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ORIGINAL ARTICLE

Pilot conversion trial from mycophenolic acid to everolimus in ABO-incompatible kidney-transplant recipients with BK viruria and/or viremia

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Summary

Immunosuppression using everolimus (EVR) plus low-dose tacrolimus (Tac) is commonly used in organ transplantation. EVR has potential antiviral effects. Herein, the long-term outcomes and impacts of Tac-EVR on the BK virus are reported in ABO-incompatible kidney-transplant recipients. The initial immunosuppressive regimen combined steroids, Tac, and mycophenolic acid (MPA). At a median of 141 (34–529) days post-transplantation, seven stable ABO-incompatible kidney-transplant recipients were converted from MPA to EVR because of active BK replication, and compared with a reference group of fourteen ABO-incompatible patients receiving classical Tac plus MPA. At 1 month before conversion, at 1, 3 months after, and at last follow-up, clinical and biological parameters were monitored. The median time from conversion to the last follow-up was 784 (398-866) days. Conversion to EVR caused no change to rejection episodes or immunological status (isoagglutinin titers, anti-HLA antibodies). At last follow-up, median eGFR was similar in the Tac-MPA versus Tac-EVR group (40 [range: 14–56] vs. 54.5 ml/min/1.73 m^2 [range: 0–128], $P = 0.07$). The major adverse event was dyslipidemia. Interestingly, conversion from MPA to EVR decreased BK viral load in five patients. ABO-incompatible kidney-transplant recipients with an active BK virus infection may benefit from conversion to EVR.

Introduction

Because of the shortage of organ donors, ABO-incompatible (ABO-i) kidney transplantation has been developed with excellent outcomes. In Japan, for instance, 5- and 10-year graft-survival rates have reached 95% and 90%, respectively, which are identical to or even better than outcomes for ABO-compatible kidney transplantations [1]. However, overcoming blood-group barriers requires immunosuppressive protocols to reduce and maintain anti-blood-group antibodies, that is, isoagglutinins at safe levels. However, desensitization programs associated with induction and maintenance therapies expose patients to elevated risks of infection [2]. An optimal combination of immunosuppressive drugs remains to be defined.

The calcineurin inhibitor (CNI) tacrolimus (Tac) is included within most pharmacologic protocols despite its well-known dose-dependent side effects (especially

long-term nephrotoxicity and cardiovascular events). Tac is most commonly associated with mycophenolate acid (MPA), which inhibits the proliferation of B and T lymphocytes. MPA-related adverse events (e.g., digestive disorders, cytopenia, viral reactivations) may lead to treatment being discontinued. An alternative to MPA is the use of inhibitor of the mammalian target of rapamycin (mTOR-I): everolimus (EVR) and sirolimus, which also inhibits antibody production in preclinical studies [3,4] and reduces allograft rejection [5].

A new approach consists of combining EVR with low-dose Tac. This appears promising for several reasons [6]. Firstly, Tac and EVR are structurally similar; they bind to FK506-binding proteins to form immunosuppressive complexes [7] and exert synergistic effects. Secondly, the lack of a pharmacokinetic interaction between Tac and EVR allows their combination without requiring dose adjustment. Thirdly, this combination is of great interest regarding viral infections, especially human polyomavirus BK (BKV). BKV viruria can lead to BKV nephropathy (BKVN), which is associated with poor graft survival [8]. BKV viruria is present in approximately 30% of renal-transplant recipients [9]. Not surprisingly, ABO-i kidney recipients are at high risk for BKVN [2]; this risk is even higher than for HLA-incompatible kidney recipients [10].

A significant risk factor for BKV viremia and BKVN is a standard-dose Tac regimen. For instance, conversion to an mTOR-I-based regimen is associated with a lower incidence of BK viremia in low-risk kidney-transplant recipients [11]: this is why the use of EVR to minimize Tac dosage can theoretically reduce the risk of BKV. Furthermore, EVR *per se* has recently been shown to decrease the risk of BKV viremia in *de novo* renal ABO-compatible transplant recipients compared with MPA [12]. In addition, recent studies indicate that immunosuppressive regimens that include an mTor inhibitor as well as minimizing Tac could preserve renal function better than standard-dose Tac [13,14] given to ABO-compatible kidney-transplant recipients.

In ABO-i transplantation, little is known about the use of EVR although, in ABO-i liver transplantation, a case of EVR-based immunosuppression has been reported [15]. Also, in stable ABO-i kidney transplantation, a pilot study performed on 16 recipients demonstrated that conversion from MPA plus standard-dose Tac to EVR plus low-exposure Tac was safe and effective [16]. However, the observation period was only 3 months and whether these benefits can be maintained over a longer period remained to be assessed.

For this reason, herein, we describe a series of seven ABO-i kidney-transplant recipients that were converted from MPA to EVR (Tac-EVR). The primary objective of this study was to describe the long-term outcomes of patients after conversion compared with fourteen ABO-i kidney-transplant recipients that received classical immunosuppression, that is, Tac-MPA. The secondary objective was to describe the potential effect of EVR on BKV status in this particular population.

Patients and methods

Patients

Between 2011 and 2013, 22 ABO-incompatible kidney transplantations were performed in the Organ Transplant Unit at Toulouse University Hospital, France. Pretransplant desensitization was based on rituximab and apheresis (immunoadsorption and/or plasmapheresis). Induction consisted of antithymocyte globulines or basiliximab. The initial immunosuppressive regimen combined steroids, Tac, and MPA. Two groups of patients were studied. In the Tac-MPA group, 14 patients received a classical immunosuppressive regimen that combined steroids, Tac, plus MPA. In the Tac-EVR group, 7 patients were converted from MPA (Myfortic®) to EVR (Certican®), at a median of 141 (34–529) days after transplantation, because they were positive with regard to BKV in their blood and/or urine. One patient, who was switched to EVR because of severe digestive troubles attributed to MPA, was excluded from the study because of a negative status for BKV (Flow chart available in Fig. 1). The target trough (C0) levels for Tac and EVR were 3–5 and 6–10 ng/ml, respectively. Steroid dose (Prednisone) was 5 mg/day. At the time of conversion, the median dose of tacrolimus was 0.11 (range: 0.02–0.28) mg/kg. Tacrolimus trough level was 6.1 (range: 3.6–10.4) ng/ml. The median dose of MPA was 19.6 (range: 10.6–25.3) mg/kg. The median dose of steroids was 0.10 (range: 0.08–0.20) mg/kg. Initial anti-A isoagglutinin median titer was 1 (range: 1/1–1/5).

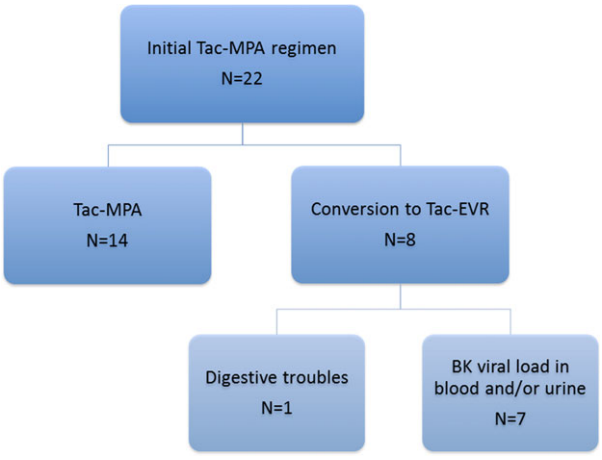

Figure 1 Flow-chart.

Methods

Clinical parameters and blood samples were collected at the last follow-up for the Tac-MPA group, and at each time point in the study (1 month before conversion, 1 and 3 months after, and at the last follow-up) for the Tac-EVR group. The median follow-up time for all patients (both TAC-MPA and TAC-EVR group) was 819 days (range 265–1448). Fasting blood samples were used for biochemical studies (serum creatinine, Tac, EVR trough levels). Estimated glomerular-filtration rate (eGFR) was calculated using the MDRD (Modification of Diet in Renal Disease) equation. Anti-A isoagglutinin titers were measured in tubes using serial dilutions and red blood cells originating from a reference blood donor (A group). Identification of anti-HLA antibodies (IgG) were assessed for class I (HLA A and B) and class II (HLA DR and DQ) using a Luminex single-antigen assay (One Lambda, Canoga Park, CA, USA). A baseline value of mean fluorescence intensity (MFI) >1000 was considered positive.

BK viral load was assessed using whole-blood samples collected in tubes with potassium EDTA. Nucleic acids were extracted from samples with a MagNA Pure 96™ instrument using MagNA Pure 96 DNA and a small-volume viral DNA kit® (Roche Molecular Diagnostics, Meylan, France), according to the manufacturer's instructions. The detection limit for BKV was 500 copies/ml. In the Tac-EVR group, within a median time of 131 (range: 0–172) days before conversion, a kidney biopsy was performed on each patient, which showed unspecific tubulopathy (three patients), interstitial fibrosis and stage-1 tubular atrophy (one patient), or no specific lesions (three patients).

The local institutional review board approved this retrospective study.

Statistics

All analyses and calculations were performed using GraphPad Prism software. The results are presented as their median values (range: min–max). D'Agostino & Pearson omnibus normality test and Shapiro–Wilk normality test were used to assess normal distribution. For variables that were not normally distributed, Mann–Whitney test was used. For variables that were normally distributed, comparisons between the Tac-MMF group and the Tac-EVR group were performed using the unpaired Student's t-test. Differences over time in the Tac-EVR group were evaluated using Wilcoxon's matched-pairs signed-rank test. A P-value of <0.05 was considered statistically significant.

Results

Pharmacological parameters for conversion from MPA to EVR

Both groups were similar in terms of their characteristics and median times between transplantation and the last follow-up (refer to Table 1). As expected, in the Tac-EVR group, conversion resulted in effective Tac reduction. According to Fig. 2, at last follow-up Tac trough level was significantly lower in the Tac-EVR group than in the Tac-MPA group [4.5 ng/ml (range: 2.5–5.9) vs. 6.1 ng/ml (range: 4.3–13.4), $P = 0.005$], although Tac doses were not statistically different between the two groups even though they were lower in the Tac-EVR group. Steroid doses were also similar. As shown in Fig. S1A, in the Tac-EVR group, Tac dosage decreased gradually, whereas EVR dosage stayed stable. As shown in Fig. S1B, the median Tac trough concentration decreased from 6.9 (range: 3.2–12) ng/ml at the time of conversion to 4.5 (range: 2.5–5.9) ng/ml at the last follow-up ($P = 0.04$).

Kidney function

Kidney function is depicted in Fig. S2. Patient-survival rates were 100% in both groups at the last follow-up. Graft loss occurred in one patient in the Tac-MPA group at 18 months after transplantation (humoral rejection). At last follow-up, median eGFR was not statistically different in Tac-EVR compared with Tac-MPA group [40 (range: 14–56) vs. 54.5 ml/min/1.73 m^2 (range: 0–128), $P = 0.07$]. No patient experienced an acute rejection in this group.

Changes in antibody titers

At the last follow-up, no significant difference was noted in anti-A titer between patients in the Tac-MPA and Tac-EVR groups (Fig. 3a). As previously described [13], conversion from MPA to EVR led to an increased anti-A isoagglutinin titer after 1 month (ns); at the last follow-up, median titer was 1/4 (range: 1/1–1/16), as depicted in Fig. 3(b). There was no change in anti-A antibody titers after conversion ($P = 0.88$). Because EVR-based immunosuppression without calcineurin inhibitors is associated with an increased risk of developing donor-specific HLA antibodies (DSAs) [17], anti-HLA antibodies were regularly screened. Figure S3 summarizes the evolution of the alloreactive status of Tac-EVR patients regarding both anti-HLA and donor-specific antibodies. Before transplantation, three patients had DSAs. At the last follow-up, DSAs were only detectable in one patient. Of note, no *de novo* DSA was detected.

Table 1. The patients' characteristics.

	Tac-MMF	Tac-EVR	*P* value
Number of patients	14	7	
Gender (male/female)	10/4	1/6	
Age at transplantation (years)	46 (24–74)	43 (21–61)	$P = 0.44$
Body mass index at transplantation (kg/m^2)	26 (16–33)	20 (18–21)	$P = 0.09$
Donor relation	Spouse 9	Spouse 1	
	Parent 3	Parent 3	
	Siblings 2	Siblings 3	
ABO incompatibility	A/O 9, B/O 3, AB/O 2	A/O 6, A/B 1	
Time from transplantation to last follow-up (days)	766 (265–1448)	1006 (432–1366)	$P = 0.29$
Time from transplantation to conversion (days)	NA	141 (34–529)	NA
Time from conversion to last follow-up (days)	NA	784 (398–866)	NA

Results are expressed as their medians (min–max). TAC, tacrolimus; MPA, mycophenolic acid; EVR, everolimus; NA, not available.

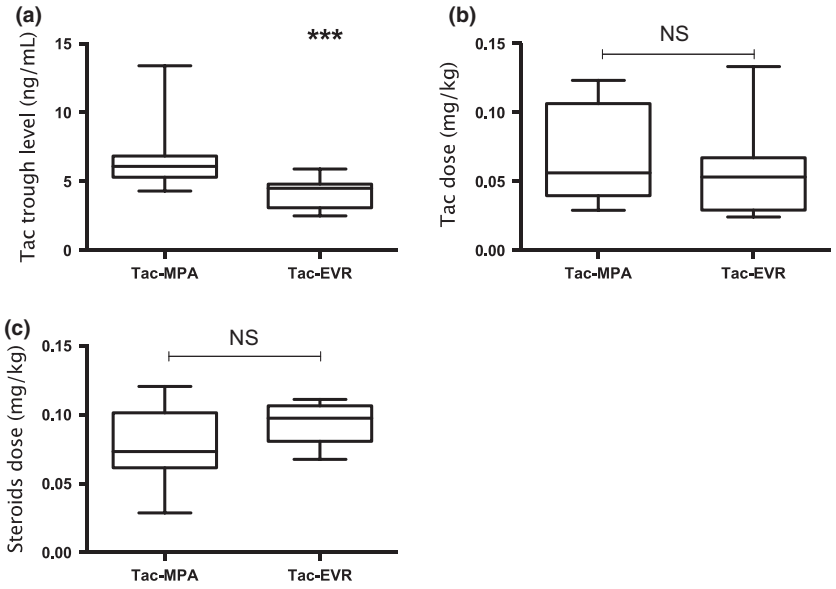

Figure 2 Everolimus-based combined immunosuppressive treatment. Tacrolimus trough levels (Panel a), Tac doses (Panel b), and steroid doses (Panel C) at the last follow-up are reported for the Tac-MPA and Tac-EVR groups. Abbreviations: Tac, tacrolimus; MPA, mycophenolic acid; EVR, everolimus. *** indicates $P < 0.001$.

Safety aspects of long-term EVR use

Because EVR may lead to classical adverse events, such as anemia, thrombocytopenia, leucopenia, dyslipidemia, hyperglycemia, and proteinuria, the presence of these parameters, was monitored.

Metabolic parameters

The metabolic parameters (cholesterol, and triglycerides levels) were initially similar in both groups (Fig. S4A), except fasting glucose that was lower in Tac-EVR patients. Hence, in the Tac-EVR group, conversion to EVR led to dyslipidemia. Median blood levels of cholesterol significantly increased from 4.0 (range: 2.8–6.3) at conversion time to 5.5 mmol/l (range: 4.2–6.8) at last follow-up $P = 0.03$. Median blood levels of triglycerides significantly increased from 1.4 (range: 1–2.9) at the conversion time to 2.6 mmol/l (range: 1.4–4.8) at the last follow-up, $P = 0.02$. At the last follow-up, the number of patients that required antilipidemic drugs in the Tac-EVR group was higher than in the Tac-MPA group (5 out of 7 vs. only 5 out of 14, respectively).

Hematological parameters

At the last follow-up, hemoglobin level was significantly lower in the Tac-EVR group compared with the Tac-MPA group (13 g/dl [range: 10.9–14.1] vs. 14.7 g/dl [range: 12.1–17.1], $P = 0.004$), as depicted in Fig. S5. However,

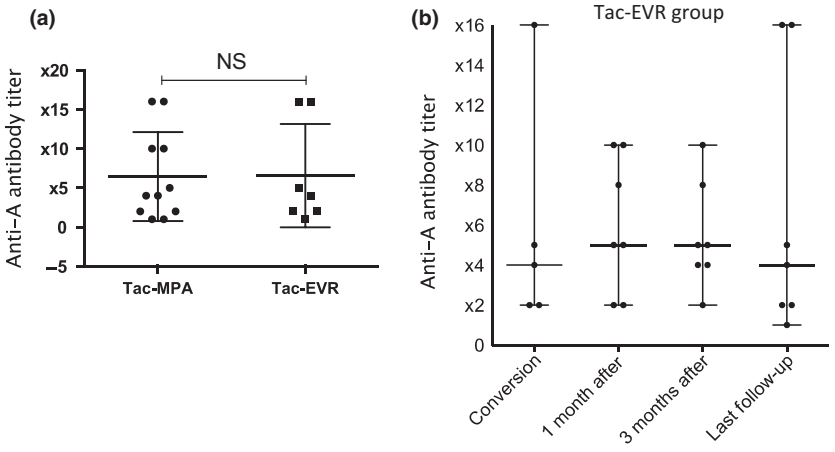

Figure 3 Changes to isoagglutinin titers. Panel a: Anti-A isoagglutinin titers at the last follow-up are reported for the Tac-MPA and Tac-EVR groups. Panel b: Anti-A isoagglutinin titers for the Tac-EVR group are depicted according to time. Abbreviations: Tac, tacrolimus; MPA, mycophenolic acid; EVR, everolimus. NS, nonsignificant.

conversion from MPA to EVR did not lead to significant anemia [hemoglobin levels at conversion and at the last follow-up were similar ($P = 0.23$)]. At the last follow-up, four patients required low doses of erythropoietin: two in the Tac-EVR group, and two in the Tac-MPA group. At the last follow-up, platelet and white blood-cell counts were similar in both groups. Mild leucopenia was observed at 1 month after conversion to EVR in two patients (2.9 and 3.4 G/l), but the patients rapidly recovered without needing granocyte stimulating agents.

Proteinuria

In the Tac-MPA group at the last follow-up, two patients had macro-albuminuria. In the Tac-EVR group, micro-albuminuria (>30 mg/g) was found in two patients before conversion and was still detectable at the last follow-up despite antiproteinuric drugs. One patient developed *de novo* microalbuminuria after conversion. The median albuminuria/creatininuria ratio at the last follow-up was 33 mg/g (range: 11–225; data not shown). Four patients were not affected by this adverse event.

Other side effects

No aphthosis was reported. One patient experienced mild lymphedema. One patient experienced CMV reactivation after conversion.

BKV status

In the Tac-MPA group, only one patient was positive for BKV in the blood and urine. In the Tac-EVR group, all patients were positive with regard to BKV in their blood and/or urine. Conversion to EVR had no impact on two of the patients. Two patients had shown biopsy-proven BKVN lesions before conversion. For five patients, the conversion from MPA to EVR was accompanied by a decrease in viral load in the urine and/or blood, as shown in Fig. 4. Viral load became negative in both the urine and blood of one patient (3D). Viral load became negative in the blood of two patients (3A, 3C). A drastic decrease in viral load in the urine was observed in four patients (3A, 3B, 3D, 3E). The median delta between viral load at conversion and viral load at the last follow-up in urine was 4.6 log (range: 0.64–7.65). Taken together, these data indicate that EVR may participate in the clearance of BKV in some patients.

Histological data

In the Tac-EVR group at conversion, the patients were in stable state with regard to kidney-allograft function. The effect of conversion from MPA to EVR on C4d deposition was evident in the kidney biopsies (Fig. S6). At the last follow-up, out of the seven patients, only one patient had active BKVN. C4d staining was positive in five patients. Global kidney structure was preserved in all the other patients.

Discussion

In this study, seven stable ABO-i kidney-transplant recipients were converted from MPA plus standard-dose Tac to EVR plus low-dose Tac (Tac-EVR) and were compared to a reference group receiving the classical Tac-MPA combination. The Tac-EVR combination was associated with a reduced burden of BKV infection in some patients. The first demonstration of using EVR in ABO-i kidney transplantation was published recently [16], but results from a longer term follow-up were lacking. Herein, we provide

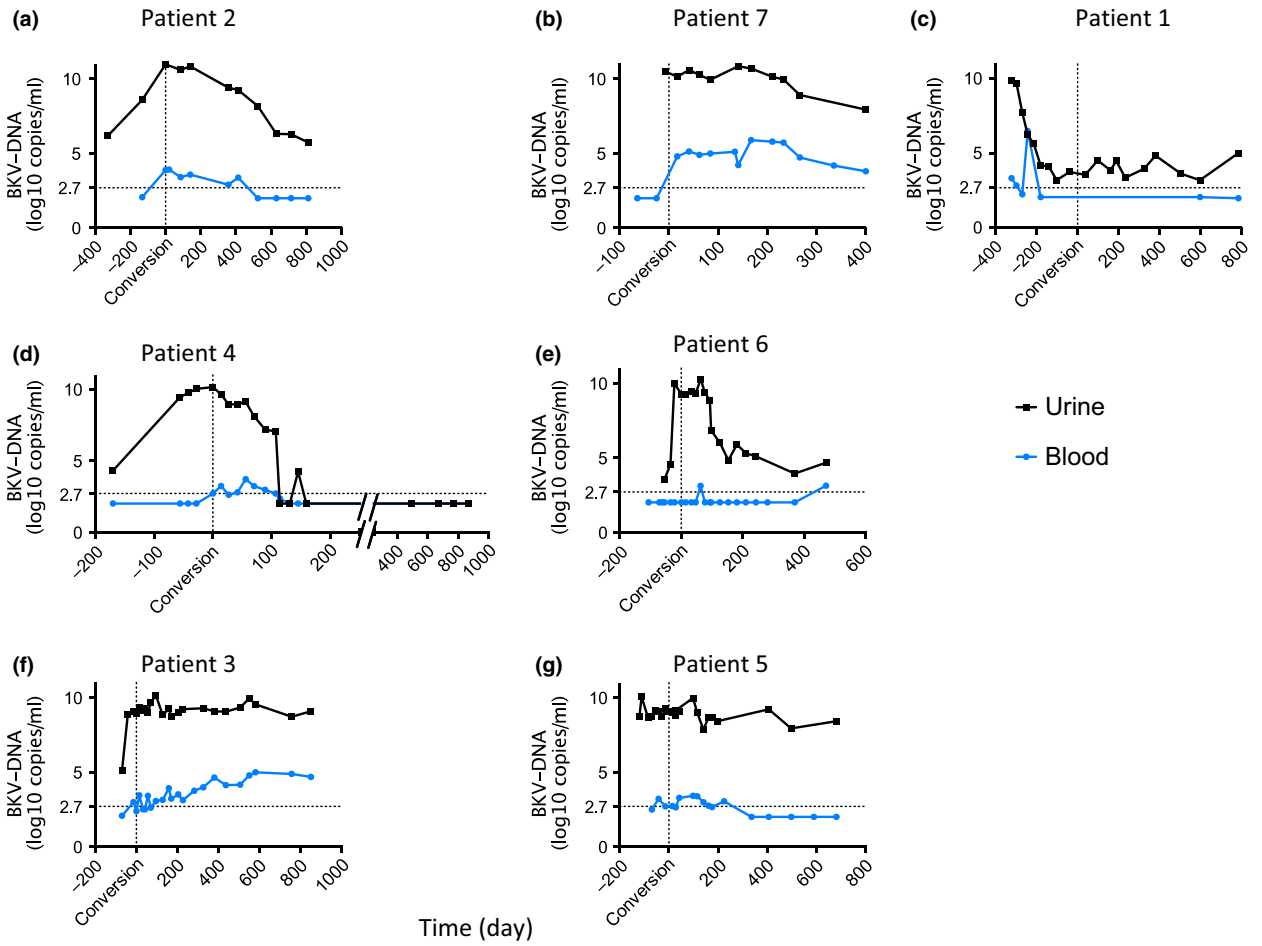

Figure 4 Decrease in BK viral loads in the blood and/or urine after conversion to everolimus for five patients. Viral loads in the blood (blue line) and urine (black line) are expressed on a log scale according to time, for seven patients. The horizontal dashed line indicates the lab-positivity threshold and the vertical one refers to the time of the conversion.

data from a longer term follow-up, that is, a median of 784 days (range: 398–866) after conversion. Moreover, in our study, conversion was implemented sooner after transplantation, that is, within a median time of 141 days (range: 34–529) versus 1 year in the Uchida study [16].

Kidney function

Several studies have reported on the preservation of renal function in ABO-compatible patients receiving a regimen that included mTOR inhibitors and CNI withdrawal or minimization. In addition, EVR and low-dose cyclosporine (CsA) have shown comparable efficacy and renal function compared with MPA and standard-dose CsA over a 2-year period, with an eGFR of approximately 50 ml/min/1.73 m^2 [18]. EVR and minimization of CsA seem to offer stable renal function after 2 years [19]. Conversion from CsA to EVR has been associated with improved renal function, which has been maintained for at least 5 years [20]. Very recently, the introduction of EVR with a CsA minimization strategy at 1 month after transplantation was accompanied by a better eGFR (68 ml/min/1.73 m^2) at 1 year post-transplantation [21].

Contrary to a unique study in ABO-i patients [16], we did not describe a significant gain in kidney function at month 3 ($P = 0.8$). This discrepancy cannot be explained by the difference in initial kidney function because our patients had similar kidney functions at baseline, that is, a median of 49 [range: 27–72] at conversion time versus 46 ml/min/1.73 m^2 in the study by Uchida *et al.* [16]. We also found a significant and progressive decrease in kidney function. The absence of an improvement in kidney function in our cohort may be explained by the higher Tac trough levels. For instance, at month 3, mean Tac trough level was 5.15 ± 1.17 ng/ml in our series versus 2.25 ± 0.1 ng/ml in the study by Uchida *et al.* [16].

Tac-related nephrotoxicity cannot be ruled out. Conversely, the rapid decline in renal function might be related to BKV infection. This could explain why the eGFR values at the last follow-up were lower in the Tac-EVR group compared with the Tac-MPA group.

Consequently, we wondered whether kidney function had been slowly altered because of rejection; however, no rejection or graft loss occurred in the Tac-EVR group. As has been also shown by Uchida *et al.*, we observed a slight (a less than fourfold increase) elevation in anti-A antibody titers at 1 month after conversion. This phenomenon was transitory, and titers at the last follow-up were globally similar to those at baseline. Although data on anti-HLA antibodies and DSAs were lacking in this pilot study, we monitored the immunological parameters of our patients. As previously shown in ABO-compatible patients, in organ transplantation [22], conversion to EVR did not modify the natural evolution of preformed anti-HLA antibodies or *de novo* DSAs. Interpretation of C4d staining is difficult in cases of ABO-i transplantation because most kidney-allograft biopsies in this setting exhibit diffuse staining.

Safety aspects

The profile for adverse events in our study was consistent with the most commonly reported adverse events associated with mTOR inhibitors. Compared with the Tac-MPA group, hemoglobin level at the last follow-up was lower in the Tac-EVR group. This did not seem to be related to conversion from MPA to EVR as the longitudinal values remained stable. One explanation for this difference could be the decreased kidney function in Tac-EVR group. Platelet and white blood-cell counts were not significantly modified. Regarding metabolic parameters, no new-onset diabetes mellitus occurred.

Hypercholesterolemia is a frequent complication after conversion to mTOR-I [23]. In our study, the frequency of dyslipidemia was higher than reported in the literature (75% vs. 66%, respectively [6]). Micro-albuminuria levels were also higher than reported by Uchida *et al.* [16]. No malignancies were noted.

Antiviral effects

Because BKV is of particular significance in ABO-i patients, who are at higher risk given their high immunosuppression burden [10], we focused on the effect of conversion to EVR regarding the BK virus. It has been shown that conversion to mTOR inhibitor-based maintenance immunosuppression after kidney transplantation is correlated with a lower incidence of BKV viremia [11] and that EVR leads to a lower risk of BKV viremia compared with MPA [12]. Patients on a Tac-MPA regimen are at higher risk of developing BKV viremia than patients receiving Tac-EVR. According to some authors, this could be attributed to the different magnitudes of $CD8^+$ and $CD4^+$ T-cell-dependent immune reactivities [24]. In our series, seven patients were converted to EVR because they presented with active BKV replication in the blood and/or urine. For four of these cases, BKV status was improved after conversion. Even if no causal link can be established, this finding suggests that EVR could have played a role in controlling BKV replication, as has been previously suggested [6]. Whether this benefit is related to a direct antiviral effect, Tac minimization, or to MPA withdrawal remains to be determined. Regarding CMV, only one patient experienced CMV reactivation after conversion.

The limitations of this study include its small size and retrospective design. Furthermore, the behavior of patients was not uniform. A larger prospective study that includes detailed immune characteristics (anti-BK immunoglobulins, lymphocytes phenotyping) would be of great interest to further define the potential antiviral effect of EVR on BKV.

Conclusion

In conclusion, conversion to EVR with a reduction in Tac was achieved in ABO-i kidney recipients over a long-term follow-up period. eGFR at the last follow-up was similar in both Tac-EVR and Tac-MPA groups. This strategy led to a good tolerance profile. Out of seven patients, complete BK virus clearance was achieved in one patient in blood and urine, in two patients in blood only. In one patient, viremia and viruria clearly decreased after the conversion. These data indicate that EVR may participate in the clearance of BKV in some patients.

If further studies confirm these data, this approach could also constitute a useful therapeutic tool in cases where there is MPA intolerance or high BKV activity in highly immunosuppressed patients, such as ABO-i recipients.

Authorship

RL: designed study. KN, EL, and RL: recruited the patients. BJ: collected the data. MC: performed the virological analysis. AA: desensitize the patients at pretransplant. SF: performed the kidney transplants. CN: performed the identifications of anti-HLA antibodies. DB: performed the isoagglutinin tests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Everolimus-based combined immunosuppressive treatment.

Figure S2. Kidney function.

Figure S3. Immunological data from the Tac-EVR group.

Figure S4. Metabolic profiles.

Figure S5. Hematological tolerance.

Figure S6. Histological data.

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