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Effect of Everolimus with Low-Dose Tacrolimus on Development of New-Onset Diabetes After Transplantation and Allograft Function in Kidney Transplantation: A Multicenter, Open-Label, Randomized Trial

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Statistical Analysis C
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Background: This randomized controlled trial aimed to investigate the effect of everolimus (EVL) with low-dose tacrolimus (Tac) on the development of post-transplantation diabetes mellitus (PTDM) in kidney transplantation (KT).

Material/Methods: Seventy-seven kidney transplant patients from 4 transplant centers were included. Patients were randomized to the "EVL group" (n=38) and the "TAC group" (n=39). The target Tac trough level was 2 to 5 ng/mL in the EVL group and 5 to 10 ng/mL in the TAC group.

Results: The 1-year cumulative incidence of PTDM in all patients was 7.8%, and no difference was found between the 2 groups (*P*=0.0819). Insulin resistance measured with the homeostatic model assessment for insulin resistance showed a significant increase only in the TAC group (1.11 to 1.30, *P*=0.0492). Allograft rejection rate and estimated glomerular filtration rate (eGFR) follow-ups every 3 months were not significantly different between the 2 groups. However, the EVL group showed a significant increase in the mean eGFR at 9 months and 12 months after KT compared to the baseline value (*P*=0.0242 and 0.0491, respectively).
The EVL group showed lower insulin resistance and higher allograft function in comparison to the TAC group.

Conclusions: EVL-based immunosuppressive therapy with lower Tac exposure could be a safer alternative for maintenance treatment.

MeSH Keywords: **Diabetes Mellitus • Graft Rejection • Kidney Transplantation**

Abbreviations: **BKVN** – BKV nephropathy; **BPAR** – biopsy-proven acute rejection; **CsA** – Cyclosporine A; **eGFR** – estimated glomerular filtration rate; **EVL** – everolimus; **HLA** – human leukocyte antigen; **HOMA-IR** – homeostatic model assessment for insulin resistance; **ITT** – intention-to-treat; **KT** – kidney transplantation; **MAU** – microalbuminuria; **MPA** – mycophenolic acid; **mTOR** – mammalian target of rapamycin; **OGTT** – oral glucose tolerance test; **PP** – per protocol; **PP2** – postprandial glucose at 2 h; **PRA** – panel reactive antibody; **PTDM** – post-transplantation diabetes mellitus; **SAE** – serious adverse events; **SD** – standard deviation; **Tac** – tacrolimus

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Background

Current immune suppression for kidney transplant (KT) recipients consists of tacrolimus (Tac), mycophenolic acid (MPA), and steroids. The introduction of a Tac-based triple therapy in the last 2 decades has significantly reduced the incidence of early acute rejection, and has increased the 1-year allograft survival [1,2]. Tac reduced the incidence of acute rejection by 17.2% compared to cyclosporine A (CsA), and the current 1-year graft survival rate after transplantation exceeds 90% [3,4].

However, Tac is known to destroy insulin-secreting beta cells, resulting in post-transplantation diabetes mellitus (PTDM). PTDM refers to diabetes diagnosed after organ transplantation. The diagnostic criteria follow the type 2 diabetes mellitus (DM) guideline presented by the American Diabetes Association (ADA) and the World Health Organization (WHO). However, it is not appropriate to diagnose PTDM with only HbA1c due to the characteristics of KT recipients, who often have impaired renal function [5]. Risk factors for PTDM development include a family history of DM, old age, obesity, and a history of impaired fasting glucose before transplantation [5].

PTDM is one of the major risk factors for patient mortality and graft loss in KT recipients [6]. After transplantation, patients with PTDM have been reported to have a 1.63-fold higher risk of graft failure and a 1.87 times higher risk of mortality [7]. PTDM is a major risk factor for cardiovascular complications [8]. In a Norwegian study of 201 KT recipients followed for 8 years, PTDM was identified as an independent predictor of major cardiac events, and increased the risk by 3.6-fold [8]. It is also well known that the incidence of PTDM is associated with reduced allograft survival [9], more infectious complications [6], and increased medical costs [10].

The prevalence of PTDM varies due to the use of inconsistent diagnostic criteria, but is known to be approximately 10–15% in the first year after KT [11]. The prevalence of PTDM is nearly 20% in KT recipients treated with Tac, which peaks around 6 months after KT [2,12,13]. Tac increases the risk of PTDM and impaired fasting glucose at 6 months after KT compared to CsA (PTDM 17% *vs.* 9% and impaired fasting glucose 34% *vs.* 26%) [14].

Several immunosuppressants have been expected to reduce the diabetogenic nature of Tac, including mammalian target of rapamycin (mTOR) inhibitors such as sirolimus and everolimus (EVL) [15–17]. In a large-scale study conducted using data from the United States Renal Data System [18], sirolimus was identified as an independent risk factor for PTDM. About 36% more PTDM occurred in patients treated with sirolimus and antimetabolite such as mycophenolate mofetil, compared to those treated with CsA and antimetabolite. When sirolimus and CsA or Tac were used in combination, 61% and 66%, respectively, more PTDM occurred than with the CsA regimen.

In a study evaluating the effects of EVL with low-dose CsA and steroids in *de novo* KT patients [19], 237 patients with EVL 1.5 mg and 256 patients with EVL 3.0 mg were analyzed. The incidence of PTDM at 6 months after KT was 4% in the EVL 1.5 mg group and 5% in the EVL 3.0 mg group. This was lower than the 9% of CsA-treated KT recipients or 17% of Tac-treated KT recipients in a previous study [14]. However, there have been no extensive studies on whether a reduction of Tac dose with the addition of EVL could prevent the development of PTDM without deteriorating graft function. In this study, we investigated whether the addition of low-dose EVL with low Tac exposure could prevent the development of PTDM.

Material and Methods

Study design, eligibility, and patient allocation

This was a multicenter, open-label, randomized, parallel study to evaluate the prevention of PTDM following EVL therapy with co-administration of low-dose Tac. The definition of PTDM conformed to the definition of the American Diabetes Association [20]. This study (NCT02036554) was conducted according to the ethical principles of the Declaration of Helsinki and the International Conference on Harmonization-Good Clinical Practice. All ethics committees and regulatory authorities approved the protocol (CRAD001AKR11T).

Patients aged ≥20 years who signed informed consent received a screening test and then were allocated with randomization into 2 groups: an EVL group and a TAC group. We added EVL 6 months after KT to avoid the influence of instability in the early KT period. Patients in the TAC group were maintained on Tac with a trough level of 5 to 10 ng/mL and MPA at the same dosage as before enrollment (usually 1500 mg for CellCept® (Myrept®), 1080 mg for Myfortic®). Azathioprine or mizoribine instead of MPA was also allowed. The Tac trough level was assessed every 3 months. Patients in the EVL group received a reduced Tac dosage to reach a trough level of 2 to 5 ng/mL, and received an additional EVL with a starting dose of 0.75 mg twice a day and then increased EVL dosage to reach a trough level of 3 to 8 ng/mL. The Tac trough level was assessed every 3 months just like that of the TAC group, and the trough level of EVL was assessed at 2 and 4 weeks and then every 3 months. The drug levels were measured at each center using patient whole blood (**Figure 1**).

Ninety-two KT recipients were enrolled from 4 transplant centers. Inclusion/exclusion criteria are shown in **Supplemenatry Table 1.**

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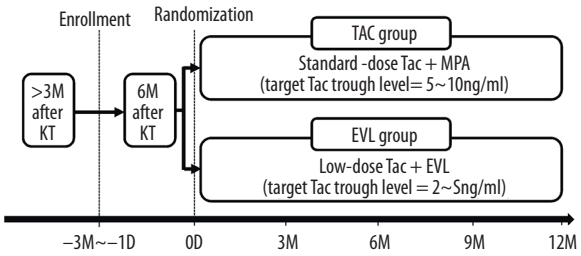

Figure 1. Overview of study protocol. The KT recipients who signed informed consents were allocated with randomization into 2 groups: "EVL group" or "TAC group," at 6 months after KT. KT – kidney transplantation; Tac – tacrolimus; EVL – everolimus; D – day; M – month.

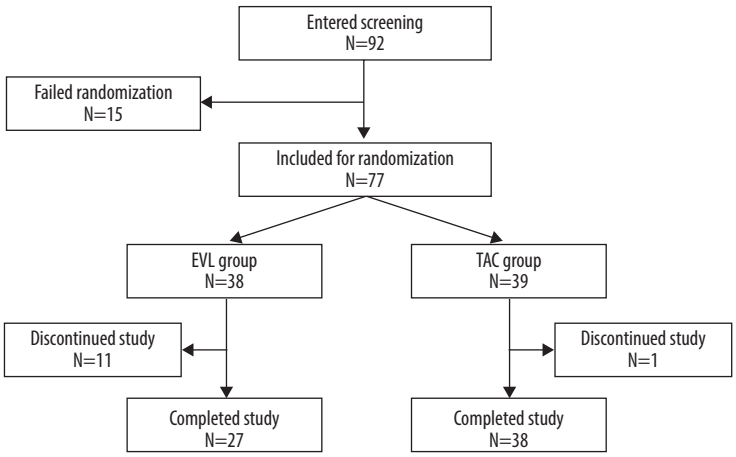

Figure 2. Allocation of the patients.

The primary outcome was incidence of PTDM at 12 months after the study allocation. The definition of PTDM was diabetes mellitus diagnosed at any time after transplantation according to the American Diabetes Association (**Supplemenatry Table 2**). Secondary outcomes were the change of homeostatic model assessment for insulin resistance (HOMA-IR) and HOMA-beta, oral glucose tolerance test (OGTT), prescription rate of anti-diabetic medication or insulin, allograft function assessed by allograft survival, patient survival, estimated glomerular filtration rate (eGFR) measured using the modification of diet and renal disease (MDRD)-equation, proportion of patients with significant proteinuria and microalbuminuria (MAU), incidence of biopsy-proven acute rejection (BPAR), all-cause hospitalization, and incidence of BK virus infection nephropathy (BKVN) at 18 months after the KT. Acute rejection included both acute T cell-mediated and acute antibody-mediated rejection, which were diagnosed by the pathologist at each center based on the Banff classification scheme [21].

Statistical analysis

The cumulative incidence of PTDM after 12 months of allocation is presented as number and percentage and compared via relative risk with a 95% confidence interval. Continuous variables with a normal distribution are presented as the mean±standard deviation (SD), and those with a non-normal distribution are presented as the median with interquartile range. The *t* test was used for analysis of continuous variables with a normal distribution, and the Mann-Whitney test was used for those with a non-normal distribution. The average categorical variables are presented as counts and percentages. The average amount of change of each parameter was assessed with the independent *t* test or Wilcoxon's rank test. Categorical variables are presented as counts and percentages, and either the chi-square test or Fisher's exact test was used for analysis of categorical variables. Univariate and multivariate Cox regression analyses were performed to investigate the independent risk factors affecting allograft survival. Allograft and patient survival were analyzed using the Kaplan-Meier method and log-rank test. A *P*-value <0.05 was considered statistically significant. We performed both intention-to-treat (ITT) and per protocol (PP) analyses. The ITT population was defined as the population of patients who received at least 1 dose of study drug. The PP population consisted of all ITT patients without any major deviations from the study protocol. The secondary outcomes were analyzed based on both the ITT and PP population. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., NC, Cary, USA).

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Table 1. Comparison of baseline characteristics (ITT population).

	EVL group (n=38)	TAC group (n=39)	P-value
Age	44.71±11.63	46.49±11.78	0.507
Male gender	19 (50.00)	20 (51.28)	0.914
Family history of diabetes	3 (7.89)	2 (5.13)	0.6748
Primary renal disease			0.2397
Hypertension	7 (18.42)	10 (25.64)	
Nephrosclerosis	1 (2.63)	1 (2.56)	
IgA nephropathy	11 (28.95)	7 (17.95)	
Other glomerulonephritis	11 (28.95)	4 (10.26)	
Polycystic kidney disease	2 (5.26)	3 (7.69)	
Unknown	5 (13.16)	11 (28.21)	
Other	1 (2.63)	2 (5.13)	
HLA-mismatch			
A (0, 1, 2)	9 (23.68), 22 (57.89), 7 (18.42)	6 (15.38), 22 (56.41),10 (25.64)*	0.5712
B (0, 1, 2)	7 (18.42), 16 (42.11), 15 (39.47)	4 (10.26), 14 (35.90), 20 (51.28)*	0.4339
DR (0, 1, 2)	5 (13.16), 20 (52.63), 13 (34.21)	7 (17.95), 18 (46.15), 13 (33.33)*	0.8627
Pre-transplant dialysis			0.3155
Hemodialysis, n (%)	23 (60.53)	27 (69.23)	
Peritoneal dialysis, n (%)	10 (26.32)	5 (12.82)	
PRA (%)	5.30±12.05	4.44±12.26	0.6069
Donor type			0.8594
Living related, n (%)	15 (39.47)	15 (38.46)	
Living-unrelated, n (%)	6 (15.79)	8 (20.51)	

* No results in one patient. Discrete variables are presented with n (%), and continuous variables with normal distributions are presented with mean±S.D. HLA – human leukocyte antigen; PRA – panel-reactive antibody.

Results

Patient disposition and baseline characteristics

Fifteen patients failed randomization during the screening period, and a total of 77 patients were randomized to either the EVL group (n=38) or the TAC group (n=39). Twelve patients dropped out during the follow-up period (**Figure 2**).

The mean age of the overall patient group was 45.7±11.6 years, and 39 patients (50.6%) were male. The proportion of patients with a family history of diabetes was 7.89% in the EVL group and 5.13% in the TAC group (*P*=0.6748). The most common primary renal disease was primary glomerulonephritis (42.9%), followed by hypertension (22.1%). There was no significant difference in immunologic profiles, including human leukocyte antigen (HLA)-mismatch number, percent PRA, and donor type, between the 2 groups (**Table 1**).

Incidence of PTDM and anti-diabetic prescription rate

The one-year cumulative PTDM incidence rate was not significantly different between the EVL and TAC groups (EVL *vs.* TAC; 13.16% *vs.* 2.56%, p=0.0819 in the ITT population; 11.11% *vs.* 2.63%, p=0.1725 in the PP population) (**Table 2**). Only 1 patient in the TAC group needed insulin at 12 months, and there was no significant difference between the 2 groups (*P*=1.0000 in both the ITT and PP populations).

Index for insulin resistance and change of OGTT

At baseline, the average HOMA-IR and HOMA-beta values were similar between the EVL and TAC groups. There was no significant difference from baseline to 1-year HOMA-IR between the 2 groups in either the ITT and PP population (EVL *vs.* TAC; 0.03 *vs.* 0.18, p=0.2688 in the ITT population; 0.13 *vs.* 0.19, *P*=0.3213 in the PP population). In the in-group analysis, however, the TAC group showed a significant increase in HOMA-IR (1.11 to 1.30, *P*=0.0492) while the EVL group did not (1.13 to

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Table 2. Comparison of development of NODAT (ITT population).

	EVL group (n=38)	TAC group (n=39)	P-value*
NODAT	5 (13.16)	1 (2.56)	0.0819
Insulin prescription	0 (0.00)	1 (2.56)	1.0000
Anti-diabetic medication	0 (0.00)	0 (0.00)	NA

Discrete variables are presented with n (%).

Table 3. Comparison of homeostatic model assessment for insulin resistance.

ITT population	EVL group N=38	TAC group N=39	P-value*
HOMA-IR			
Baseline	1.26±0.89	1.12±0.39	0.6176
12 months	1.29±0.91	1.30±0.56	0.5376
Mean change from baseline	0.03±0.99	0.18±0.57	0.2688
p-value**	0.9238	0.0532	
HOMA-beta			
Baseline	94.29±42.53	85.64±27.58	0.5309
12 months	97.46±44.74	92.45±31.16	0.9756
Mean change from baseline	3.17±24.31	6.81±26.02	0.3228
p-value**	0.5915	0.1105	
PP population	N=27	N=38	
HOMA-IR			
Baseline	1.13±0.40	1.11±0.40	0.8593
12 months	1.26±0.93	1.30±0.56	0.3756
Mean change from baseline	0.13±1.05	0.19±0.57	0.3213
p-value**	0.7792	0.0492	
HOMA-beta			
Baseline	89.96±30.85	86.11±27.80	0.6006
12 months	90.82±28.23	93.47±30.91	0.7244
Mean change from baseline	0.86±26.05	7.37±26.13	0.3253
p-value**	0.7792	0.0905	

* Wilcoxon's rank sum test; ** Wilcoxon's signed rank test. HOMA-IR=fasting insulin (uU/mL)×fasting glucose (mg/dL)/405. HOMA-%B=(20×fasting insulin (uU/mL))/(fasting glucose (mg/dL)/18–3.5). Discrete variables are presented with n (%), and continuous variables with normal distributions are presented with mean±S.D. HOMA – homeostatic model assessment; IR – insulin resistance.

1.26, *P*=0.7792) in the PP population. The change in HOMA-beta showed no significant difference in inter- and intra-group analyses (**Table 3**).

At baseline, the average values of fasting glucose and post-prandial glucose at 2 h (PP2) were not significantly different between the EVL and the TAC groups. In addition, the difference of fasting and PP2 was also not different between the groups in the ITT or PP population (**Supplementary Table 3**).

Estimated glomerular filtration rate

In the ITT population, eGFR showed no significant difference between the 2 groups and did not differ according

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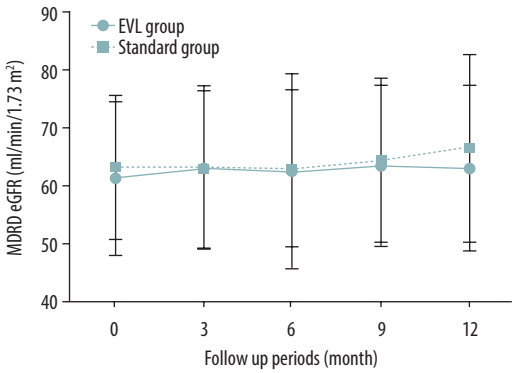

Figure 3. Change of allograft function in ITT population. The figure shows the course of the mean MDRD eGFR from the baseline to 12 months in the ITT population. Vertical error bars indicate the respective standard deviations. MDRD – modified diet and renal disease; eGFR – estimated glomerular filtration rate; ITT – intention-to-treat.

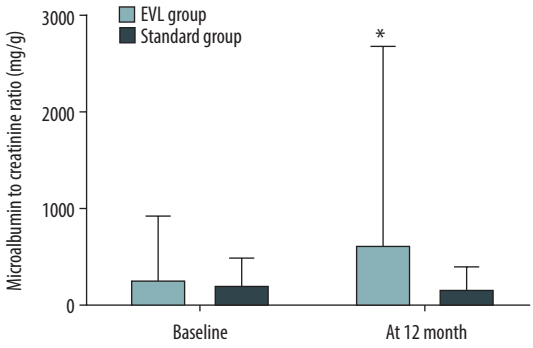

Figure 4. Change of microalbuminuria. The figure shows the difference in MAU from the baseline to 12 months in the EVL (black column) and standard group (white column). Vertical error bars indicate the respective standard deviations. MAU – microalbuminuria; EVL – everolimus * $P<0.05$ compared to baseline.

to post-transplant period from baseline to 12 months. In the PP population, no average eGFR values at baseline, 3, 9, and 12 months were significantly different between the 2 groups. However, eGFR values at 9 months (64.94 mL/min/1.73 m^2) and 12 months (64.90 mL/min/1.73 m^2) were significantly higher than the value at baseline in the EVL group (60.84 mL/min/1.73 m^2) (9 months, p=0.0242; 12 months, p=0.0491) (**Figure 3, Table 4**).

Tac and EVL trough serum concentrations

The mean serum trough concentration of Tac in the EVL group was 4.43 ng/mL and 5.68 ng/mL in the TAC group, which was significantly higher in the TAC group ($P<0.0001$). The EVL serum trough concentration was measured only in the EVL group, with an average of 3.40 ng/mL and a standard deviation of 1.07.

Allograft rejection and survival rate

One case of BPAR was detected in the EVL group in the ITT population, while no rejections were identified in either group in the PP population. Allograft loss did not occur during the study period (**Table 4**).

Microalbuminuria and overt proteinuria

The average MAU values at baseline and 12 months were not different between the EVL and TAC groups. However, the EVL group showed a significant increase in MAU at 12 months after KT (225.47 to 584.04 mg/g Cr, $P<0.00001$), and the degree of difference from baseline to 12 months was also different between the 2 groups (EVL *vs.* TAC, 358.58 *vs.* –28.47, $P=0.0035$) in the ITT population. Only 1 patient in the EVL group showed overt proteinuria greater than 1 g/gCr at 12 months, and there was no difference in the inter- or intra-group analyses in the ITT population. The PP population showed similar results to the ITT population (**Figure 4, Table 4**).

Compliance and safety issues

Adherence to drug was 99.9±0.42 in the EVL group and 99.97±0.13 in the TAC group during the total study period ($P=0.5520$). Among the 77 subjects in the ITT population, at least 1 adverse event occurred in the 37 patients of the EVL group and the 38 patients of the TAC group ($P=1.0000$), and serious adverse events (SAE) occurred in 9 patients of the EVL group and 8 patients of the TAC group ($P=0.7373$). Three cases of SAE in the EVL group were suspected to have allograft rejection, and these were excluded from the PP population. All-cause hospitalization was similar between the 2 groups. No deaths were reported during the study period (**Table 5**).

Discussion

This study was designed to investigate the effect of EVL treatment with a reduced exposure to Tac on the development of PTDM and allograft function. The primary endpoint, PTDM prevalence, was similar in patients who took EVL with low-dose Tac (Tac at levels from 2 to 5 ng/mL) and patients who were maintained on a standard dose of Tac at levels from 5 to 10 ng/mL. Allograft function and the incidence of BPAR were also not different between the 2 groups. These findings suggest that the addition of EVL with a low dose of Tac is an acceptable strategy for maintenance immunosuppression in KT.

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Table 4. Comparison of the allograft function.

	EVL group	TAC group	P-value*
ITT population	**N=38**	**N=39**	
MDRD eGFR (ml/min/1.73 m²)			
Baseline	61.39±13.32	62.98±12.46	0.6209
12 months	63.12±14.36	66.56±16.04	0.6103
Mean change from baseline	1.73±10.61	3.58±13.46	0.8905
p-value**	0.3216	0.1878	
Urinary ACR (mg/g)			
Baseline	225.47±688.86	172.98±307.80	0.3633
12 months	584.04±2087.43	141.75±245.63	0.4789
Mean change from baseline	358.58±1814.96	–28.47±241.46	0.0035
p-value**	<0.0001	0.4399	
Overt proteinuria			
Baseline	0 (0.00)	0 (0.00)	NA
12 months	1 (0.38)	0 (0.00)	0.4935
BPAR	1 (2.63)	0 (0.00)	0.3110
Allograft loss	0 (0.00)	0 (0.00)	NA
PP population	**N=27**	**N=38**	
MDRD eGFR (ml/min/1.73 m²)			
Baseline	60.84±12.84	62.85±12.60	0.5667
12 months	64.90±13.02	66.89±16.12	0.9416
Mean change from baseline	4.06±10.22	4.04±13.32	0.5802
p-value**	0.0491	0.1094	
Urinary ACR (mg/g)			
Baseline	145.56±297.15	176.46±311.28	0.5589
12 months	649.40±2371.10	144.32±248.39	0.1898
Mean change from baseline	503.84±2147.56	–29.23±244.74	0.0016
p-value**	<0.0001	0.4691	
Overt proteinuria			
Baseline	0 (0.00)	0 (0.00)	NA
12 months	1 (3.70)	0 (0.00)	0.4242

* Wilcoxon's rank sum test; ** Wilcoxon's signed rank test. BPAR – biopsy-proven acute rejection, cumulative rate for 12 months. Allograft loss, cumulative rate for 12 months. Discrete variables are presented with n (%), and continuous variables with normal distributions are presented with mean±S.D. ACR – albumin-to creatinine ratio.

The Kidney Disease: Improving Global Outcomes guidelines suggest that standard dose may be defined as it is recommended by the producer; the dose achieving 5–15 ng/mL of TAC trough levels. They also suggest using the lowest planned dose by 2-4 months after transplantation in stable KT recipients to minimize toxicity, with a low quality of evidence [22]. The results of the Symphony study showed that low-dose tacrolimus (target Tac trough level 3-7 ng/mL) may be advantageous for renal function, allograft survival, and acute rejection rates [23]. It has been reported that patients who maintained the target tac trough level of 3-7 ng/mL at 6-12 months of KT developed fewer BPARs than those maintained at 2–5 ng/mL [24]. In the present study, the target Tac trough level of the EVL group was set to 2–5 ng/mL. We expected reduced

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Table 5. Serious adverse events in ITT population.

	EVL group (n=38)	TAC group (n=39)
Total events, n (%)	13 (100.00)	10 (100.00)
Gastrointestinal disorder		
Diarrhea	1 (7.69)	0 (0.00)
Immune system disorder		
Suspicious allograft rejection	3 (23.08)	0 (0.00)
Infection		
Gastroenteritis	1 (7.69)	0 (0.00)
Herpes zoster	0 (0.00)	1 (10.00)
Influenza	1 (7.69)	0 (0.00)
Urinary tract infection	6 (46.15)	4 (40.00)
Varicella	0 (0.00)	1 (10.00)
Viral meningitis	0 (0.00)	1 (10.00)
Neoplasm		
Gastric adenoma	0 (0.00)	1 (10.00)
Urinary tract disorder		
Ureterolithiasis	0 (0.00)	1 (10.00)
Hematologic disorder		
Bleeding time prolongation	1 (7.69)	0 (0.00)
Reproductive system and breast disorder		
Fibrocystic breast disease	0 (0.00)	1 (10.00)

Discrete variables are presented with n (%).

BPAR risk through the combination of EVL and Tac, and wanted to minimize Tac exposure.

The effect of EVL with low-dose Tac on PTDM development has been controversial. Some studies reported that the use of mTOR inhibitors instead of Tac reduced the prevalence of PTDM [17,19,25,26]. On the other hand, mTOR inhibitors, including EVL, are known diabetogenic drugs [18,27–29], and conversion from calcineurin inhibitors (CNI) to mTOR inhibitor even showed a trend toward increased risk of PTDM in KT recipients [30]. These discrepancies in results may be related to the timing of conversion and the dose of mTOR inhibitors and Tac.

In the present study, we added EVL 6 months after KT to avoid the influence of instability in the early KT period. The use of EVL in the early post-operative period was associated with a high incidence of wound healing adverse events [31]. In the early conversion of CNI to EVL study [32], the incidence of BPAR was 13.0% in the EVL group and 4.8% in the CsA group (P=0.015). Further, we tried to minimize exposure to Tac to avoid diabetogenicity [33] and nephrotoxicity [34]. The incidence of hyperglycemia was high due to the use of high-dose immunosuppressants in the early stages of transplantation, but not all patients progressed to PTDM. In an early basal insulin therapy study [35], the incidence of PTDM was significantly lowered at 6 months after KT through early basal insulin therapy.

There was no statistically significant difference in HOMA-IR and HOMA-beta between baseline and 12 months in both groups in the ITT population, but HOMA-IR tended to increase in the TAC group. In addition, in the PP population, a significant increase in HOMA-IR was observed only in the TAC group. These findings can be interpreted as having relatively little effect on insulin resistance of the EVL group compared to the TAC group. However, this encouraging result did not lead to a reduction in PTDM development in the EVL group. There were 6 cases of PTDM that developed during the 1-year follow-up, 5 cases that occurred at 3 months after allocation in the EVL group, and 1 case at 12 months after allocation in the TAC group. In the safety analysis of a previous study [36], early Tac reduction plus EVL at 3 months after KT did not show a significant difference in PTDM prevalence compared to the conventional Tac dose treatment. The present study was primarily designed to evaluate the effect of EVL with low-dose Tac treatment in PTDM development and showed a similar result in the difference of PTDM prevalence. However, the cumulative incidence

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of PTDM seems to be low in this study (7.8%) because KT recipients who already had diabetes before 6 months after KT were excluded. PTDM has been reported to show a peak incidence of approximately 15 to 20% within the first year after transplantation, especially in the initial 6 months after KT [10,37,38]. This small number of PTDM events in this study may have reduced its statistical power.

In view of renal function preservation, EVL showed favorable results in that eGFR at 9 and 12 months were improved compared to baseline in the EVL group, with no improvement in the standard-dose TAC group. Tac-induced nephrotoxicity led to a demand for treatment that was less nephrotoxic and not inferior to Tac. Moreover, the mTOR inhibitor was identified as an alternative immunosuppressant due to its renoprotective effect in its early periods. Several studies have reported that EVL with reduced CNIs improves renal function [36,39–43].

However, a key hurdle for any alternative drug used for the reduction of Tac exposure is associated with the relatively high risk of acute rejection in current medical settings. Several reports have warned of the possible increase of acute rejection risk with EVL plus Tac withdrawal or low-dose Tac versus the standard Tac regimen [44,45]. In the present study, 3 cases of allograft rejection were reported in the EVL group, and all were excluded from the PP population due to drug discontinuation based on the judgement of the clinician. Although the total number of allograft rejections was numerically higher in the EVL group (n=4) relative to the TAC group (n=0), the difference was not statistically significant, and the relationship of rejection with the use of EVL and BPAR was not certain in 3 of these 4 cases.

The safety profiles of the 2 groups were generally comparable. The incidence of BKVN was similar between the groups (P=0.494). The MAU rate was higher in the EVL group compared to the TAC group, which agrees with previous studies [41,46,47]. Lipid profiles, including total cholesterol and triglyceride, showed higher levels in the EVL group, but these levels did not lead to drug discontinuation. Other laboratory data showed anticipated results for each drug, and new safety concerns were not detected (**Supplementary Table 4**).

There are some limitations to this study. First, the small number of participants with a small number of events may have reduced the power of the study to identify statistical differences. We enrolled patients in the first 6 months after KT to avoid bias resulting from possible instability in the early post-transplant period, like infection or post-operative complications. Therefore, all patients with PTDM events that occurred before 6 months after KT were excluded from the study. Second, the follow-up period of the study was 1 year, which is too short to reveal the long-term allograft outcomes and PTDM prevalence with EVL therapy.

Conclusions

EVL-based immunosuppressive therapy with low-dose Tac could be a safer alternative for maintenance treatment. The EVL group showed lower insulin resistance and higher allograft function in comparison to the TAC group, which suggests that we can consider this regimen for patients with a high risk of PTDM. Further long-term follow-up research with a large cohort is necessary to determine the effect of EVL-facilitated Tac reduction on PTDM and allograft function.

Conflicts of interest

None.

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Supplementary Data

Supplementary Table 1. Inclusion and exclusion criteria.

1) Inclusion criteria
a) Age ≥20
b) At least 3 months from KT
c) Patients who have been on Tac±anti-proliferating agents+steroid for at least 3 months
d) MDRD eGFR ≥50 ml/min/1.73 m^2 or serum creatinine <2.0 mg/dL
e) The change of serum creatinine less than 30%
f) Spot urine protein/creatinine ratio > 0.3
g) Patients who do not meet the criteria of PTDM at baseline (6 months from KT)
h) Patients who agree to participate in this study
2) Exclusion criteria
a) Combined non-renal transplantation
b) Re-transplantation
c) ABO incompatible transplantation
d) highly sensitized patients before transplantation (pre-transplant PRA titer >50% (either class I or class II), or pre-transplant T cell cytotoxicity crossmatch (+))
e) HLA-identical living related donor
f) Diabetes mellitus before transplantation or PTDM at baseline
g) Acute rejection within within 3 months
h) Hypersensitivity to everolimus
i) Gastrointestinal malabsorptive condition due to gastrectomy or diabetic gastroparesis
j) HIV, HBsAg or HCV Ab (+)
k) AST, ALT or total bilirubin >3 fold than normal value
l) Absolute neutrophil count <1.5×10^9/L or WBC 2.5×10^9/L or platelet <75×10^9/L
m) Clinically significant infection within 4 weeks at 6 months from KT
n) Active malignancy, skin cancer, post-transplant lymphoproliferative disease
o) Pregnancy, lactation, plan for another pregnancy within 12 months
p) Major surgery within 4 weeks at 6 months from KT
q) Another clinical study

MDRD – modification of diet in renal disease; eGFR – estimated glomerular filtration rate; PTDM – post-transplantation diabetes mellitus; PRA – panel-reactive antibody.

Supplementary Table 2. The definition of PTDM.

Diabetes mellitus after transplantation may be diagnosed at any time after transplantation by any of the following
a) Symptoms of diabetes plus random plasma glucose ≥200 mg/dL (11.1 mmol/L). Symptoms include polyuria, polydipsia, and unexplained weight loss.
b) Fasting plasma glucose ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least eight hours. An abnormal fasting blood glucose should be confirmed on another day.
c) Two-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (OGTT). The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.
d) a HbA1c ≥6.5 percent.

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Supplementary Table 3. Comparison of the oral glucose tolerance test.

	EVL group (n=38)	TAC group (n=39)	P-value
OGTT (Fasting) (mg/dL)			
Baseline	97.47±9.48	98.87±10.11	0.5333
12 months	96.79±13.44	100.18±15.53	0.2493
Mean change from baseline	–0.68±11.26	1.31±11.09	0.6057
p-value	0.3248	0.9693	
OGTT (PP2) (mg/dL)			
Baseline	121.24±30.12	117.00±32.71	0.4538
12 months	117.24±27.49	117.46±38.21	0.5242
Mean change from baseline	–4.00±24.14	0.46±35.28	0.5185
p-value	0.3138	0.9353	

OGTT – oral glucose tolerance test; PP2 – 2 hours post-prandial.

Supplementary Table 4. Comparison of lipid profiles.

	EVL group (n=38)	TAC group (n=39)	P-value
Total cholesterol (mg/dL)			
Baseline	169.00±36.68	171.62±31.14	0.4120
12 months	185.84±42.45	169.13±27.17	0.0444
Mean change from baseline	16.84±29.75	–2.49±25.50	0.0056
p-value	0.00001	0.5460	
Triglyceride (mg/dL)			
Baseline	118.82±55.85	119.10±76.62	0.8785
12 months	145.87±75.78	115.59±95.08	0.0081
Mean change from baseline	27.05±79.52	–3.51±48.47	0.0249
p-value	0.0393	0.2824	
HDL-cholesterol (mg/dL)			
Baseline	58.87±16.20	58.28±16.07	0.9675
12 months	63.18±16.39	60.15±15.14	0.4786
Mean change from baseline	4.32±10.23	1.87±8.27	0.2520
p-value	0.0133	0.1656	
LDL-cholesterol (mg/dL)			
Baseline	89.08±35.58	91.41±30.17	0.5786
12 months	100.58±37.26	89.03±29.39	0.2331
Mean change from baseline	11.50±27.78	–2.38±20.29	0.0215
p-value	0.0030	0.9509	

Continuous variables with normal distributions are presented with mean±S.D. HDL – high-density lipoproteins; LDL – low-density lipoproteins

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