study, ordered logistic regression models did not show a significant association ($P = 0.2321$) between the severity of IF/TA at 12 months and the mean Tac trough concentrations during each month throughout the 12-month follow-up period. Geissler and Schlitt have reported the potential beneficial effects of mTOR inhibitors on kidney fibrosis.³¹ Kidney fibrosis is mainly induced by the actions of transforming growth factor- β , a well-known primary mediator, which induces fibroblast proliferation and myofibroblast transition. It has been suggested that the Akt/mTOR complex 1 axis is activated by transforming growth factor- β through a PI3K/Akt/TSC2-dependent pathway.³² Additionally, the use of rapamycin (an mTOR inhibitor) to block mTOR complex 1 led to decreasing renal IF in an obstructive nephropathy rodent model through reductions in the numbers of interstitial fibroblasts and myofibroblasts.³³ In contrast, no significant reduction in fibrosis was observed after 1 year when patients were converted from CNIs to rapamycin at 12 weeks after KT.³⁴ This suggests that the negative effects of CNIs can occur early and cannot be prevented after several months. Our results support the hypothesis that EVR administration for de novo KT reduces CNI nephrotoxicity.

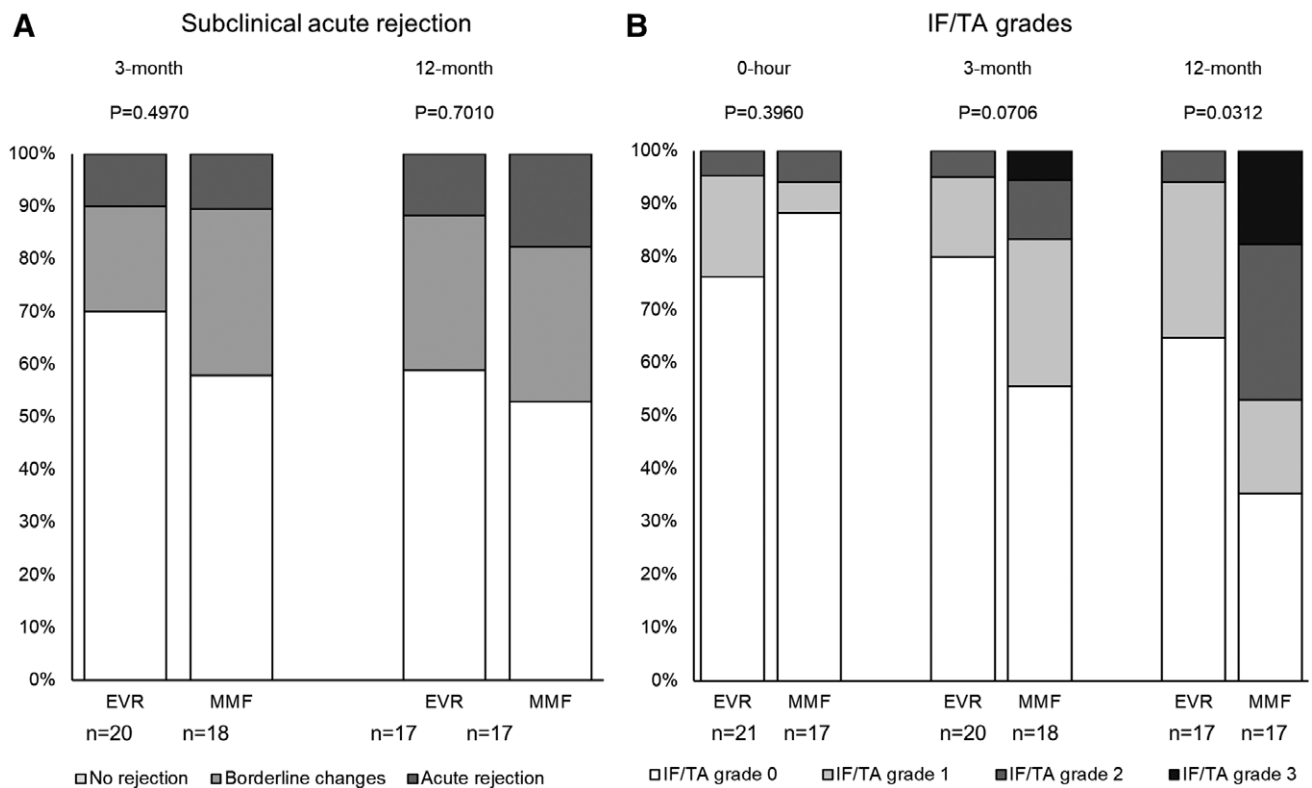


FIGURE 4. Rates of (A) acute rejection and (B) IF/TA without any specific etiology in the 3- and 12-month protocol biopsies. The incidence of borderline changes/acute rejection (Banff grade 1a or higher) and severity of IF/TA did not differ significantly between the 2 groups at 3 months. The severity of IF/TA was significantly milder in the EVR group than in the MMF group at 12 months ($P = 0.0312$). EVR, everolimus; IF, interstitial fibrosis; MMF, mycophenolate mofetil; TA, tubular atrophy.

The antiproliferative properties of mTOR inhibitors may interfere with the wound-healing process post transplantation; therefore, some clinicians are hesitant to incorporate mTOR inhibitors into immunosuppressive regimens immediately posttransplantation. Ueno et al also reported that patients treated with basiliximab and EVR had a 1.7-fold greater risk of clinical and subclinical adverse wound-healing events than patients treated with basiliximab and mycophenolate sodium.³⁵ However, another study showed comparable risks of adverse wound-healing events between EVR- and MMF-based regimens.¹⁴ Our study showed that there were no differences in surgical complications between the 2 groups. Specifically, 2 patients in the EVR group had surgical complications: 1 had ureteral stenosis requiring nephrostomy, while the other had lymphorrhea that caused ureteral compression requiring ureteral stenting. One patient in the MMF group had postoperative bleeding that required repeat laparotomy.

Although there were no significant differences between the 2 groups in spot urine protein/creatinine ratio, 2 patients discontinued EVR because of severe proteinuria; moreover, the mean spot urine protein/creatinine ratio tended to be higher in the EVR group than in the MMF group, which is similar to the results of other studies.^{14,36} Microalbuminuria predicts graft loss and all-cause mortality.³⁷ However, the impact of mTOR-inhibitor-induced proteinuria/microalbuminuria on graft outcome remains unclear. As reported previously,¹⁴ the low-density lipoprotein level was significantly higher in the EVR group than in the MMF group. There was no significant difference between the 2 groups in terms of the adverse effect of bone marrow suppression, as indicated by white blood cell counts and hemoglobin levels. The TRANSFORM

study showed no difference in anemia, but demonstrated that patients in the MMF group had a higher rate of leukopenia than those in the EVR and low-dose Tac group.¹⁴ One possible explanation of our results is that rituximab administration and CNI dose might be more potent factors for patients with leukopenia, compared with the administration of EVR or MMF. One patient in the EVR group was withdrawn because of leg edema, which is a specific adverse effect of EVR.¹⁴ Treatment discontinuation as a result of adverse effects was higher in the EVR group ($n = 4$) than in the MMF group ($n = 1$). However, in the MMF group, another 5 patients had dose reduction of MMF because of adverse effects, including recurrent infection ($n = 3$) and gastrointestinal symptoms ($n = 2$). Dose reduction was used because EVR had not been approved at that time and we did not have effective options for resolution without reducing the dose of MMF. The adverse effects appeared to be comparable between the 2 groups.

The limitations of this study should be noted. First, this was a retrospective observational study; despite the use of propensity scores, unmeasurable confounders may have affected our results. To match propensity scores as closely as possible, 111 of 153 transplantation patients were excluded from this study, thereby reducing the sample size and power. Moreover, the 2 groups did not undergo KT during the same period. Patients in the EVR group underwent KT more recently than those in the MMF group. Despite the consistently low immunological risk of all patients in this study, the recency of KT in the EVR group may have led to improvement relative to the MMF group. Therefore, there may have been a time period bias. Long-term interventional trials would be ideal, but these are costly and time-consuming. It is at least necessary to validate our results in a

separate, large observational cohort. Second, the sample size was small and the observational period was short. Third, we did not assess de novo DSA, which constitutes a potent risk factor for graft survival. However, Narumi et al reported that, at 10 years after KT, the mean fluorescence intensity of de novo DSA was lower in patients who underwent EVR-based therapy for KT than in patients who underwent standard MMF-based therapy.³⁸

In conclusion, this study suggests that renal function and safety of EVR and standard-dose Tac in recipients of de novo ABOi LDKT are comparable with those of MMF and standard-dose Tac. Furthermore, this study may be an effective means to introduce EVR protocols with low-dose Tac for ABOi KT in the future. Prospective investigations with a randomized controlled design, a large population, and a long study period are needed to confirm these findings.

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