De novo everolimus for recipients of kidney transplants from HLA identical donors

Uso de everolimo de novo em receptores de transplante renal com doador vivo HLA idêntico

ABSTRACT

Introduction: Kidney transplant recipients from HLA-identical living donor have lower risk of acute rejection and greater graft survival compared to other types of kidney transplantation. Immunosuppressive regimens without calcineurin inhibitors (CNI) can further improve these results by reducing cardiovascular, metabolic and toxic events related to this drug class. Objective: This study aimed to evaluate efficacy and safety of a new immunosuppressive regimen with planned suspension of CNI. Methods: This was a prospective, single center and single treatment arm study to evaluate HLA-identical kidney transplant recipients receiving everolimus (EVR), tacrolimus (TAC) and corticosteroids, followed by TAC discontinuation 30 days after transplantation. TAC discontinuation was later postponed to the third month after an interim efficacy analysis. Results: Thirty-nine patients were included. Although mean TAC and EVR blood concentrations have remained within the proposed therapeutic ranges, five patients had biopsy-proven acute rejection and one patient had an episode of C4D-positive glomerulitis. This result led to the end of the inclusions. Interestingly, the proportion of patients with proteinuria greater than 0.5 g/L has not reached more than 22% of patients in any visit. Adverse events related to EVR use were the most incident in this population: oral ulcers, dyslipidemia and peripheral edema. Conclusion: The proposed scheme was not effective for this population, particularly due to a high incidence of acute rejection. Safety profile showed that prolonged exposure to a high concentration of blood EVR increases the incidence of adverse events related to this drug.

Keywords: immunosuppression; kidney transplantation; living donors.

RESUMO

Introdução: Receptores de rim de doadores vivos HLA-idêntico apresentam menor risco para rejeição aguda e maior sobrevida do enxerto, quando comparado a outros tipos de transplante. Um regime imunossupressor sem inibidor de calcineurina (ICN) pode melhorar ainda mais esses resultados, através da redução de eventos cardiovasculares, metabólicos e tóxicos secundários a esse fármaco. Objetivo: Avaliar eficácia e segurança do novo tratamento imunossupressor com suspensão planejada do ICN. Métodos: Estudo prospectivo, aberto, braço único de tratamento em único centro para avaliar resultados do transplante renal HLA-idêntico em pacientes que recebem everolimo (EVR), tacrolimo (TAC) e corticoide, seguido da descontinuação do TAC 30 dias pós-transplante. Após análise interina de eficácia, a descontinuação do TAC foi postergada para o terceiro mês pós-transplante, através de emenda ao protocolo. Resultados: Trinta e nove pacientes foram incluídos. Apesar de as médias das concentrações de TAC e EVR terem respeitado os intervalos propostos, cinco pacientes tiveram rejeição aguda com aprovada por biópsia e um paciente apresentou um episódio de glomerulite com depósitos de C4D. Esse resultado demandou o fim das inclusões. A proporção de pacientes com proteinúria > 0.5g/L não atingiu mais que 22% dos pacientes em nenhuma visita. Os eventos adversos mais frequentes foram relacionados ao uso de EVR: úlceras orais, dislipidemia e edema periférico. Conclusão: O regime proposto não foi eficaz para essa população, principalmente pela alta incidência de rejeição aguda. O perfil de segurança mostrou que a exposição prolongada a altas concentrações sanguíneas de EVR aumenta a incidência dos eventos adversos relacionados ao fármaco.

Palavras-chave: doadores vivos; imunossupressão; transplante de rim.

Authors
Marina Pontello Cristelli 1
Alexandra Ferreira 1
Pedro Hannun 1
Claudia Felipe 1
Wilson Aguiar 1
Marcello Franco 1
Hélio Tedesco-Silva 1
José Medina-Pestana 1

1 Hospital do Rim, Fundação Osvaldo Ramos - Universidade Federal de São Paulo.
Everolimus in HLA-identical kidney transplantation

INTRODUCTION

In the last two decades, there has been a clear improvement in the outcomes of kidney transplant, especially after the introduction of calcineurin inhibitors (CNI). HLA-identical renal transplantation, which intrinsically has the lowest risk of acute rejection, graft loss and death, also achieved these improvements.

Since it is well established that CNI exposure and acute rejection are well correlated in the first 3 months after transplantation, and that the majority of acute rejection episodes occurs within the first month post-transplant, maintaining CNI in the first 30 days is critical to the success of transplantation. By the other side, due to the increased life expectancy, HLA-identical renal transplant recipients usually remains exposed to CNI for long periods of time, and are at increased risk of morbidity and mortality from cardiovascular events and malignancy. Despite the evident role of immunosuppression, there is still no viable strategies to prevent the development of cardiovascular neoplastic events. The introduction of new immunosuppressive agents, mainly everolimus (EVR), emerged as a real possibility to overcome this challenge.

EVR-based immunosuppressive regimens allow minimization or withdrawal of CNI, thereby reducing cardiovascular and neoplastic risks associated with chronic use of these agents, in addition to bringing additional benefits such as lower incidence of viral infections. The purpose of this study was, therefore, to develop an alternative immunosuppressive regimen capable of maintaining efficacy and resulting in better long-term safety profile in HLA-identical renal transplant recipients.

OBJECTIVES

The aim of this study was to investigate outcomes of HLA-identical renal transplantation with an EVR-based immunosuppressive regimen.

PRIMARY OBJECTIVE

To evaluate the efficacy and safety of a new EVR-based immunosuppressive regimen with planned withdrawal of calcineurin inhibitor.

SECONDARY OBJECTIVE

To evaluate the incidence of acute rejection, renal function, wound healing events, adverse events and laboratory changes in 12 months of follow-up.

METHODS

STUDY DESIGN

This was a prospective, single center, open-label, single arm study. The protocol was reviewed and approved by the Ethics Committee of UNIFESP/EPM. Written informed consent was obtained from all patients following approval from the Ethics Committee and before inclusion. The study was conducted in compliance with Good Clinical Practice and the Declaration of Helsinki.

All study participants underwent renal transplantation with HLA-identical living donors, and began the immunosuppressive regimen consisting of tacrolimus (TAC)/prednisone (PRED)/EVR in the first day after surgery. One month after transplantation, TAC was discontinued, and the patients remained in use of EVR/PRED. Doses of immunosuppressive drugs were adjusted according trough blood concentrations and the occurrence of adverse events. An amendment to the protocol postponed discontinuation of TAC from one month to three months after transplantation, due to the occurrence of frequent episodes of acute rejection.

INCLUSION AND EXCLUSION CRITERIA

The study included adult kidney transplant recipients from a HLA identical living donor. The study excluded patients who (1) were in use of immunosuppressive drugs before transplantation; (2) were in use of an investigational drug within the last 12 months before transplantation, and (3) had previous known history of hepatitis B, hepatitis C and HIV infection and/or malignancy, except non-melanoma skin cancer. Women at childbearing age were advised to and agreed with the use of contraceptive methods.

TREATMENT

All patients received 1g of methylprednisolone intraoperatively, just before graft revascularization. On the first day after transplantation, these patients received an initial dose of TAC 0.1 mg/kg b.i.d, EVR 1.5 mg b.i.d. and PRED 0.5 mg/kg/day (maximum of 30 mg/day). PRED was tapered (5 mg/week) to 5 mg/day on day 30 after transplantation. The first TAC and EVR trough blood concentrations were measured on the third day after transplantation. The doses of TAC and EVR were adjusted according to the trough blood concentrations to maintain therapeutic levels between 3 ng/mL and 8 ng/mL and 4 ng/mL to 8 ng/mL, respectively.
The dose of TAC was reduced by half after the first month of transplantation, and permanently discontinued the following week. After an interim analysis of efficacy, there was observed a high incidence of acute rejection (22%), when compared to historical data of HLA-identical population in the center (around 6%). Thus, an amendment to the protocol was made to postpone the reduction and discontinuation of TAC until the third month after transplantation. The other drugs was kept unchanged after discontinuation of TAC, and the dose of EVR was adjusted to a new therapeutic target between 6 ng/mL and 10 ng/mL. Adjustments due to adverse reactions and poor tolerability were allowed, and were conducted at an individual basis.

**Prophylaxis**

All patients received prophylaxis against *Pneumocystis jirovecii* with trimethoprim-sulfamethoxazole. No other prophylaxis was administered to these patients.

**Visits and Procedures**

Study visits were performed at the immediate pre-transplant day, at days 1 and 3 after transplantation; at weeks 1, 2 and 3 after transplantation; and at months 1, 2, 3, 6, 9 and 12 after renal transplantation.

**Primary Outcomes**

Efficacy was evaluated by incidence of treatment failure, defined as the occurrence of the first episode of biopsy proven acute rejection, graft loss, death, or discontinuation of treatment. The number of discontinuations of the proposed treatment defined safety outcomes.

**Secondary Outcomes**

**Acute Rejection**

We evaluated all acute rejection episodes according if clinical and biopsy-proven acute rejection; time from transplantation, severity on Banff 2009 Classification (for episodes greater than or equal to IA); type of treatments and outcomes. Allograft biopsies were performed as if clinically indicated, except for a protocol histological analysis by optical microscopy and immunofluorescence at 12 months after transplant (only for those who agreed).

**Renal Function**

At each visit, data regarding renal function were collected: estimated glomerular filtration rate (eGFR) based on creatinine and according to the Modification of Diet in Renal Disease (MDRD) formula, and protein/creatinine ratio in spot urine.

**Wound Healing**

Wound healing complications related to transplant surgery were assessed by physical evaluation and protocol ultrasound after 30 days post transplantation.

**Adverse Events**

At each visit, adverse events were collected through a specific form. Serious adverse events were reported to the Ethics Committee and to the study sponsor. The events were described and graduated according to the Common Terminology Criteria for Adverse Events, CTCAE, version 4.0.

**Laboratorial Data**

Local hematology and biochemistry assessment (lipid profile, liver enzymes, and electrolytes) and tacrolimus and everolimus trough blood concentrations ($C_0$) were collected at each visit. Virological assessment (cytomegalovirus (CMV), Epstein Barr virus (EBV) and polyomavirus blood test viral load) was performed at a monthly basis.

**Statistical Analysis**

Analysis of the primary and secondary endpoints were performed for the intent-to-treat (ITT) population and for the on-therapy population (defined as all patients who remained on the assigned treatment).

Statistical comparisons between the two treatment groups were performed using the Fisher’s exact test for categorical variables and repeated measures ANOVA tests for continuous variables. All p-values were two-sided, and a $p$-value $< 0.05$ was considered statistically significant.

**Results**

**Demography**

Patients were included between 04/09/2012 and 04/14/2014, and were followed until 09/04/2015. Although the number of patients initially proposed was 100 patients, only 47 were selected and, of those, 39 received at least one dose of study medication (Figure 1).

Mean age of the included patients was 43 years, 53.8% were male, 61.5% were Caucasian, and 92.3%
were recipients of a first kidney transplant (Table 1). Most patients had unknown cause of chronic kidney disease (51.3%). Among the specific causes, the most frequent one was glomerulonephritis (15.3%). Mean time on dialysis was 22 months (22.6 ± 20.0 months).

**IMMUNOSUPPRESSION**

On days 30, 60, 90, 180 and 365, mean EVR $C_0$ in the ITT population was $5.3 \pm 2.0$ ng/mL, $6.4 \pm 2.1$ ng/mL, $7.2 \pm 1.9$ ng/mL, and $7.5 \pm 1.7$ ng/mL, respectively. Mean TAC $C_0$ on days 30, 60 and 90 was $6.9 \pm 4.1$ ng/mL, $5.1 \pm 4.4$ ng/mL, and $4.7 \pm 4.0$ ng/mL, respectively. TAC was discontinued in 14 patients after the first month post transplantation, as indicated by the initial protocol. In these patients, mean EVR $C_0$ on days 60 and 90 was $6.2 \pm 1.9$ ng/mL and $7.15 \pm 2.5$ ng/mL, respectively (Table 2).

Mean EVR $C_0$ in the on-therapy population, in the same visits as mentioned above, was $5.5 \pm 2.2$ ng/mL, $6.3 \pm 2.1$ ng/mL, $7.5 \pm 1.8$ ng/mL, and $7.6 \pm 1.5$ ng/mL, respectively. For the 14 patients who discontinued TAC by month 1, mean EVR level at month 3 was 7.6 ng/mL (Table 2).

Mean doses of PRED was toward the recommended target dose of 5 mg/day after the first month (M2: 6.1 ± 1.7 mg/day; M3: 5.7 ± 4.1 mg/day; M6: 5.0 mg/day; and M9: 5.9 ± 5.7 mg/day) (Table 2).

**Efficacy**

The cumulative incidence of treatment failure was 28.2%, and was mostly secondary to the occurrence of biopsy proven acute rejection (incidence of 15.4%, Table 3).

The rejection episodes occurred on average at four months after TAC discontinuation of TAC (4.1 ± 3.7 months, ranging from 1 to 10 months after discontinuation of the drug), and were mostly classified as mild to moderate (Table 3). However, one patient developed a severe rejection episode. This patient had been discontinued of TAC after three months of transplantation, kept EVR $C_0$ slightly below the range proposed by the protocol (EVR...
around 5 ng/mL, and had stable renal function before the episode of acute rejection (creatinine 1.7 mg/dL, protein/creatinine ratio 0.3).

Nine months after transplantation, there was a worsening of renal function associated with an increasing of proteinuria (up to creatinine 2.0 mg/dL and protein/creatinine ratio 4.02). Histological analysis showed IIA acute rejection, which required treatment with polyclonal antibody for seven days. TAC promptly switched EVR, and mycophenolate sodium was associated to the regimen approximately one month later. At follow-up, patient renal function returned to baseline (creatinine 1.8 mg/dL in the next visits).

Besides the episodes of biopsy-proven acute rejection, there were two episodes of acute graft dysfunction in one patient: the first one classified as clinical acute rejection, and the second one classified as borderline rejection. The first episode occurred fifteen days after transplantation, and was characterized by an abrupt increase of creatinine (from 0.87 mg/dL to 6.09 mg/dL), new onset of proteinuria (protein/creatinine ratio 4.25) and histological findings of glomerulitis and C4d deposition. Anti-HLA specific donor antibodies (anti-A, anti-B and anti-DR) were absent.

The concentrations of immunosuppressive drugs were within the appropriate range (TAC 6 ng/mL and EVR 3.1 ng/mL). There was a rapid clinical response after methylprednisolone pulse (declining creatinine to 1.05 mg/dL). The second episode occurred six months and a half after the first one, and presented with a less severe dysfunction (creatinine from 0.9 mg/dL to 1.3 mg/dL, no proteinuria). At this time, the patient was in use of EVR and PRED, and the EVR C0 was within the recommended range (7.7 ng/mL). Histological analysis showed borderline rejection and grade I-II IF/TA. The patient received with methylprednisolone pulse for 3 days, with complete clinical response (creatinine 1.16 mg/dL during follow-up).

There was only one graft loss due to renal artery thrombosis. There were no deaths during the study period. Patient and graft survivals at 12 months were 100% and 97.4%, respectively.

Only eight patients underwent protocol biopsy at 12 months. Of these, only two presented histological abnormalities. In one of the patients, there was grade I-II IF/TA. In the other, there were mesangial IgA deposits, consistent with IgA nephropathy. All biopsies were negative for C4d deposition.

### Renal Function

Mean eGFR in the ITT population remained above 60 mL/min/1.73m² during follow-up. However, there was a significant decline of eGFR over time. Specifically, eGFR at first month post transplantation was 73.4, versus 62.6 mL/min/1.73m² at month 12 (p = 0.001). Patients who discontinued TAC in the first month had a more apparent impairment in renal function compared to those who had discontinued TAC later.

### Table 1: Demographic Characteristics of the Included Population

| Characteristic                              | Value          |
|--------------------------------------------|----------------|
| Recipient age, years (mean ± SD)           | 43.3 ± 12.5    |
| First transplantation, N (%)               | 36 (92.3)      |
| Male gender, N (%)                         | 21 (53.8)      |
| Ethnicity, N (%)                           |                |
| Caucasian                                  | 24 (61.6)      |
| Black                                      | 7 (17.9)       |
| Mixed                                      | 7 (17.9)       |
| Other                                      | 1 (2.6)        |
| Cause of end stage renal disease, N (%)    |                |
| Glomerulonephritis                         | 6 (15.4)       |
| Hypertension                               | 1 (2.6)        |
| Diabetes Mellitus                          | 2 (5.1)        |
| Unknown                                    | 20 (51.3)      |
| Other                                      | 10 (25.6)      |
| Time on dialysis (mean ± SD)               | 22.6 ± 20.0    |
| Type of therapy, N (%)                     |                |
| Hemodialysis                               | 34 (87.2)      |
| Peritoneal dialysis                        | 3 (7.7)        |
| Panel reactive antibodies, (%)             |                |
| Class I (mean ± SD)                        | 18.2 ± 34.1    |
| Class II (mean ± SD)                       | 74 ± 23.3      |
| EBV positive IgG, N (%)                    | 37 (94.9)      |
| HCV positive IgG, N (%)                    | 0 (0)          |
| HBV positive IgG, N (%)                    | 0 (0)          |
| CMV positive IgG, N (%)                    | 36 (92.3)      |
| Donor age, years (mean ± SD)               | 41.8 ± 10.3    |
| Male donor, N (%)                          | 24 (61.5)      |
| Donor ethnicity, N (%)                     |                |
| Caucasian                                  | 25 (64.1)      |
| Black                                      | 6 (15.4)       |
| Mixed                                      | 7 (17.9)       |
| Other                                      | 1 (2.6)        |

Mean eGFR in the ITT population remained above 60 mL/min/1.73m² during follow-up. However, there was a significant decline of eGFR over time. Specifically, eGFR at first month post transplantation was 73.4, versus 62.6 mL/min/1.73m² at month 12 (p = 0.001). Patients who discontinued TAC in the first month had a more apparent impairment in renal function compared to those who had discontinued TAC later.
Temporal decline of renal function was even more accentuated among on-therapy population. Specifically, eGFR at first month post transplantation was 73.4, versus 63.1 mL/min/1.73m² at month 12 (p = 0.001). Patients who discontinued TAC in the first month also had a worse renal function compared to those who had discontinued TAC at month 3 (Table 4). There were no significant changes on the protein excretion over time, as assessed by protein/creatinine ratio, for both the ITT (month 1: and on-therapy population (Table 4).

**Safety**

All patients had at least one adverse event. Among the ITT population, the incidence of serious adverse events was 35.9%, and the incidence of adverse events leading to discontinuation of the drug was 5.1%. The most common events were oral ulcers (17.9%), hyperlipidemia (46.2%), and peripheral edema (48.7%). Proteinuria, new onset diabetes after transplantation, anemia and thrombocytopenia reported as adverse events had an incidence of 8%, 5.2%, 5.2% and 2.6%, respectively. From all
patients, 23.7% had at least one clinical event related to surgical wound healing complication, and the most prevalent one was lymphocele (25.6% of all wound related events). Only two patients underwent surgical procedure because of wound-related events. One third of all patients had subclinical findings at ultrasound protocol, mostly consisting of small perigraft fluid collections (Table 5).

During 12 months of follow-up, one patient developed a squamous cell skin carcinoma, and one patient developed CMV infection. There was no pattern of CMV, EBV, or polyomavirus replication. Between one and three patients had detectable viral load of CMV and EBV in at least one visit, without any clinical manifestation. No patient developed polyomavirus viremia or disease.

The most striking laboratory finding was the progressive increase of serum triglycerides levels (M1: 200 ± 117 mg/dL vs. M3: 218 ± 103 mg/dL vs. M6: 231 ± 122 mg/dL vs. M12: 257 ± 258 mg/dL, p = 0.001). Hematologic and glycemic profiles did not show significant changes during follow-up.

**Treatment Discontinuation**

Five patients stopped the study treatment due to lack of efficacy (60% of them had been discontinued TAC with only one month of transplantation). All these patients were restarted on TAC, or remained on the initial treatment consisted by TAC/PRED/EVR. One patient had EVR switched to mycophenolate, taking into account the severity of the acute rejection episode. One patient stopped the study treatment due to wound healing complications after valve replacement surgery (secondary to infective endocarditis), and another one due to IgA nephropathy. These patients remained only with TAC/PRED.

| Table 4 | Renal function according follow-up visits. Intent-to-treat and on-treatment population |
|---------|-------------------------------------------------------------------------------------|
|         | Month 1 | Month 2 | Month 3 | Month 6 | Month 9 | Month 12 |
| Intent-to-treat population (N = 39) |         |         |         |         |         |         |
| Creatinine, mg/dL (mean ± SD) | 1.2 ± 0.3 | 1.3 ± 0.6 | 1.2 ± 0.3 | 1.3 ± 0.3 | 1.3 ± 0.3 | 1.3 ± 0.4 |
| eGFR, mL/min/1.73m² (mean ± SD) | 73.3 ± 21.3 | 68.6 ± 23.1 | 71.4 ± 24.2 | 66.4 ± 20.4 | 65.8 ± 18.6 | 62.6 ± 17.1 |
| Protein/creatinine urinary ratio, (mean ± SD) | 0.4 ± 0.6 | 0.3 ± 0.3 | 0.2 ± 0.2 | 0.3 ± 0.4 | 0.3 ± 0.5 | 0.3 ± 0.5 |
| Proteinuria > 0.5 mg/L, N (%) | 9 (23.1) | 4 (10.3) | 1 (2.7) | 4 (10.3) | 5 (12.8) | 7 (17.9) |
| On-treatment population (N = 32) |         |         |         |         |         |         |
| Creatinine, mg/dL (mean ± SD) | 1.2 ± 0.3 | 1.2 ± 0.4 | 1.2 ± 0.3 | 1.2 ± 0.3 | 1.2 ± 0.3 | 1.3 ± 0.3 |
| eGFR, mL/min/1.73m² (mean ± SD) | 73.4 ± 22.5 | 70.3 ± 22.3 | 72.4 ± 24.2 | 67.9 ± 21.2 | 65.8 ± 18.2 | 63.1 ± 17.0 |
| Protein/creatinine urinary ratio, (mean ± SD) | 0.4 ± 0.7 | 0.3 ± 0.3 | 0.2 ± 0.2 | 0.2 ± 0.3 | 0.3 ± 0.4 | 0.3 ± 0.6 |
| Proteinuria > 0.5 mg/L, N (%) | 7 (21.9) | 4 (12.5) | 0 | 3 (9.4) | 4 (12.5) | 6 (19.8) |

| Table 5 | Safety analysis, intent-to-treat population |
|---------|------------------------------------------|
| N = 39 |                                        |
| Patients with any adverse event, N (%) | 39 (100) |
| Patients with at least one infectious complication, N (%) | 25 (64.1) |
| Patients with any serious adverse event, N (%) | 14 (35.9) |
| Patients with at least one serious infectious complication, N (%) | 7 (17.9) |
| Wound healing complications |                                      |
| Patients with at least one clinical event, N (%) | 9 (23.1) |
| All reported events, N (%) | 13 (33.4) |
| Lymphocele | 5 (12.8) |
| Urinary fistula | 1 (2.6) |
| Surgical site infection | 2 (5.1) |
| Incisional hernia | 1 (2.6) |
| Wound dehiscence | 1 (2.6) |
| Perigraft hematoma | 2 (5.1) |
| Graft hydronephrosis | 1 (2.6) |
| Patients with event detected by protocol ultrasound, N (%) | 13 (33.4) |
| Patients requiring surgical intervention, N (%) | 2 (5.1) |
| All reported events requiring surgical intervention, N (%) | 3 (7.7) |
| Lymphocele | 1 (2.7) |
| Incisional hernia | 1 (2.7) |
| Perigraft hematoma | 1 (2.7) |

**Discussion**

Most immunosuppressive regimens described for HLA-identical renal transplants include
Everolimus in HLA-identical kidney transplantation

corticosteroids (CS) and CNIs,\textsuperscript{11-24} although previous studies have already demonstrated that MPA monotherapy or azathioprine could achieve satisfactory results.\textsuperscript{1} To date, there is no reported studies using EVR in this population.

Acute rejection rates among HLA-identical renal transplant recipients vary widely among the studies: 8.1\% to 55\% with azathioprine-CS;\textsuperscript{11,14,19,24} 0\% to 21\% when adding a CNI to azathioprine or MMF plus CS;\textsuperscript{11,14,19,21} 3.2\% to 49\% in regimens with cyclosporine-CS;\textsuperscript{11,18,24} and 16.7\% using cyclosporine monotherapy.\textsuperscript{24} Despite the diversity of these results, these rates are considerably higher than the current ones, lower than 10\% among living-donor transplant recipients receiving triple therapy.\textsuperscript{25} In the present analysis, the new proposed regimen was associated with an incidence of rejection of 15.4\%, and all episodes occurred after discontinuation of the CNI. This prohibitive high rate demanded the end of the inclusions.

It is possible that this high rate of acute rejection in patients with that low-immunological risk could be explained by immunological response directed towards minor histocompatibility antigens (mHAgs), and/or non-HLA graft antigens.\textsuperscript{26-28} According Gerrits and colleagues, recipient T-cell responses were directed against mHAgs and/or other non-HLA antigens of the donor in 67\% of HLA-identical renal transplants, and this finding was detectable up to a year after transplantation.\textsuperscript{27}

Therefore, modification of a stable immunosuppressive regimen might have disturbed the delicate immune balance, and led to development of acute rejection.\textsuperscript{29,30} From our cohort, eight patients (5.1\%) had panel reactive antibodies (PRA) greater than 50\% before transplantation (actually, four patients had PRA > 90\%), strongly suggesting that immune response and acute rejection developed against these types of antigens. During the period in which patients were on triple therapy, our study showed that maintenance therapy with low-dose CNI plus EVR provided an effective and safe regimen for HLA-identical renal transplant recipients.

Regarding safety, the proportion of adverse events related to the EVR was higher than reported in recent clinical studies. Compared to the A2309 trial,\textsuperscript{8} in what immunosuppressive regimens with EVR resembled the initial scheme of the present study, there was a more than doubled incidence of hyperlipidemia (20.8\% for EVR 0.75 mg b.i.d. and 21.6\% for EVR 1.5 mg b.i.d.) and oral ulcers (3.3\% for EVR 0.75 mg b.i.d. and 5.0\% for EVR 1.5 mg b.i.d.). This finding was probably due to the compulsory increase in EVR C\textsubscript{0} after discontinuation of CNI. Interestingly, there were no high rates of proteinuria or adverse events related to wound healing, which could be explained by the low initial exposure to the mTOR inhibitor.

This study has some limitations. In the first place, its single-center feature prevents generalizability to other populations. Second, the absence of a control group limits the interpretation of the results obtained, although we have tried to mitigate this by analyzing historical published cohorts. Finally, the short period of follow-up could not have been long enough to capture the outcomes being evaluated, mainly recipient death and graft loss.

\section*{Conclusion}

In this study, the new EVR-based immunosuppressive regimen with planned withdrawal of calcineurin inhibitor was not effective for HLA-identical renal transplant recipients, particularly due to a high incidence of acute rejection. The safety profile showed that a prolonged exposure to high blood concentrations of EVR resulted in frequent adverse events related to this drug. However, a regimen with low-dose TAC in combination with EVR proved to be able to provide the desired efficacy.

\section*{Competing interests}

This academic study is financially supported by Novartis. The authors do not receive any reimbursement or financial benefits and declare that they have no competing interests. Novartis played no role in the design, methods, data management or analysis or in the decision to publish.

\section*{References}

1. Shimmura H, Tanabe K, Ishida H, Miyamoto N, Tokumoto T, Ishikawa N, et al. Long-term results of living kidney transplantation from HLA-identical sibling donors under calcineurin inhibitor immunosuppression. Int J Urol 2006;13:502-8. DOI: http://dx.doi.org/10.1111/j.1442-2042.2006.01350.x
2. Takiff H, Cook DJ, Himaya NS, Mickey MR, Terasaki PI. Dominant effect of histocompatibility on ten-year kidney transplant survival. Transplantation 1988;45:410-5. PMID: 3278435 DOI: http://dx.doi.org/10.1097/00007890-198802000-00033
3. Terasaki PI, Cho Y, Takemoto S, Cecka M, Gjertson D. Twenty-year follow-up on the effect of HLA matching on kidney transplant survival and prediction of future twenty-year survival. Transplant Proc 1996;28:1144-5. PMID: 8658601
4. Terasaki PI. The HLA-matching effect in different cohorts of kidney transplant recipients. Clin Transpl 2000;497-514. PMID: 11512361

5. Israe AK, Riad SM, Leduc R, Oetting WS, Guan W, Schlaf D, et al.; DeKAF Genomics Investigators. Tacrolimus trough levels after month 3 as a predictor of acute rejection following kidney transplantation: a lesson learned from DeKAF Genomics. Transplant Int 2013;26:982-9. DOI:http://dx.doi.org/10.1111/tri.12155

6. Galante NZ, Tedesco HS, Machado PG, Pacheco-Silva A, Medina-Pestana JO. Acute rejection is a risk factor for long-term survival in a single-center analysis of 1544 renal transplants. Transplant Proc 2002;34:508-13. DOI:http://dx.doi.org/10.1016/S0041-1345(02)02613-1

7. Campbell SB, Hothersall E, Preston J, Brown AM, Hawley CM, Wall D, et al. Frequency and severity of acute rejection in live- versus cadaveric-donor renal transplants. Transplantation 2003;76:1452-7. PMID: 14657685 DOI:http://dx.doi.org/10.1097/01.TP.0000083895.64198.10

8. Tedesco Silva H Jr, Cibrik D, Johnston T, Lackova E, Mange K, Panis C, et al. Everolimus plus reduced-exposure CsA versus mycophenolic acid plus standard-exposure CsA in renal-transplant recipients. Am J Transplant 2010;10:1401-13. DOI:http://dx.doi.org/10.1111/j.1600-6143.2010.03129.x

9. Blagosklonny MV. Immunosuppressants in cancer prevention and therapy. Oncoimmunology 2013;2:e26961. DOI:http://dx.doi.org/10.4161/oni.26961

10. Brennan DC, Legendre C, Patel D, Mange K, Wiland A, McCague K, et al. Cytomegalovirus incidence between everolimus versus mycophenolate in de novo renal transplants: pooled analysis of three clinical trials. Am J Transplant 2011;11:2453-62. DOI:http://dx.doi.org/10.1111/j.1600-6143.2011.03674.x

11. Moon JI, Kim YS, Chung SY, Kim MS, Kim SI, Park K. Long-term results of kidney transplantation between HLA-identical siblings. Surg Today 2001;31:123-8. DOI: http://dx.doi.org/10.1007/s005950170195

12. de Mattos AM, Bennett WM, Barry JM, Norman DJ, et al. HLA-identical sibling renal transplantation-a 21-yr single-center experience. Clin Transplant 1999;13:158-67. PMID: 10202612 DOI:http://dx.doi.org/10.1034/j.1399-0012.1999.130202.x

13. Peddi VR, Weiskittel P, Alexander JW, Woodle ES, First MR. HLA-identical renal transplant recipients: immunosuppression, long-term complications, and survival. Transplant Proc 2001;33:3411-3. PMID: 11750460 DOI:http://dx.doi.org/10.1016/S0041-1345(01)02470-8

14. Kettel E, Santos AF, Alves MA, Neto JP, Schafer PG, Bittar AE, et al. Immunosuppression protocols for HLA identical renal transplant recipients. Transplant Proc 2003;35:1074-5. DOI:http://dx.doi.org/10.1007/10.1016/S0041-1345(03)00313-0

15. Ascher NL, Simmons RL, Noreen H, VanHooke J, Howard RJ, Sutherland DE, et al. 100 HLA-identical sibling transplants. Prognostic factors other than histocompatibility. Ann Surg 1979;189:209-16. PMID: 371556 DOI:http://dx.doi.org/10.1097/00000658-197902000-00013

16. Sumrani N, Delaney V, Ding ZK, Butt K, Hong J. HLA-identical renal transplants: impact of cyclosporine on intermediate-term survival and renal function. Am J Kidney Dis 1990;16:417-22. DOI:http://dx.doi.org/10.1016/S0272-6386(12)80053-9

17. Sumrani N, Delaney V, Ding Z, Davis R, Butt K, Hong J. Is cyclosporine indicated in HLA-identical renal transplant recipients? Transplant Proc 1991;23:1239-40. PMID: 1989199