ORIGINAL ARTICLE

Prospective randomized study comparing everolimus and mycophenolate sodium in *de novo* kidney transplant recipients from expanded criteria deceased donor

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SUMMARY

The optimal immunosuppressive regimen for recipients of expanded criteria donor (ECD) kidneys has not been identified. In this single-center study, 171 recipients of ECD kidney transplants were randomized to receive antithymocyte globulin induction, and delayed introduction of reduced dose tacrolimus, prednisone and everolimus (r-ATG/EVR, $n = 88$), or mycophenolate (r-ATG/MPS, $n = 83$). No cytomegalovirus (CMV) pharmacological prophylaxis was used. The primary endpoint was the incidence of CMV infection/disease at 12 months. Secondary endpoints included treatment failure [first biopsy-proven acute rejection (BPAR), graft loss, or death] and safety. Patients treated with EVR showed a 89% risk reduction (13.6 vs. 71.6%; HR 0.11, 95% CI 0.06–0.220, $P < 0.001$) in the incidence of first CMV infection/disease. Incidences of BPAR (16% vs. 5%, $P = 0.021$), graft loss (11% vs. 1%, $P = 0.008$), death (10% vs. 1%, $P = 0.013$), and treatment discontinuation (40% vs. 28%, $P = 0.12$) were higher in the r-ATG/EVR, leading to premature study termination. Mean glomerular filtration rate was lower in r-ATG/EVR (31.8 ± 18.8 vs. 42.6 ± 14.9, $P < 0.001$). In recipients of ECD kidney transplants receiving no CMV pharmacological prophylaxis, the use of everolimus was associated with higher treatment failure compared with mycophenolate despite the significant reduction in the incidence of CMV infection/disease (ClinicalTrials.gov.NCT01895049).

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Key words
antithymocyte globulin, everolimus, expanded criteria deceased donor, kidney transplant, mycophenolate

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Introduction

Performing kidney transplants from expanded criteria donors (ECDs) is a worldwide trend to increase the numbers of deceased donor kidneys available [1]. ECD kidneys are associated with a 1.7 higher relative risk of graft loss compared with standard criteria donor kidneys [2].

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The optimal immunosuppression for recipients of ECD kidneys has not been defined. Evidence from clinical trials has demonstrated that kidney transplanted from ECDs has higher incidence of delayed graft function (DGF) and acute rejection (AR) episodes and is more susceptible to nephrotoxic events [3,4]. To mitigate these interconnected risks, highly effective induction therapy with rabbit antithymocyte globulin (r-ATG) and delayed introduction of calcineurin inhibitors (CNI) has been used. Furthermore, potential nephron-protecting strategies including reduction in cold ischemia time and pulsatile perfusion preservation and adequate infection prophylaxis are necessary to promote and preserve full recovery of renal function [5].

Cytomegalovirus (CMV) is the most prevalent opportunistic infection after kidney transplantation, and it is associated with a higher incidence of other infections, graft loss, and death [6]. Randomized trials suggested that regimens containing r-ATG are associated with increased risk of CMV infection compared with noninduction therapy [7] and basiliximab (BAS) [8]. Similarly, the use of mycophenolate (MPS) has also been associated with higher incidence of CMV infection compared with mammalian target of rapamycin inhibitors (mTORi) [9], even in patients receiving r-ATG induction [10].

Our transplant system is characterized by high incidence of DGF (60%), with mean time of dialysis of more than 15 days [11]. Also, access to CMV pharmacological prophylaxis is limited because of costs and lack of reimbursement. Considering these local environment characteristics, we devised the rational of this study where recipients of ECD kidneys received r-ATG induction, delayed and reduced tacrolimus (TAC) exposure, fast corticosteroid taper and everolimus (EVR), or MPS, in the absence of CMV pharmacological prophylaxis, aiming to obtain similar efficacy but lower incidence of CMV infection/disease.

Methods

Study design

This was a single-center, prospective, randomized, 12-month open label-controlled trial in *de novo* kidney transplant recipients from ECD comparing the safety and efficacy of EVR versus MPS. It was conducted in compliance with Good Clinical Practice guidelines, in accordance with the Declaration of Helsinki, and was approved by local ethics committee. All patients signed a written informed consent. The trial is registered at ClinicalTrials.gov, as NCT01895049. Novartis and Sanofi partially funded this study.

Population

Patients older than 18 years were eligible. Key exclusion criteria included ABO-incompatibility, positive crossmatch, class I or class II panel reactive antibody equal to or above 50% by flow cytometry, leukocytes count $< 2000/mm^3$, platelet count $< 100\ 000/mm^3$, malignancy within the past 2 years, positive HIV serology, and any other active systemic infections.

Randomization

A computer-generated randomization (1:1) sequence was generated using 20 blocks of 10 patients each and placed in sequentially numbered opaque envelops. Eligible patients were randomly assigned in a 1:1 after the transplant surgery to receive either EVR or MPS.

Treatments

All patients received 1 g methylprednisolone before graft revascularization and initial prednisone (Pred) dose of 0.5 mg/kg/day (maximum of 30 mg/day), tapered to 5 mg/day by day 45. Induction therapy consisted of four fixed 1.5 mg/kg dose of r-ATG at days 1, 3, 5, and 7 after transplantation. Patients randomized to r-ATG/EVR group received an initial 1.5 mg dose of EVR *Bis in die* (twice a day) (BID) starting at day 1 to maintain whole blood trough concentrations (C0) between 4 and 8 ng/ml. Patients randomized to r-ATG/MPS group received an initial 720 mg dose BID starting at day 1, and plasma MPA trough concentrations (C0) were measured. TAC (0.05 mg/kg BID) was initiated at day 8 or one day after the last dose of r-ATG. TAC doses were adjusted to maintain whole blood trough concentrations (C0) between 3 and 5 ng/ml.

Prophylaxis against infections

No pharmacological prophylaxis for CMV infection was used. All patients received pre-emptive strategy which consisted of weekly monitoring of CMV viral replication (pp65 CMV antigenemia test) from the third week to the third month, every other week until the fourth month and monthly until the sixth month after transplantation. All patients received albendazole for 5 days, oral nystatin for 30 days, and trimethoprim–sulfamethoxazole for 12 months.

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Definitions

Delayed graft function was defined as the need for dialysis during the first week after transplantation [12]. Biopsy-proven acute rejection (BPAR) episodes were graded according to Banff 2009 classification [13]. Treated acute rejection (tAR) included BPAR and those treated episodes (at least 500 mg of methylprednisolone for 3 consecutive days) without biopsy confirmation, including borderline changes.

Primary endpoint

The primary endpoint was the cumulative incidence of first CMV infection/disease during the first year after transplantation. CMV infection was based on detection of CMV viral replication (at least five positive cells per 200 000 cells by CMV pp65 antigenemia) in asymptomatic patients. CMV disease was based on the evidence of CMV infection with related symptoms. CMV infection/disease was treated with intravenous ganciclovir with weekly monitoring of viral replication. Treatment was prolonged for 1 week after the first negative antigenemia test. Recurrent CMV infection/disease was defined as a new CMV event. Because CMV infection or disease was the primary endpoint in this study, all episodes were reported as serious adverse event.

Secondary endpoints

Secondary outcomes included treatment failure, defined as a composite endpoint of BPAR, graft loss, death, and loss to follow-up; incidence, severity, timing, and treatment of all tAR episodes and donor-specific antibodies (DSAs). Serum anti-HLA antibodies were analyzed before transplantation and at month 12 with a screening test (LABScreen, One Lambda) and Luminex platform. *De novo* DSA was the presence of DSA with MFI > 300 after the transplant in the absence of pretransplant DSA. All donors and recipients were typed for HLA-A, HLA-B, and DRB1. Typing of additional loci was performed in cases of detection of antibodies other than HLA-A, HLA-B, or DRB1. We have also evaluated the incidence and duration of DGF, renal function (eGFR by MDRD formula [14]), and spot urine protein-to-creatinine ratio (U p/c). Safety analysis included incidence of adverse events.

Protocol amendments

After starting the study, two amendments were implemented. The first amendment was implemented after three of the first six randomized patients developed immediate graft function. Thereafter, patients with immediate or early recovery of graft function were discontinued from r-ATG dosing schedule and started receiving TAC. The main reason for this strategy was to improve safety by limiting the total dose of r-ATG in those patients with early graft function. The second amendment was implemented after randomization of 164, with 112 of them already followed up for one than 1 year. Preliminary analysis showed a higher number of graft losses (9 vs. 1) and deaths (7 vs. 1) in the r-ATG/EVR compared with r-ATG/MPS group, respectively. Because of safety concerns, we limited the use of r-ATG to only two doses on days 1 and 3, followed by introduction of TAC on day 4. After this amendment, seven additional patients were enrolled, reaching 171 patients (88 in the r-ATG/EVR group and 83 in the r-ATG/MPS group). Given that two additional deaths and one graft loss occurred subsequently, the study was prematurely terminated before reaching the calculated sample size. All patients included were followed up for 12 months.

Statistical analysis

Sample size was calculated for the primary endpoint of CMV infection/disease during the first year after transplantation. The pretest hypothesis considered a 30% reduction in the incidence of CMV infection/disease, from 69% in the r-ATG/MPS group, based on historical data, to 48% in the r-ATG/EVR group. The estimated sample size of 85 patients per group using a bicaudal test and a significance level of 5% provided 80% power. Considering a 20% dropout rate, the final sample size was 100 patients per group. Categorical variables were summarized in frequency distributions, and groups were compared using chi-square or Fisher exact tests. Continuous variables were described by mean and standard deviation or median and interquartile range (IQR) after assessment of normal distribution. The differences between groups were analyzed using Student's *t*-test or Mann–Whitney. The Cox regression model was used to calculate the cumulative incidence of first CMV infection/disease and 95% confidence interval (CIs) during the first 12 months. A sensitive analysis was also performed including death and graft loss (all events that might have occurred before the first CMV event) as competing risk. Cause-specific hazard regression model for competing events was fitted after no evidence against the assumption of proportionality was revealed by analysis of Schoenfeld residuals. The effect of covariates on cause-specific hazard was estimated with Cox

proportional hazard regression and 95% confidence interval. To evaluate risk factors for CMV infection/disease, a logistic regression with multivariable analysis was used. Kaplan–Meir test assessed patient and graft survival as well as AR-free survival. Groups were compared using the log-rank test. For renal function evaluation, estimated glomerular filtration rate was assessed by MDRD4 equation. The imputation of the last eGFR was done, using the method of last observation carried forward (LOCF) analysis for patients who died or lost follow-up. For patients who lost the graft, eGFR was imputed as zero. A sensitive analysis was performed attributing the eGFR value of 10/ml/min/1.73 m^2 for all patients who died or were loss to follow-up during the first year. These analyses were done in intention-to-treat population (ITT) and in the per-protocol population (PP). Inferior renal function was defined as the eGFR below the median value of the total study population at 12 months. Variables included in the model were based on previously published risk factors known to influence renal function at 12 months. The relative small sample size persuaded us to use GFR as a dichotomous rather than continuous variable and to limit the number of variables (most relevant) included in the multivariable logistic regression model. Statistical analyses were performed using SPSS version 19, SPSS Inc, Chicago, IL, USA and a level of 0.05 of significance was used in all tests.

Results

Population

Between November 8, 2013, and March 25, 2016, 171 of the 283 patients screened were randomized to receive the first study drug dose and 169 were treated after the transplant surgery (r-ATG/EVR = 88; r-ATG/MPS = 81). Two patients were excluded from the ITT analysis in the r-ATG/MPS group, one because of severe urinary tract infection diagnosed during the transplant surgery (exclusion criteria) and one because of withdrawal of the informed consent on day 7. After 1 year, 148 (87.5%) patients completed the study (78% vs. 97%, $p < 0.001$) and 111 (65%) completed study receiving randomized therapy (60% vs. 71%, $P = 0.145$, Fig. 1). Mean donor age was over 60 years, 78% (133) died because of cerebrovascular accident, and mean Kidney donor profile index (KDPI) was 89%. There were no significant differences in KDPI distribution (Fig. S1), but the proportion of deceased donors with final creatinine above 1.5 mg/dl was higher in the r-ATG/EVR group [58% ($n = 51$) vs. 42% ($n = 35$), $P = 0.047$]. Mean recipient age was 52 years, and the prevalence of diabetes mellitus as the primary cause of chronic kidney disease was higher in the r-ATG/EV [32% ($n = 28$) vs. 17% ($n = 14$), $P = 0.026$]. Only 6% were high-risk donor positive recipient-negative CMV IgG. The majority of the patients were nonsensitized with a mean cold ischemia time over 22 h (Table 1).

Immunosuppression

The mean cumulative dose of r-ATG was 4.2 ± 1.5 mg/kg per patient with no differences in the proportion of patients receiving one or more doses of r-ATG (Fig. S2). Initial mean EVR C0 concentration was 3.3 ± 0.9 at day 3, progressively increasing till day 90 (Table 2). EVR C0 was below 4 ng/ml in 70% ($n = 70$), 70% ($n = 55$), 62% ($n = 53$), 38% ($n = 28$), and 17% ($n = 11$) of patients on days 3, 7, 14, 30, and 90, respectively (Table S1, Fig. 2a). Median MPA C0 plasma concentration was 3 μg/ml during the first year (Table 2, Fig. S3) [15]. In the r-ATG/EVR group, 45 (51%) patients started TAC before day 7 (day 3 = 5; day 4 = 19; day 5 = 18; day 6 = 3) compared with 40 (50%) patients in the r-ATG/MPS group (day 4 = 18; day 5 = 13; day 6 = 9). Tacrolimus concentrations obtained at day 5 for patients initiating TAC on day 3 were 3.7, 3.8, 3.9, 6.4, and 8.1 ng/ml. For patients initiating TAC on days 4, 5, and 6, concentrations were obtained at day 7 or thereafter. Mean TAC concentrations at day 7 were 5.6 ± 3.4 and 6.0 ± 4.5, respectively. Median TAC concentrations were maintained between 5 and 6 ng/ml throughout the first year after transplantation (Table 2, Fig. 2b).

Primary endpoint

The pre-emptive monitoring complied with the protocol defined visits, with one (1%) missing CMV pp65 data on day 7 in the r-ATG/EVR group and 2 (2.4%) in the r-ATG/MPS group on days 42 and 56. The incidence of CMV infection/disease was lower in patients receiving EVR compared with MPS [13.6% ($n = 12$) vs. 71.6% ($n = 58$) $P = 0.001$, Table 3]. Treatment with EVR was associated with 89% risk reduction compared with MPS (Fig. 3). Cox proportional hazard regression using death and graft loss as competing cause-specific risks confirmed that patients receiving MPS were at a higher risk to develop CMV infection/disease (HR = 8.863, 4.637–16.26 95% CI, $P < 0.001$). The incidence of first episode of CMV disease was 8% (7/88) in the EVR group and 35% (28/81) in the MPS group. The mean time to the first CMV event was delayed by 13 days in the r-ATG/EVR,

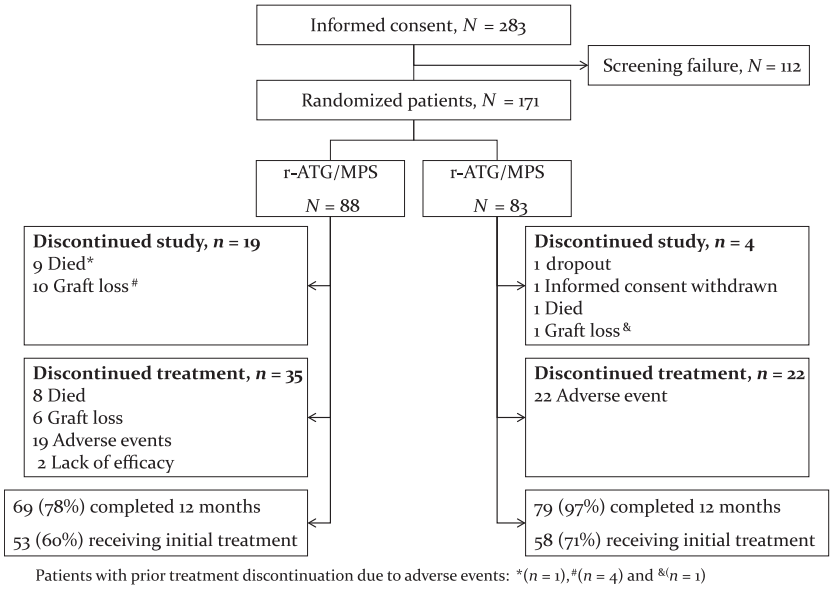

Figure 1 Patient disposition.

but duration of treatment was similar in both groups. The incidence of CMV infection among high-risk donor (+)/recipient (−) CMV combination was 75% ($n = 3$) in r-ATG/EVR and 100% ($n = 6$) in r-ATG/MPS group. Of the 12 events of CMV infection or disease in the r-ATG/EVR group, six occurred after tAR and three after discontinuation of EVR. The proportion of patients with more recurrences was lower in the r-ATG/EVR group. Overall, a significantly lower number of CMV treatments were required in the r-ATG/EVR group. Importantly, there was no tissue-invasive CMV disease in any group (Table 3).

Secondary endpoints

Efficacy

The incidence of treatment failure was higher in the r-ATG/EVR group [30.7% ($n = 27$) vs. 6.1% ($n = 5$), $P < 0.001$] (Table 4). The incidence of BPAR was significantly higher in the r-ATG/EVR group [16% ($n = 14$) vs. 5% ($n = 4$), $P = 0.021$]. There was a significant difference in the Kaplan–Meier estimates for rejection-free survival with the higher rate in r-ATG/MPS group (95.1% vs. 84.1, $P = 0.019$, log-rank test). Rejection episodes occurred earlier in r-ATG/EVR compared with BAS/MPS group (47 ± 52 vs. 158 ± 141 days, $P = 0.002$). The observed difference in the incidence of first treated AR was due to an excess rate of AR during the first month in the r-ATG/EVR group (14 vs. 3 episodes). Mean (±SD) of EVR concentrations among patients with AR during the first 15 days was 2.8 ± 0.6 ng/ml, below the proposed therapeutic range (Table S4). The majority of BPAR were successfully treated with methylprednisolone. Five patients required antibody therapy, four in the r-ATG/EVR and one in the r-ATG/MPS group (Table 4). Among 169 patients, DSAs against HLA class I were detected in four patients (r-ATG/EVR: 1; r-ATG/MPA: 3), but not found at month 12. DSAs against HLA class II were detected in one patient in each group, which persisted at month 12. From 148 alive patients at month 12, 147 (99%) were re-evaluated for DSA and three patients developed *de novo* DSA against HLA class II (r-ATG/EVR: 1; r-ATG/MPA: 2). There were ten graft losses in r-ATG/EVR group [lack of efficacy ($n = 3$), venous thrombosis ($n = 2$), nonimmunological IFTA ($n = 2$), focal segmental glomerulosclerosis, primary nonfunction, thrombotic microangiopathy], and there was one in r-ATG/MPS group because of lack of efficacy. The overall (78.4 vs. 97.5%, $P = 0.000$, Fig. S4A) and death-censored (88.6 vs. 98.8%, $P = 0.007$, Fig. S4B) graft survivals were higher in the r-ATG/MPS group as well as patient survival (89.9 vs. 98.8%, $P = 0.014$, Fig. S4C). Of the 10 patients who died during the study period, nine occurred in r-ATG/EVR group (five because of cardiovascular events and four because of infection) and there was one death in MPS group because of infection (Table 4). Key demographic characteristics associated with death and graft loss are shown in Table S2. Of nine deaths in the EVR group, two occurred after the treatment of AR episode. In the MPS group, the only death occurred in a patient without previous rejection.

Table 1. Demographic characteristics of the transplant population

Variables	r-ATG/EVR (*N* = 88)	r-ATG/MPS (*N* = 82)
Donor age, *years* (mean ± SD)	60.7 ± 6.5	60.3 ± 6.3
Donor gender, *male, N* (%)	42 (47.7)	39 (47.5)
Donor ethnicity, *N* (%)		
White	53 (60)	44 (54)
Black/mixed	34 (38.6)	38 (46.3)
Other	1 (1)	0
Death due to cerebrovascular accident	66 (75.0)	67 (81.7)
Final creatinine, mg/dL, median (IQR)	1.9 (1.1–2.9)	1.3 (1.0–2.4)
Final creatinine > 1.5 mg/dl, *N* (%)*	51 (57.9)	35 (42)
History of hypertension, *N* (%)	77 (87.5)	69 (84)
Kidney Donor Profile Index (KDPI), %, median (IQR)	91 (84–97)	90 (82–96)
KDPI, *N* (%)		
<79	11 (12)	11 (13)
80–85	12 (14)	20 (24)
86–90	13 (15)	12 (15)
91–95	24 (27)	17 (21)
>96	28 (32)	22 (27)
Recipient age, years (mean ± SD)	52.3 ± 11.8	51.8 ± 10.8
Recipient ethnicity, *N* (%)		
White	41 (46.5)	40 (48)
Black/mixed	44 (50)	42 (51)
Other	3 (3.4)	0
Body Mass Index (kg/m^2)	24.4 ± 5.2	24 ± 5
Cause of chronic kidney disease, *N* (%)		
Glomerulonephritis	9 (10.2)	3 (3.6)
Hypertension	0	1 (1.2)
Diabetes Mellitus	28 (31.8)	14 (17)
Undetermined	37 (42)	54 (65.8)
Other	14 (16)	10 (12)
Time on dialysis, months, median (IQR)	35.5 (20–57.8)	35.5 (22–60.3)
Type of treatment, Hemodialysis *N* (%)	85 (96.5)	80 (97.5)
Class I PRA		
Zero	77 (87)	67 (81)
1–25%	10 (11)	13 (16)
26–50%	1 (1)	2 (2)
Class II PRA		
Zero	87 (99)	81 (99)
1–25%	1 (1)	1 (1)
26–50%	0	0
CMV IgG serologic status, *N* (%)		
Donor (+)/Recipient (+)	79 (89.8)	72 (87.8)
Donor (−)/Recipient (+)	3 (3.4)	1 (1.2)
Donor (+)/Recipient (−)	4 (4.5)	6 (7.3)
Donor(unknown)/Recipient (+)	2 (2.3)	3 (3.7)
HLA mismatches, median (IQR)	2 (2–3)	2 (2–3)
Zero HLA-DR mismatches, *N* (%)	82 (93)	71 (86)
Cold ischemia time, hours (mean ± SD)	22.3 ± 5.7	22.4 ± 5.8
Cold ischemia time > 24 h, *N* (%)	23 (26)	24 (29)

PRA, panel reactive antibodies.

*P = 0.047, **P = 0.026.

Renal function

The incidence of DGF was similar between groups (65% vs. 73%, P = 0.197); however, patients in the r-ATG/EVR group showed a higher median duration of DGF (6 vs. 4 days, P = o.004, Table 5). Mean eGFR at 12 months (LOCF imputation) was lower in r-ATG/EVR compared

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with r-ATG/MPS group in the ITT (31.8 ± 18.8 vs. 42.6 ± 14.9 ml/min/1.73 m^2, $P < 0.001$) or in the PP (36.2 ± 18.6 vs. 44.8 ± 14.0 ml/min/1.73 m^2, $P = 0.005$). A sensitive analysis imputing the eGFR value of 10/ml/min/1.73 m^2 for all patients who died or were loss to follow-up during the first year confirmed this observation (Table S3). Mean $U_{P/C}$ was comparable in the ITT (0.48 ± 0.52 vs. 0.56 ± 1.1, $P = 0.518$) and PP (0.45 ± 0.42 vs. 0.54 ± 1.11; $P = 0.609$) population (Table 5). At 1 and 12 months, the proportion of patients with lower eGFR categories was higher in the r-ATG/EVR compared with the r-ATG/MPS group (Fig. 4). Among traditional risk factors known to influence renal function, donor age, tAR, and EVR use were independently associated with inferior eGFR at 12 months (Table 6).

Safety

Almost all patients experienced at least one adverse event. The incidence of SAEs was higher in the r-ATG/MPS group [67% ($n = 59$) vs. 85% ($n = 69$), $P = 0.006$], primarily because all CMV infection (Table 7). On the other hand, treatment discontinuation was higher in the r-ATG/EVR group. The most common reason for EVR discontinuation was infection and unsatisfactory renal function and for MPS discontinuation was CMV infection. While the proportion of patients with wound complications were comparable [63% ($n = 55$) vs. 58% ($n = 47$)], the proportion requiring surgical reintervention was higher in the r-ATG/EVR group [47% ($n = 26$) vs. 30% ($n = 14$), $P = 0.071$] (Table 7).

Discussion

This open, prospective, and randomized clinical trial investigated the efficacy and safety of EVR versus MPS in recipients of ECD kidney transplants receiving r-ATG induction therapy, low TAC exposure, and fast steroid taper. The incidence of CMV infection/disease and recurrent events was significantly lower in those receiving EVR compared with MPS, despite the use of up to 6 mg/kg doses of r-ATG and no CMV pharmacological prophylaxis. According to the last international consensus guidelines on the management of CMV in solid-organ transplantation, universal prophylaxis and pre-emptive therapy are both recommended in patients with intermediate risk to develop CMV disease after kidney transplantation [6]. Historically, we have chosen to use pre-emptive therapy for all patients considering the following reasons: (i) In our population, the prevalence of the high-risk CMV D+/R- group accounts for only 5–6% of the total population; (ii) a significant proportion of patients show self-limited and spontaneously resolved CMV viremia; (iii) high cost and drug-related adverse reactions associated with pharmacological prophylaxis; and (iv) high incidence of late CMV infection/disease after stopping CMV pharmacological prophylaxis. The lack of differences in mean r-ATG doses and TAC exposure further supports the inhibitory effect of EVR on viral replication. These data confirm previous findings with a lower 3 mg/kg r-ATG fixed dose [10]. Evidence from several studies has demonstrated that the use of EVR decreases the incidence of CMV infection after transplantation [16,17]. A recent Spanish study in CMV+ recipients receiving mTORi suggested that pharmacological prophylaxis may be discontinued even in those patients receiving high-intensity immunosuppression with r-ATG or desensitization therapy [18]. Another EVR study showed low incidence (6.1%) of CMV infection with pre-emptive therapy used only in high-risk D+/R− patients, after tAR and discontinuation of EVR [19]. In ECD kidney recipients receiving r-ATG induction for 10 consecutive days with MMF, the infection rate was 23% with prophylaxis [20].

The analysis of the secondary endpoints highlights the difficulties in using the combination of CNI and mTORi in transplant recipients with expected lower renal function, even using more recent and optimized strategies [21,22]. The use of r-ATG and delayed introduction of reduced TAC exposure was associated with delayed and incomplete recovery of renal function, higher incidence of AR, higher treatment discontinuation, and ultimately higher incidence of graft loss and death among patients receiving EVR compared with MPS, all leading to early termination of the trial.

The overall incidence of DGF was 69%, very similar to that observed in a previous analysis [23], significantly higher to that observed in the United States [24] and Europe [25]. Patients receiving EVR showed longer DGF period and lower eGFR reached at month 1. In two recent large prospective multicenter trials, no significant differences in the incidence and duration of DGF were observed [21,26]. Even in patients at high risk of DGF, no difference in the incidence and duration was observed comparing immediate or delayed introduction of EVR [27]. The population included in this study has several demographic characteristics that may account for these findings. The higher incidence of DGF with slower recovery phase might be associated, at least in part, to inadequate donor management [28] and long cold ischemia time inherent to our allocation system that prioritizes HLA matching (90% zero HLA-DR mismatch), regardless of the donor type [29]. Furthermore, 51% of the

Table 2. Immunosuppressive drug doses and concentrations

	r-ATG/EVR (*n* = 88)	r-ATG/MPS (*n* = 81)
Day 3		
EVR – MPS dose, mg/day (*N*)	3 (87)	720 (81)
[EVR] ng/ml – [MPA] μg/ml (*N*)	3.3 ± 0.9 (87)	–
Day 7		
TAC dose, mg/day (*N*)	7.4 ± 1.6 (56)	7.0 ± 1.4 (49)
[TAC] ng/ml (*N*)	5.6 ± 3.4 (39)	6.0 ± 4.5 (34)
EVR – MPS dose, mg/day (*N*)	3 [3–4] (86)	1440 [1440–1440] (80)
[EVR] ng/ml – [MPA] μg/ml (*N*)	3 [3–4.5] (78)	3 [1.3–5] (80)
PRED dose, mg/day (*N*)	30 [30–30] (64)	30 [20–30] (57)
Day 14		
TAC dose, mg/day (*N*)	8.0 ± 3.4 (84)	7.3 ± 2.7 (80)
[TAC] ng/ml (*N*)	6 [4–8] (84)	6 [4–7] (80)
EVR – MPS dose, mg/day (*N*)	4 [3–4] (85)	1440 [1440–1440] (80)
[EVR] ng/ml – [MPA] μg/ml (*N*)	4 [3–4.5] (85)	3 [1.3–5] (72)
PRED dose, mg/day (*N*)	20 [20–20] (85)	20 [20–20] (81)
Day 30		
TAC dose, mg/day (*N*)	6 [4–8] (79)	6 [4–8] (79)
[TAC] ng/ml (*N*)	5 [4–7] (79)	5 [4–7] (79)
EVR – MPS dose, mg/day (*N*)	4.5 [4–6] (73)	1440 [1440–1440] (78)
[EVR] ng/ml – [MPA] μg/ml (*N*)	4.5 [4–6] (73)	3 [2–4] (78)
PRED dose, mg/day (*N*)	10 [10–15] (81)*	10 [10–10] (79)
Day 90		
TAC dose, mg/day (*N*)	4 [2–6] (78)	4 [3–6] (79)
[TAC] ng/ml (*N*)	5 [4–7] (78)*	6 [4–8] (79)
EVR – MPS dose, mg/day (*N*)	4 [3–6] (66)	720 [720–1440] (72)
[EVR] ng/ml – [MPA] μg/ml (*N*)	6 [4.4–7] (66)	3 [2–5] (72)
PRED dose, mg/day (*N*)	5 [5–5] (78)	5 [5–5] (79)
Day 180		
TAC dose, mg/day (*N*)	4 [2–6] (74)	4 (3–6] (79)
[TAC] ng/ml (*N*)	5 [4–7] (74)	6 (5–7] (79)
EVR – MPS dose, mg/day (*N*)	3 [2–5.3] (58)	720 [720–1440] (63)
[EVR] ng/ml – [MPA] μg/ml (*N*)	5 [4–7] (58)	3 [2–4] (63)
PRED dose, mg/day (*N*)	5 [5–5] (75)	5 (5–5)(79)
Day 270		
TAC dose, mg/day (*N*)	4 [2–8] (69)	4 [3–6] (78)
[TAC] ng/ml (*N*)	5 [4–7] (69)	6 [4–7] (78)
EVR – MPS dose, mg/day (*N*)	3 [2–4] (56)	720 [720–720] (61)
[EVR] ng/ml – [MPA] μg/ml (*N*)	5.7 ± 2.1 (56)	-
PRED dose, mg/day (*N*)	5 [5–5] (70)	5 [5–5] (79)
Day 360		
TAC dose, mg/day (*N*)	3 [2–6] (68)	4 [3–6] (79)
[TAC] ng/ml (*N*)	5 [4–7] (68)*	7 [5–8] (79)
EVR – MPS dose, mg/day (*N*)	3 [2–4] (53)	720 [720–1440] (58)
[EVR] ng/ml – [MPA] μg/ml (*N*)	6 [5–7] (53)	3 [2–5] (58)
PRED dose, mg/day (*N*)	5.0 ± 0.5 (69)	5 ± 0.5 (79)

TAC, tacrolimus; EVR, everolimus; MPS, mycophenolate sodium; MPA, mycophenolic acid; PRED, prednisone; [TAC], tacrolimus whole blood trough concentration; [EVR], everolimus whole blood trough concentration; [MPA], mycophenolic acid plasma trough concentration.

Data are summarized as mean ± standard deviation or median (interquartile range).

*$P < 0.005$, Mann–Whitney test.

donors had terminal creatinine > 1.5 mg/dl and 69% of the transplants were performed with kidneys with KDPI above 85%. These characteristics have been associated with higher discard rates [30]. Experimental studies suggest that the combination of CNI and mTORi interferes with cellular aerobic metabolism,

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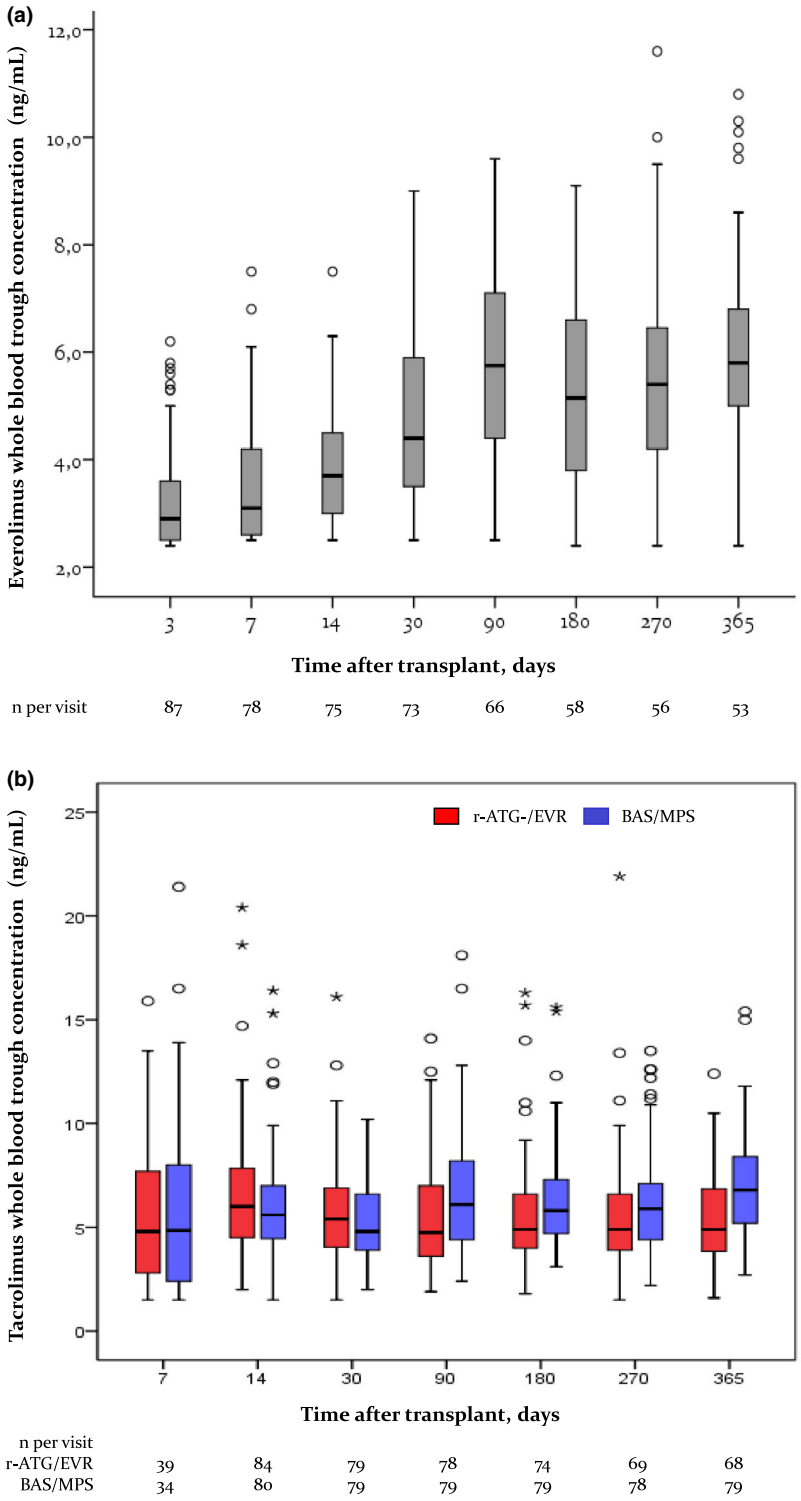

Figure 2 (a) Box plot distribution of everolimus whole blood trough concentrations in each visit during the first year after transplantation. The percentage of patients with everolimus concentration below the lower therapeutic range (4 ng/ml) in each visit is shown at the bottom (*n* = number of determinations in each visit). (b) Mean and standard deviation of tacrolimus whole blood trough concentrations in each visit during the first year after transplantation in each group.

potentially limiting recovery from ischemia-reperfusion injury in grafts with less functional reserves such as high KDPI kidneys [31,32].

Patients receiving EVR showed a higher incidence of BPAR compared with MPS, but there were no obvious differences in severity. The recent TRANSFORM trial

Table 3. Incidence of CMV infection/disease

	r-ATG/EVR (*N* = 88)	r-ATG/MPS (*N* = 81)
Incidence of first CMV event, *N* (%)*	12 (13.6)	58 (71.6)
Infection	5 (41.7)	30 (51.7)
Disease	7 (58.3)	28 (48.3)
Pretransplant CMV serostatus, *N* (%)†		
Donor (+) Recipient (+)	8 (10.1)	49 (68)
Donor (−) Recipient (+)	0	1 (100)
	3 (75)	6 (100)
Donor (+) Recipient (−)		
Donor (unknown) Recipient (+)	1 (50)	2 (66.6)
Time to first CMV event, days (mean ± SD)**	48 ± 23	35.3 ± 14.4
Duration of treatment, days, median [IQR]***	12.5 [5.5–13]	17 [13–23.3]
First CMV event after treated acute rejection, *N* (%)	6 (50)	1 (1.7)
First CMV event after EVR discontinuation, *N* (%)	3	-
Patients with recurrent CMV events*	2	26
Patients with 1 recurrence	*1*	*15*
Patients with 2 recurrences	*0*	*9*
Patients with 3 recurrences	*1*	*1*
Patients with 4 recurrences	*0*	*1*
Total number of recurrent CMV events	4	40
Total number of CMV events	16	98
CMV incidence density (*n*/1000 patients-year)	1.78	8.92

*P = 0.000, chi-square test; **P = 0.030, Student's t-test; ***$P < 0.001$ Mann–Whitney test.

†Percentage was calculated based on the prevalence of each CMV serostatus combination described in Table 1.

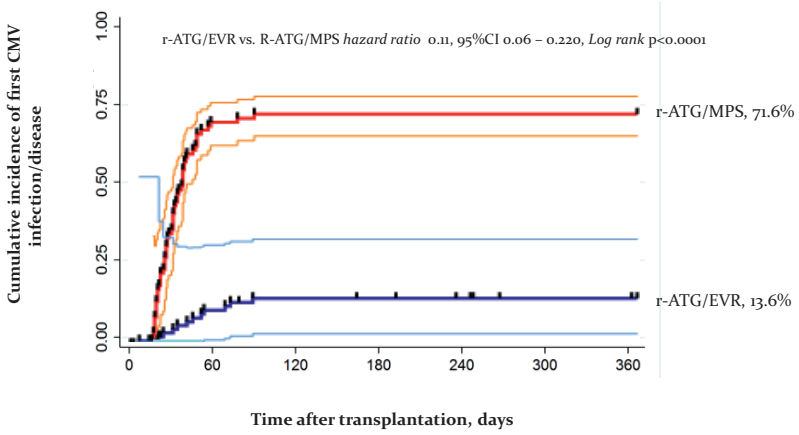

Figure 3 Comparison of the cumulative incidence of first CMV infection/disease (95% confidence intervals) during the first 12 months in each group using the Cox regression model.

showed that the incidence of BPAR is not different comparing EVR and MPA, regardless of the type of induction therapy [21]. Two factors inherent to our study design may account for the observed difference: first, the delayed introduction of TAC and second, the proportion of patients with EVR exposure below the lower therapeutic range during the first weeks after transplantation. In a study to identify the optimal dose of EVR associated with CNI, Felipe *et al.* showed that there was a significant proportion of patients with EVR concentrations below 3 ng/ml during the first week after renal transplantation [33]. The r-ATG/EVR group had 14 episodes of first BPAR, 50% occurred within the first 30 days post-transplantation and 65% of the patients had EVR below 4 ng/ml. Yet, the incidence of *de novo* DSA was low and similar in both groups.

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Table 4. Treatment failure at 12 months

Parameters, *n* (%)	r-ATG/EVR*N* = 88	r-ATG/MPS*N* = 81
Treatment failure*	27 (30.7)	5 (6.1)
Biopsy-proven acute rejection**	14 (16)	4 (5)
Graft loss***	10 (11)	1 (1)
Death†	9 (10)	1 (1)
Loss to follow-up	0	0
First treated acute rejection	22 (25)	12 (15)
Acute antibody-mediated rejection	1	1
T-cell-mediated rejection	13	3
IA	2	1
IB	5	2
IIA	4	0
IIB	0	0
III	2	0
Borderline changes	4	4
Clinical acute rejection	4	4
Time to first treated acute rejection, days mean (±SD)††	47 ± 52	158 ± 141
All treated acute rejection	25	13
Incidence density (per 1000 patients-year)	3.27	1.85
Acute antibody-mediated rejection	1	1
T-cell-mediated rejection	13	3
IA	2	1
IB	5	2
IIA	4	0
IIB	0	0
III	2	0
Borderline	7	5
Clinical acute rejection	4	4
Treated acute rejections per patient		
1	20 (22)	11 (13)
2	1 (1)	1 (1)
3	1 (1)	0
Treatment, *N* (%)	24 (27)	13 (16)
Methylprednisolone	19 (21)	11 (13)
Methylprednisolone and r-ATG	2 (1.1)	1 (0.9)
r-ATG	2 (2)	0
Methylprednisolone and plasma exchange	1 (1)	1 (1)
Patients with dnDSA, n/n tested	69/69	78/79
Class I	0	0
Class II	1	2
MFI 300–3000	0	1
MFI > 3000	1	1
Graft loss	10	1
Lack of efficacy	3	1
Venous thrombosis	2	0
Nonimmunological IF/TA	2	0
Focal segmental glomerulosclerosis	1	0
Primary nonfunction	1	0
Thrombotic microangiopathy	1	0
Death	9	1
Cardiovascular events	5	0
Acute myocardial infarction	2	0
Arrhythmia	1	0
Sudden death	1	0
Hemorrhagic stroke	1	0

Table 4. Continued.

Parameters, *n* (%)	r-ATG/EVR*N* = 88	r-ATG/MPS*N* = 81
Infections	4	1
Pneumonia	2	1
Disseminated cryptococcosis	1	0
Clostridium difficile infection	1	0

DSA, anti-HLA donor-specific antibody; MFI, mean fluorescence intensity; IF/TA, interstitial fibrosis/tubular atrophy.
*P=0.000, **P = 0.021, ***P = 0.008, $^{\dagger}P$ = 0.013, chi-square test, $^{\dagger\dagger}P$ = 0.002 Student's t-test.

Table 5. Renal function parameters during the first 12 months.

Parameters	r-ATG/EVR (n = 88)	r-ATG/MPS (n = 81)
DGF, *n* (%)	57 (65)	59 (73)
Time in DGF, days, median (IQR)[#]	6 (2–5)	4 (2–8)
Number of dialysis, medina (IQR)[&]	3 (1–6)	2 (1–4)
Renal function, ITT, eGFR ml/min/1.73 m^2, mean ± SD		
Day 7	15.5 ± 14.1	15.5 ± 16.2
Day 14	22.2 ± 18.9	26.5 ± 18.3
Month 1*	31.8 ± 22.2	39.5 ± 17.8
Month 3*	34.7 ± 20.4	44.9 ± 14.6
Month 6*	32.8 ± 19.5	45.1 ± 14.8
Month 9*	32.7 ± 19.4	42.4 ± 16.5
Month 12*	31.8 ± 18.8	42.6 ± 14.9
Urine protein/creatinine ratio, mean ± SD, month 12	0.48 ± 0.52 (n = 67)	0.56 ± 1.1 (n = 77)
Renal function, PP, eGFR ml/min/1.73 m^2, mean ± SD		
Day 7	15.5 ± 14.1	15.5 ± 16.2
Day 14	22.4 ± 19.0	26.7 ± 18.3
Month 1*	33.2 ± 22.3	39.8 ± 17.3
Month 3*	36.8 ± 20.5	45.8 ± 14.3
Month 6*	36.9 ± 19.4	46.9 ± 13.5
Month 9*	37.0 ± 19.2	44.3 ± 15.3
Month 12*	36.2 ± 18.6	44.8 ± 14.0
Urine protein/creatinine ratio, mean ± SD, month 12	0.45 ± 0.42 (n = 52)	0.54 ± 1.11 (n = 61)

DGF, delayed graft function; IQR, interquartile range; ITT, intention to treat population; PP, per protocol population.
Mean and standard deviation (SD) of estimated glomerular filtration rate in each group was calculated using the imputation method of last observation carried forward (LOCF) analysis for patients who died or lost follow up. For patients who lost the graft, eGFR was imputed as zero.
$^{\#}P$ = 0.004, $^{\&}P$ = 0.009, *P < 0.005.

Previous studies have shown that renal function at 12 months was similar in patients receiving EVR or MPA in combination with TAC [22,34,35]. The incomplete recovery of renal function observed from months 1 to 12 occurred despite comparable and low exposure to TAC. It is possible that the small imbalance in donor characteristics (higher proportion of donors with creatinine greater than 1.5 mg/dL in the r-ATG/EVR group) and the higher incidence of AR compared with r-ATG/MPS group might account for this observation. In fact, donor age, AR, and EVR use were independent risk factors associated with inferior renal function at 12 months. Unexpectedly, there was no difference in the proportion of patients with proteinuria or in the magnitude of proteinuria between the groups. Late conversion trials showed higher incidence of proteinuria in patients with lower GFR [36] and with chronic histological lesions [37], raising the possibility that the combined use of CNI might have a protective effect [38].

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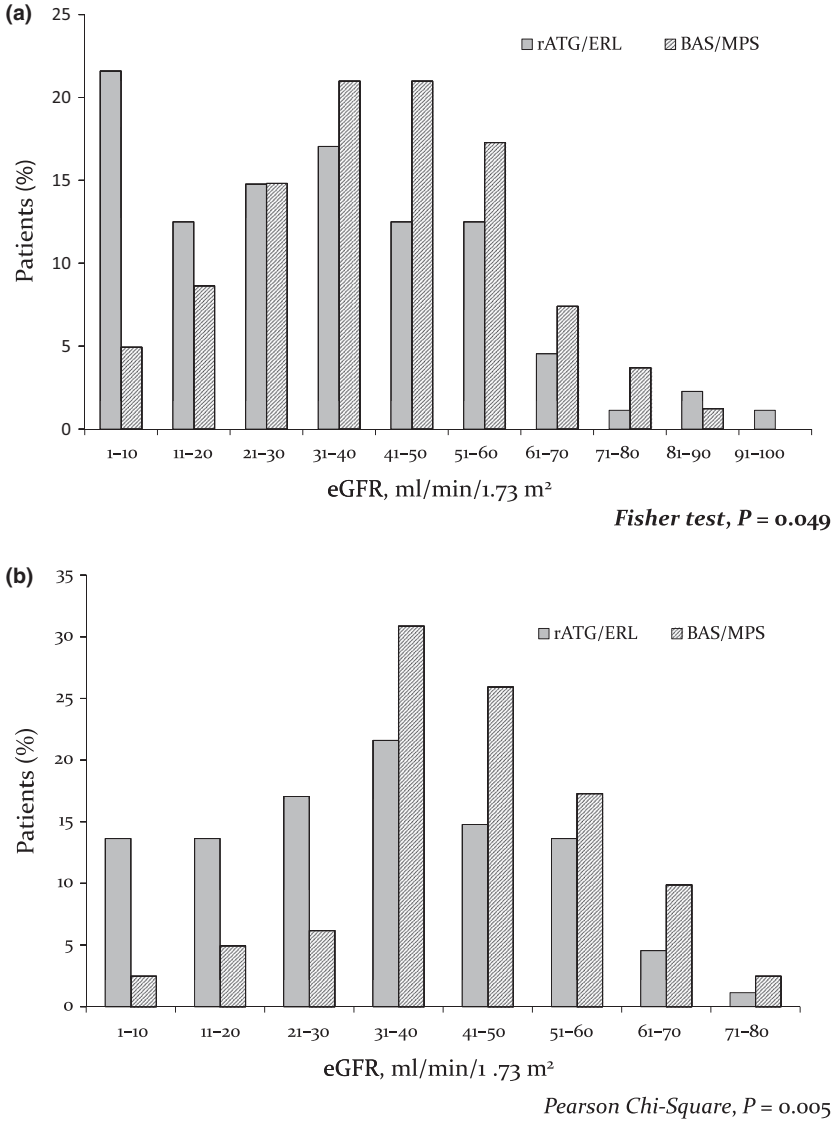

Figure 4 Proportion of patients stratified by eGFR categories at 1 month (a) and 12 months (b) after transplantation.

Regardless of recipient age, ECD kidney transplantation is associated with increased mortality and risk of graft loss [39]. These patients were more vulnerable to developing cardiovascular events, infections, and neoplasms and were more susceptible to developing drug-related adverse effects and drug toxicity [40]. The incidences of death and graft loss were higher in the r-ATG/EVR group and were the leading causes for study termination. Infection and cardiovascular events accounted for all deaths, suggesting that this regimen is not suitable for an older population with longer time on dialysis and more comorbidities such as the higher prevalence of diabetes mellitus in the r-ATG/EVR group [5].

The incidence of graft loss was also high in the r-ATG/EVR group, and the causes were either rejection or class-related adverse events. Several registry data have shown that the use of iMTOR was associated with a high mortality and graft loss [41]. Yet, a retrospective analysis of 581 patients in different clinical trials, comparing SRL or EVR or AZA or MMF combinations in ICN, showed no difference in the incidence of AR, graft loss, and death [42]. Therefore, it is plausible to speculate that the interaction of several risk factors including donor characteristics, prolonged cold ischemia time, ischemia-reperfusion injury, and pre-existing kidney histological lesions increased the susceptibility to the potential nephrotoxic effects of the r-ATG/EVR regime [43].

Table 6. Risk factors for inferior renal function (eGRF <37 ml/min/1.73 m^2) at month 12.

	Univariable analysis				Multivariable analysis			
Variable	OR	95% CI		*P*	OR	95% CI		*P*
Recipient characteristics								
Age, years	0.996	0.969	1.023	0.743				
Gender, male	0.526	0.269	1.027	0.060	0.456	0.197	1.053	0.066
Diabetes mellitus	0.467	0.227	0.961	0.039	0.543	0.214	1.383	0.201
HLA mm	0.837	0.602	1.164	0.291				
Time on dialysis, months	1.009	0.999	1.018	0.087	1.009	0.997	1.022	0.156
CMV IgG serologic status, Donor (+)/Recipient (−)	0.658	0.179	2.422	0.529				
Donor characteristics								
Age, years	1.084	1.031	1.140	0.002	1.096	1.006	1.193	0.035
Gender, male	1.023	0.559	1.871	0.942				
History of hypertension	1.835	0.910	3.699	0.090	2.197	0.887	5.440	0.089
Cold ischemia time, hours	0.979	0.928	1.032	0.422				
Kidney Donor Profile Index	1.066	1.025	1.108	0.001	1.011	0.951	1.076	0.721
Transplant characteristics								
First treated acute rejection, yes	9.592	3.182	28.914	0.000	9.894	2.860	34.222	0.000
Cytomegalovirus, yes	0.625	0.337	1.158	0.145				
Delayed graft function, yes		1.587	0.861	2.927	0.139			
Delayed graft function duration, days	1.050	1.004	1.098	0.033	1.821	0.828	4.007	0.136
Treatment, everolimus	3.31	1.76	6.22	0.000	4.346	1.969	9.593	0.000

Inferior renal function was defined as the eGFR below the median value (37 ml/min/1.73 m^2) of the total study population at 12 months.

Two recent systematic reviews and meta-analyses have been published recently: the first, involving eleven randomized controlled trials with 850 renal transplant recipients receiving EVR plus low-dose CNI, showed similar efficacy and safety compared with mycophenolate plus standard-dose CNI regimen (10.5414/CN109287). The other one, including eleven randomized controlled trials with 4930 patients receiving either sirolimus or EVR in combination with CNI, showed an increased risk of graft loss, even when combined with a reduced dose of CNI, compared with mycophenolate and CNI combination (10.1186/s12882-015-0078-5). Clearly, trial design, transplant vintage, donor and recipient demographic characteristics, type of mTOR inhibitor, drug dosing, and exposure may account for this apparent discrepancy. Importantly, these trials did not a representative proportion of recipients of kidneys recovered from ECDs. In this regard, the recent ATHENA trial, where 25% of the donors were over 65 years of age, showed that the use of similar standard TAC concentration (4–8 ng/ml) resulted in inferior renal function at month 12 among patients receiving EVR compared with mycophenolate (10.1016/j.kint.2019.01.041).

Premature study drug discontinuation was higher in the r-ATG/EVR group. Infection, unsatisfactory renal function, thrombotic events, and rejection were the predominant reasons. These findings demonstrate that the use of EVR in recipients of kidneys with high KDPI and high risk to develop DGF should be avoided. In the r-ATG/MPS, CMV infection was the leading cause of study drug discontinuation, clearly associated with the lack of pharmacological prophylaxis. Overall, 60% of the patients presented at least one wound complication, with a higher incidence in the r-ATG/EVR group that was confirmed by the higher proportion of patients requiring surgical reintervention compared with the r-ATG/MPS group. Studies investigating the incidence of wound complication in patients receiving mTORi have shown conflicting results [44,45]. Overall, it appears that the use of EVR is another risk factor for wound complication after kidney transplantation, perhaps interacting with several other known demographic characteristics such as diabetes mellitus, obesity, nutritional status, surgical procedure, and steroid dosage [46,47].

This single-center trial has several particularities limiting the extrapolation of the observed finding. The first and more relevant are the demographic characteristics

Table 7. Safety analysis including adverse events, graft loss, death, treatment discontinuations and alternative immunosuppressive regimens.

Parameters, *n* (%)	r-ATG/EVR*N* = 88	r-ATG/MPS*N* = 81
Patient with at least 1 AE, *N* (%)	83 (97)	81 (100)
Patient with at least 1 SAE, *N* (%)*	59 (67)	69(85)
Study drug discontinuation, *N* (%)	35 (40)	23 (28)
Graft loss	6	0
Venous thrombosis	*2*	–
Thrombotic microangiopathy	*1*	–
Primary non-function	*1*	–
Death	8	1
Cardiovascular events	*4*	–
Infection	4	1
Lack of efficacy	2 *(TAC/MPS/P)*	0
AEs	19	22
Infection	*4 (TAC/P)*	*16 (10 TAC/EVR/P; 6 TAC/P)*
Unsatisfactory renal function	*8 (6 TAC/P; 2 TAC/MPS/P)*	–
Thrombotic microangiopathy	*2 (EVR/MPS/P)*	*1 (MPS/P)*
Dyslipidemia	*1 (TAC/MPS/P)*	–
Leukopenia	*1 (TAC/P)*	–
Edema	*1 (TAC/MPS/P)*	–
Delayed graft function	*1 (TAC/MPS/P)*	–
Focal and segmental glomerulosclerosis	*1 (TAC/MPS/P)*	–
Anemia	–	*1 (TAC/P)*
Gastrointestinal disorders	–	*4 (2 TAC/P; 2 TAC/AZA/P)*
Patient with at least WHC, *N* (%)	55 (63)	47 (58)
All reported WHC, *N*	129	101
Hematoma	36 (28)	24 (24)
Lymphocele	25 (20)	12 (12)
Lymphorrea	17 (13)	23 (22)
Dehiscence	24 (18)	17 (17)
Infection	7 (5)	12 (12)
Hyperemia	15 (12)	11 (11)
Urine leak	5 (4)	2 (2)
Surgical reinterventions, *N* (%)	26 (47)	14(30)

AE, adverse event; EVR, everolimus; MPS, mycophenolate; P, prednisone; SAE, serious adverse event; TAC, tacrolimus; WHC, wound healing complications.
**P* = 0.006, chi square test.

of the donor and recipient, leading to a significantly high incidence of DGF. Second, at the time this study was conducted and pp65 antigenemia test was still a valid option to monitor CMV replication to trigger pre-emptive treatment based on a clinically validated threshold. Currently, monitoring of CMV replication is using commercially available and standardized quantitative nucleic acid amplification test. Third, while the study was powered to, and met the primary endpoint in reducing the incidence of CMV infection/disease, the obvious difference in the incidence of death and graft loss was sufficient to terminate the trial.

In summary, in these low immunological risk recipients of ECD kidneys receiving induction therapy with r-ATG, low TAC exposure, faster taper of prednisone, and no CMV pharmacological prophylaxis, coadministration of EVR was associated with a lower incidence of CMV infection/disease compared with MPS. This potential benefit was surpassed by the higher incidence of AR, graft loss, and death, possibly associated with delayed and incomplete recovery of renal function. Therefore, under the conditions of this trial, the use of EVR in ECD kidney transplant recipients should be avoided.

Authorship

ANF, CRF, and HT-SJ: designed the study, performed the study, collected the data, analyzed the data, and wrote and revised the manuscript. MC, LV, JM, MdP, DW, WA, and JM-P: performed the study and wrote and revised the manuscript. RdM: performed the study, collected the data, analyzed the data, and wrote and revised the paper. MG-D and HP: performed the study, analyzed the data, and wrote and revised the paper.

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Conflict of interest

The institution "Hospital do Rim" received research grants from Novartis and Sanofi to conduct this study.

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SUPPORTING INFORMATION

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

Figure S1. Proportion of patients receiving kidneys with increasing KDPI percentage range in each group.

Figure S2. Proportion of patients with 1–4 doses of 1.5 mg/kg of r-ATG in each group.

Figure S3. Box-plot distribution of MPA plasma concentrations in each visit during the first year after transplantation (n = number of determinations in each visit).

Figure S4. Kaplan Meier estimates and 95% confidence intervals for overall graft survival (A), death-censored graft survival (B) and patient survival (C). Differences between the two groups were identified using the Log-rank test.

Table S1. Proportion of patients with EVR and TAC whole blood trough concentrations below the lower limit of therapeutic range in each study visit.

Table S2. Individual data of patients with graft loss or death.

Table S3. Renal function parameters during the first 12 months.

Table S4. Timing and drug concentrations of patients with first treated acute rejection episodes.

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