Everolimus-based Immunosuppression Possibly Suppresses Mean Fluorescence Intensity Values of De Novo Donor-specific Antibodies After Primary Kidney Transplantation

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ABSTRACT

Purpose. We evaluated de novo donor-specific antibody (DSA) production of everolimus (EVR)-based immunosuppression for primary kidney transplant recipients involved in the A1202 study at our institute.

Methods. From March 2008 to August 2009, 24 recipients were prospectively randomized into 2 groups. The EVR group received reduced cyclosporin A and EVR. The standard protocol (STD) group received standard cyclosporin A and mycophenolate mofetil. Both groups received basiliximab and steroids. De novo DSA was identified using LABScreen single antigen beads (One Lambda, Canoga Park, Calif., United States). Mean fluorescence intensity (MFI) values >1000 were considered positive. $P < .05$ was considered significant.

Results. Graft survival was 100% in the EVR group and 90.9% in the STD group. All patients remained on the primary protocol in the EVR group, but 3 patients in the STD group (27.3%) were converted to tacrolimus due to DSA and non-adherence. Estimated glomerular filtration rate was similar in both groups. No EVR group recipients and 9.1% of STD group recipients were treated for T-cell-mediated rejection. No recipients of the EVR group exhibited peritubular capillaritis, while 9.1% in STD group developed chronic active antibody-mediated rejection. LABScreen revealed an accumulative class II DSA production rate of 15.4% in the EVR group and 18.3% in the STD group at 10 years. When the MFI cut-off level was set to 6000, anti-HLA antibody and de novo DSA-free survival was significantly better in the EVR group.

Conclusions. EVR-based immunosuppression provided equivalent or even better clinical outcomes. EVR suppressed de novo DSA production at 10 years follow-up; however, further follow-up is inevitable.

SINCE 2000, graft survival following kidney transplantation has improved. These improvements are largely attributable to the introduction of mycophenolate mofetil (MMF), thymoglobulin, and inhibitors of the mammalian target of rapamycin (mTOR). Prospective multicenter open-labeled trials of everolimus (EVR) such as A2309 [1], A1202 [2,3], ASSET [4,5], US92 [6], and

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Table 1. Demography of Recipients and Donors

	Factors	EVR Group (n = 13)	STD Group (n = 11)	*P* Value
Recipient	Sex (men/women)	8/5	5/6	.682
	Age (y; mean ± SD)	43.7 ± 15.3	35.0 ± 8.8	.086
	BMI (kg/m^2; mean ± SD)	21.7 ± 3.1	21.0 ± 3.1	.777
	Hemodialysis period (mo; mean ± SD)	18.0 ± 31.5	17.6. ± 23.1	.345
	Preemptive transplantation	4 (30.8%)	2 (18.2%)	.649
	HLA mismatch class I	1.9 ± .7	1.6 ± .7	.408
	HLA mismatch class II	1.2 ± .6	.8 ± .6	.168
Donor	Sex	8/5	4/7	.414
	Age (y; mean ± SD)	55.2 ± 8.5	56.9 ± 6.6	.583
	BMI (kg/m^2; mean ± SD)	23.6 ± 2.3	22.8 ± 2.9	.489
	Preoperative eGFR (mL/min/1.73 m^2; mean ± SD)	83.0 ± 14.3	73.6 ± 16.4	.151

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; EVR, everolimus; SD, standard deviation; STD, standard protocol.

Transform [7] have been reported. In those studies, the incidence of de novo anti-donor specific antibody (DSA) production did not statistically differ and obvious superiority was not demonstrated for graft survival and function over both short- and middle-term observation. We herein describe the correlation of EVR and DSA production, over a 10-year follow-up study at a single institute, from A1202.

IMMUNOSUPPRESSION PROTOCOL

Basiliximab induction and steroids were administrated to all patients, who were divided into 2 groups. The EVR group received cyclosporine 3 mg/kg twice a day and EVR .75 mg twice a day. The target range of EVR was 3–8 ng/mL for trough level. Trough levels of cyclosporine were targeted to 100–200 ng/mL within 2 months,75–150 ng/mL between 2 and 4 months, 50–100 ng/mL between 4 and 6 months, and 25–50 ng/mL after 6 months. The standard protocol (STD) group received cyclosporine 4 mg/kg twice a day and MMF 1000 mg twice a day. The target trough ranges of cyclosporine were 200–300 ng/mL within 2 months and 100–250 ng/mL after 2 months. MMF was maintained throughout the observation period, if adverse effects were not identified due to over-immunosuppression. Routine kidney graft biopsies were conducted 1 year after transplantation.

PATIENTS AND METHODS

Between March 2008 and August 2009, 24 patients who underwent living donor adult kidney transplantation at our hospital were enrolled in a 2-year, multicenter, randomized phase-3 study (RAD001A1202 study). Thirteen patients were prospectively assigned to the EVR group and 11 were assigned to the STD group. Each year, we examined anti-HLA antibody (IgG antibody against human leukocyte antigen class I and class II) using the Flow PRA or the LABScreen Mixed Kit (One Lambda, Canoga Park, Calif., United States) for screening. DSA was identified using LABScreen single antigen beads (One Lambda). Graft kidney biopsies for diagnosing chronic antibody-mediated rejection were conducted in the recipients with de novo DSA, following acquisition of written informed consent.

Statistical analyses were performed using the independent samples *t*-test or Mann-Whitney U test for continuous data and χ^2 or Fisher's exact test for categorical variables. *P* values $< .05$ were considered statistically significant. Analyses were performed using SPSS for Windows version 13.0 (IBM, Armonk, NY) statistical software.

RESULTS

There were no statistical differences in donors' and patients' demography (Table 1). Patient survival was 100% in both groups, and graft survival was 100% in the EVR group and 90.9% in the STD group (mean observation 10.1 years: 9.4–10.8). All patients remained on the primary protocol in the EVR group but 3 patients in STD group (27.3%) were converted to tacrolimus due to DSA production or non-adherence. Graft function measures, represented by estimated glomerular filtration rate, were similar in both groups. No members of the EVR group and 9.1% of STD group members were treated for clinical T-cell-mediated rejection. Banff borderline changes, assessed in 12-month protocol biopsies, were observed in 7.7% of the EVR group and 18.2% of the STD group. No members of the EVR group exhibited peritubular capillaritis, while 9.1% of MMF group members developed chronic active antibody-mediated rejection (CAAMR). LABScreen revealed accumulative class II DSA production rates of 15.4% in the EVR group and 18.3% in the STD group at 10 years. There were no significant between-group differences in anti-human leukocyte antigen antibody or DSA-free survival (Fig 1). The mean highest mean fluorescence intensity (MFI) of anti-HLA antibody was significantly less in the EVR group (average 1837 vs 12399). When the MFI cut-off level was set to 6000, anti-HLA antibody and DSA-free survival were significantly better in the EVR group (Fig 2).

DISCUSSION

Improved immunosuppression dramatically improved graft survival following kidney transplantation, especially after 2000 when MMF became available [8]. Since 2010, EVR, a second-generation mTOR inhibitor, has been available. Prospective randomized studies of de novo EVR use with reduced calcineurin inhibitor (CNI) have demonstrated no

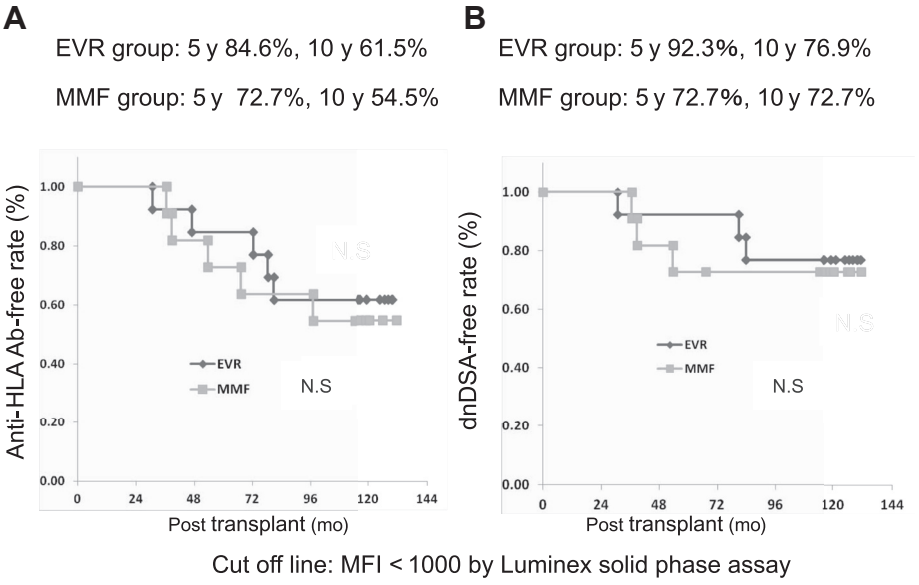

Fig 1. Anti-HLA antibody and DSA-free survival (MFI $<$ 1000). **((A)** Anti-HLA antibody and **(B)** DSA survival were not significantly different when the cut-off level was set to MFI $<$ 1000.

inferiority of EVR for short- and middle-term graft function and survival [1–5,7,9]. A meta-analysis study concluded that EVR plus a low-dose CNI regimen was similar in efficacy and safety to the MMF plus standard-dose CNI regimen after kidney transplantation [10]. DSA is still associated with an increased risk of graft rejection in patients who undergo kidney transplantation [11]. The DSA production rate was reportedly not superior to EVR in those studies.

Episodes of T-cell-mediated acute rejections were associated with antibody-mediated rejection (AMR) during long-term follow-up. The ZEUS CNI elimination study reported a 6% higher rate of acute rejection in the EVR group. Interestingly, most rejections occurred early after conversion, were treatable, and patients with rejections all had similar renal function at 1 year in this study [12]. EVR-based immunosuppression without CNI is reportedly associated with an increased risk of DSA and AMR. Higher rates of rejections and AMR may reflect underimmunosuppression resulting from the EVR-based regimen, suggesting that the combination of 2 antiproliferative immunosuppressants (EVR and MMF) is not sufficient for adequate rejection prophylaxis with insufficient downregulation of T- and B-cell-mediated immunity [13]. As seen in our previous study and other research, de novo use of EVR with low CNI did not increase T-cell-mediated immunity and AMR [1–6]. Once de novo DSA is produced, leading to CAAMR, there are no

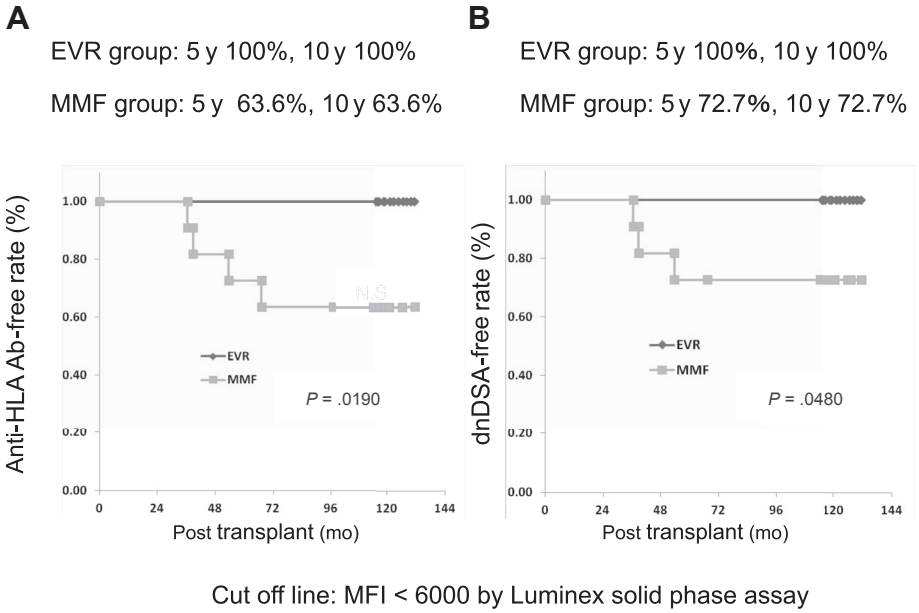

Fig 2. Anti-HLA antibody and DSA-free survival (MFI $<$ 6000). When the cut-off level was set to MFI $<$ 6000, both anti-HLA antibody **(A)** and DSA **(B)** survival were significantly better in the EVR group.

effective modalities for maintaining graft function. Bortezomib was once reported effective for elimination of DSA [14]. However, further studies failed to demonstrate decreased DSA following a single use of bortezomib [15,16]. Therefore, preventing DSA production seems most important for long-term graft survival. Even when de novo DSA production was produced, the intensity of DSA and MFI >6000 were reportedly associated with worse graft outcomes [17]. In our series, when cut-off MFI was set to 6000, DSA and HLA-antibody-free survival were significantly better in the EVR group according to Kaplan-Meyer analysis. Multivariate analysis, conducted using the Cox hazard proportional model, failed to confirm the benefit of EVR because event of DSA >6000 was not observed in EVR group. The mean MFI of the max HLA-antibody was also significantly lower; furthermore, no patients in the EVR group developed CAAMR in our series. During liver transplants, mTOR inhibitors prevent the development of DSA [18]. The mTOR-inhibitor also inhibited HLA-DR expression by itself [19], suggesting a basic mechanism for inhibiting the anti-HLA antibody.

So far, long-term follow-up data have not demonstrated the superiority of EVR, as related to graft function [20,21]. Close follow-up should be necessary, especially with regard to the effects of DSA. Our study had several limitations. This was a single-center investigation involving a limited number of patients and few study events. At this point, we are unable to observe differences in graft function and survival. Further long-term follow-ups are therefore needed.

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