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Renal Protective Effect of Everolimus in Liver Transplantation: A Prospective Randomized Open-Label Trial

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Background. Renal dysfunction is associated with poor long-term outcomes after liver transplantation. We examined the renal sparing effect of everolimus (EVR) compared to standard calcineurin inhibitor (CNI) immunosuppression with direct measurements of renal function over 24 months. **Methods.** This was a prospective, randomized, open-label trial comparing EVR and mycophenolic acid (MPA) with CNI and MPA immunosuppression. An Investigational New Drug Application (IND # 113882) was obtained with the Food and Drug Administration as EVR is only approved for use with low-dose tacrolimus. Serum creatinine, 24-hour urine creatinine clearance, iothalamate clearance, Cockcroft-Gault creatinine clearance (CrCl), and Modification of Diet in Renal Disease estimated glomerular filtration rate were prospectively measured at 4 study visits. Nonparametric statistical tests were used for analyses, including the Mann-Whitney U test for continuous outcomes and Pearson's chi-square test for binary outcomes. Effect size was measured using Cohen's *d*. Patients also completed quality of life surveys using the FACT-Hep instrument at each study visit. Comparison between the 2 groups was performed using the Student *t* test. **Results.** Each arm had 12 subjects; 4 patients dropped out in the EVR arm and 1 in the CNI arm by 24 months. Serum creatinine ($P=0.015$), Modification of Diet in Renal Disease estimated glomerular filtration rate ($P=0.013$), and 24-hour urine CrCL ($P=0.032$) were significantly better at 24 months with EVR. Iothalamate clearance showed significant improvement at 12 months ($P=0.049$) and a trend toward better renal function ($P=0.099$) at 24 months. There was no statistical significance with Cockcroft-Gault CrCl. Adverse events were not significantly different between the 2 arms. The EVR group also showed significantly better physical, functional, and overall self-reported quality of life ($P=0.01$) at 24 months. **Conclusions.** EVR with MPA resulted in significant long-term improvement in renal function and quality of life at 24 months after liver transplantation compared with standard CNI with MPA immunosuppression.

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INTRODUCTION

Acute kidney injury and chronic kidney disease (CKD) are known to be associated with poor long-term outcomes and survival after liver transplantation.[1-3] Serum creatinine is heavily weighted in the Model for End-Stage Liver Disease (MELD) equation. As a consequence, more patients are reaching liver transplantation with baseline renal dysfunction. Additionally, standard long-term calcineurin inhibitor (CNI)-based immunosuppression has a known nephrotoxic effect.[4] Significant renal impairment has been observed in liver transplant recipients at 5 years postoperatively, with up to 18.1% reaching end-stage renal disease.[1,5] Deterioration or a decrease in the estimated glomerular filtration rate (eGFR) ≥30% within the first year of liver transplantation has been reported as a strong predictor for CKD and death after 1 year post-transplantation.[3,6] The incidence of acute kidney injury after liver transplantation can range from 17% to 94% and has been associated with prolonged hospitalization, a higher risk of developing CKD stage 4–5 and increased mortality.[2,4] Given the significant impact of renal dysfunction on patient survival after liver transplantation, new renal protective strategies in long-term immunosuppression are necessary. Mammalian target of rapamycin (mTOR) inhibitors such as everolimus (EVR) promise such an intervention. The following study reports the results of a prospective, randomized, open-label, single-center study that examines the long-term impact of the renal sparing effect of EVR compared with a CNI-based immunosuppressive protocol using both calculated and gold-standard renal function measures.

MATERIALS AND METHODS

Study Design

This was a prospective, randomized, open-label single-center study, performed at an Academic University Hospital in the United States (Penn State Health Milton S Hershey Medical Center), assessing the long-term impact and superiority of the renal sparing effect in liver transplantation of EVR (EVR arm) with mycophenolic acid (MPA) compared with CNI immunosuppression with MPA (standard of care [SOC] arm). Steroids were weaned by 3 months after liver transplantation, and patients were randomized to study arms between 90 and 120 days post–liver transplantation.

An Investigational New Drug (IND) application was submitted to the Food and Drug Administration (FDA) on November 11, 2011 because EVR was only approved in the United States for utilization in conjunction with low-dose tacrolimus post–liver transplantation. The IND request was approved (IND # 113882) on April 19, 2012. Institutional review board (IRB) approval for the study protocol (IRB Protocol # 38115) was obtained on August 15, 2012, after the FDA IND assignment. This was an investigator-initiated study; funding by Novartis Pharmaceutical Corporation was finalized on March 1, 2013. The study was registered in ClinicalTrials.Gov (NCT01936519), and it complied with the requirements of the Declaration of Helsinki and the Declaration of Istanbul. Study data were collected and managed using a Research Electronic Data Capture tool (REDCap) at Penn State Health Milton S. Hershey Medical Center and Penn State College of Medicine. REDCap is a secure, web-based application designed to support data capture for research studies, providing an intuitive interface for validated data entry, audit trails for tracking data manipulation and export procedures, automated export procedures for seamless data downloads to common statistical packages, and procedures for importing data from external sources.[7] Once a RedCap database was created, subject screening began in May 2013, and the first patient enrollment occurred on December 16, 2013. The last subject study visit was on July 31, 2019. Utilization of the Penn State Clinical and Translational Science Institute resources for this study was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR002014.

Participants and Randomization

Twenty-four liver transplant recipients were included in the study with 12 patients in each arm (EVR versus SOC). A total of 105 liver transplant patients were screened, and those who agreed to participate underwent an initial spot urine protein-creatinine ratio to rule out the presence of proteinuria before signing a written study consent form and randomization. A ratio ≥0.7 g/g was considered exclusionary.

For inclusion in the study, candidates had to be 18 to 70 years of age, recipients of a full deceased donor liver transplant on standard CNI immunosuppression with corticosteroids and MPA during the first 3 months posttransplant, have a functioning liver allograft with liver function tests ≤3× the upper limit of normal value, an abbreviated Modification of Diet in Renal Disease estimated glomerular filtration rate (MDRD eGFR) ≥30 mL/min/1.73 m^2, and patients had to be able to take oral medication at the time of randomization.

Exclusion criteria included recipients of multiple organ transplants and partial liver grafts, hepatocellular cancer beyond United Network for Organ Sharing criteria, administration of antibody induction at time of transplant, presence of hypersensitivity to immunosuppressants used, and a hypercoagulable state or need for anticoagulation. Enrollment exclusion criteria included severe hyperlipidemia (hypercholesterolemia >350 mg/dL or hypertriglyceridemia >500 mg/dL), thrombocytopenia <50 000/ mm^3, absolute neutrophil count <1000/mm^3 or white blood cell count of <2000/mm^3, HIV-positive test, presence of a clinically significant systemic infection, renal failure with renal replacement therapy within the past 7 days, hepatic artery thrombosis (HAT), rejection requiring antibody therapy, multiple steroid-sensitive rejection episodes, and presence of proteinuria by spot urine protein-to-creatinine ratio ≥0.7 g/g.

Subjects were randomized 1:1 in each group using a simple randomization procedure by allocation concealment with the primary investigator blindly picking either an EVR or SOC assignment slip from a closed container with a second person present and witnessing the randomization procedure. For arm A, EVR was administered initially at 0.75 mg twice daily, and the CNI was discontinued once a serum EVR level of 6–8 ng/mL was achieved. The mean transition time from the start of EVR to discontinuation of CNI was 31.2 days. The dosing of MPA remained unchanged. Patients in arm B (SOC) continued on their standard CNI with MPA. No subjects were on corticosteroids, which are weaned off by 3 months post–liver transplantation.

Once a patient agreed to participate in the study and fulfilled criteria, a written consent was obtained. Randomization

was performed after consent, and patients underwent a baseline study visit, which included the following:

1. Serum creatinine level
2. Calculated Cockcroft-Gault creatinine clearance (CrCl) and eGFR by isotope dilution mass spectrometry-traceable MDRD study equation
3. 24-hour urine CrCl
4. Iothalamate clearance study
5. 24-hour urine protein

Subjects in each arm were then followed for a period of 24 months and underwent repeat study visits at 6, 12, and 24 months.

Procedures

Iothalamate Clearance Test

The iothalamate clearance test was performed by administering a bolus followed by a continuous pump infusion of 30% iothalamate meglumine (Conray 30 from Mallinckrodt and later Guerbet Company). Iothalamate dosages were based on the patient's calculated Cockcroft-Gault CrCl on the study day. Urine and blood samples were collected at baseline, and 1 hour after intravenous iothalamate bolus was administered. The urine output was then measured and recorded every 30 minutes while blood and urine samples were collected at the same 30 minute time points. Urine and blood samples were labeled on the basis of their respective time of collection and were assayed for iothalamate concentration using a high-pressure liquid chromatography system with an UV light detector. Effective iothalamate clearance was calculated as the average of 4 individual clearances.

Twenty-four-hour Urine Creatinine Clearance and Protein Level

Each subject was provided a container and brought the total urine collected to their study visit with a documented start and end time of each 24-hour urine collection. This was then sent to the hospital laboratory for 24-hour urine CrCl and protein level.

Calculated Renal Function Parameters

Serum creatinine level was measured on the day of the study visit, and Cockcroft-Gault CrCl and eGFR by isotope dilution mass spectrometry-traceable MDRD study equation were calculated.

Health-related Quality of Life Surveys

Measurements of health-related quality of life (HRQoL) were prospectively collected at each study visit using the Functional Assessment of Cancer Therapy-Hepatobiliary Cancer (FACT-Hep) instrument. FACT-Hep is liver specific and was designed to measure HRQoL in patients with hepatobiliary cancers, including liver, bile duct, and pancreas, but has been successfully applied in populations treated with hepatic resection.[8] It is a 45-item, self-report questionnaire that measures quality of life across 4 specific domains: physical well-being, social well-being, emotional well-being, and functional well-being.[9] In addition to these domains, the FACT-Hep includes a disease-specific hepatobiliary cancer subscale that assesses quality of life related to pain, gastrointestinal symptoms, weight loss, and jaundice. Two additional summary scores are available in the FACT-Hep: the target of intervention summary combines the results of the hepatobiliary subscale with the physical and functional well-being subscales, and a total FACT-Hep score combines the results of all subscales.

Outcomes

The primary endpoint of this study was to examine the renal sparing impact of implementing a strategy of conversion to EVR from a CNI-based immunosuppressive protocol between 90 and 120 days post–liver transplant. Secondary outcome measures were the evaluation and the incidence of adverse events, and the prospective assessment of subject HRQoL during the 24-month period of follow-up. Primary monitoring was performed by the study team: the principal investigator and study team met on a weekly basis to review real-time accrual of adverse events and any medication dose limiting toxicities or side effects. Oversight was also provided by a Data Safety Monitoring Board, and assessment reports were sent directly to the IRB. An annual FDA report was submitted outlining study results and progress. Per the protocol, serious adverse events that qualified for removal from the study included the following:

- Refractory leucopenia ≤500mm^3 unresponsive to drug dose reduction/interruption or stimulation by neupogen (filgrastim).
- Persistent thrombocytopenia at <50 000/mm^3, unresponsive to study medication dose reduction/interruption.
- Development of refractory severe hyperlipidemia (cholesterol >350 mg/dL or triglycerides >500 mg/dL) unresponsive to treatment and to study medication dose reduction/interruption.
- Refractory anemia (hemoglobin ≤6 g/dL) not related to surgical bleeding, and unresponsive to blood transfusion and to study medication dose reduction/interruption.
- Development of hemolytic uremic syndrome.
- Development of any thrombotic events such as liver graft hepatic arterial or portal/hepatic venous thrombosis, or thrombotic microangiopathy or thrombotic thrombocytopenic purpura, or radiologically documented pulmonary embolism.
- Refractory seizures or neurologic side effects unresponsive to treatment or reduction/interruption of study medication.
- Life-threatening infectious complications.

Statistical Analysis

The study was powered to measure significant improvements in renal function. To estimate the required sample size, we assumed that the eGFR would improve from 34 to 43 mL/min/1.73 m^2 as reported in a recent study by Fairbanks et al[10] This was a conservative estimate because the renal sparing effects of EVR are larger than those of sirolimus. Based on these assumptions, it was determined that a sample size of 12 in each group would have 80% power to detect a difference in means of –9.0 (the difference between a group 1 mean of 34.0 and a group 2 mean of 43.0) assuming that the common SD was 7.5 using a 2-group *t* test with a 0.05 2-sided significance level.

Because of the relatively small sample size, nonparametric statistical tests were used for all analyses, including the

Mann-Whitney U test for continuous outcomes and Pearson's chi-square test for binary outcomes. In addition, because there were no significant differences in patient characteristics between the 2 groups, univariate statistical tests were used for all comparisons. To provide information about effect size, we also included Cohen's *d* for all comparisons. There was some attrition over time, including 2 patients at 12 months and 2 additional patients (a total of 4 patients) at 24 months in the EVR arm, and 1 patient at 12 months (no additional patients at 24 mo, a total of 1 patient) in the SOC arm. As a robustness check, we imputed missing values. Results from imputation were largely the same in terms of both effect and significance, so we report results without imputation of missing values. Imputation results are provided in Table S1 (SDC, http://links.lww.com/TXD/A328).

The primary endpoint of the trial was renal function as assessed by 24-hour urine CrCl. CrCl was compared between the 2 arms of the study using the Mann-Whitney U test at baseline and each follow-up period. Comparisons of secondary endpoints (Cockcroft-Gault CrCl, MDRD eGFR, iothalamate clearance, and 24-h urine protein) between the EVR and SOC groups were also made using the Mann-Whitney U test. Serum outcomes (white blood cell count, absolute neutrophil count, and testosterone) were compared using a Mann-Whitney U test.

Safety endpoints included rejection and infection episodes, and medication, gastrointestinal, cardiovascular, neurological, and metabolic side effects. These were compared at baseline and at each follow-up between arms using a Pearson chi-square test.

For assessment of subject HRQoL, FACT-Hep measurements were taken at baseline, while all patients were receiving CNIs, and again after randomization at the 6-, 12-, and 24-month follow-up study visits for subjects in both arms. Comparisons were made between patients in each treatment group (SOC and EVR) at each time period using the Student *t* test.

The statistical and data analyses were performed by C.S.H. and the primary investigator Z.K. All analyses were performed using Stata software (version 15, College Station, TX).

Oversight of the study was provided by a Data Safety Monitoring Board. The study was registered in ClinicalTrials.Gov (NCT01936519).

RESULTS

One hundred five (105) patients were screened. Patients were excluded for the following reasons: patient refusal (n = 21), need for anticoagulation (n = 18), noncompliance posttransplant (n = 5), renal failure (n = 5), segmental graft (n = 5), combined liver-kidney transplant (n = 5), retransplantation (n = 2), recurrent hepatitis C with liver dysfunction (n = 1), HAT (n = 1), hepatocellular cancer beyond Milan Criteria (n = 1), leukopenia (n = 2), iothalamate allergy (n = 2), rejection (n = 2), age >70 years (n = 1), language barrier (n = 2), neurologic disorder (n = 1), proteinuria (n = 3), and diagnosis of posttransplant lymphoproliferative disease (n = 1). Three patients were screen failures as they opted to withdraw from the study after consent but before randomization. The first patient enrolled in the study on the EVR arm on December 16, 2013, and the study was completed on July 31, 2019, with the last subject study visit.

Twenty-four patients were enrolled and 19 subjects completed the study with follow-up. Four subjects were withdrawn in the EVR arm for noncompliance (n = 1), development of significant proteinuria (n = 1), late HAT (n = 1), and an allergy to MPA (n = 1). In the SOC arm, 1 subject withdrew for foley catheter-related bladder spasms that did not allow iothalamate clearance testing (n = 1). Figure 1 shows the study trial profile summary, and the subject demographics are detailed in Table 1.

Renal function parameters measured at study visits showed a significantly superior renal function in the EVR cohort at 12 and 24 months (Table 2; Figure 2). At 12 months, serum creatinine ($P = 0.024$), MDRD eGFR ($P = 0.020$), and iothalamate clearance ($P = 0.049$) were significantly better in the EVR arm. At 12 months, the total number of patients in each study arm was 10 in the EVR cohort and 11 in the SOC cohort. At 24 months, the EVR arm showed significantly better renal function based on serum creatinine ($P = 0.015$), MDRD eGFR ($P = 0.013$), and 24-hour urine CrCl ($P = 0.032$). The iothalamate clearance also showed a persistent trend toward better renal function in the EVR arm ($P = 0.099$). The Cockcroft-Gault CrCl did not show statistical significance at any time point. The incidence of proteinuria was not significantly different between the 2 arms at 24 months. One subject in the EVR arm was removed from the study after 6 months due to the development of significant refractory proteinuria.

No induction agents were administered to study subjects. Tacrolimus was progressively weaned in the SOC arm per our center's clinical practice to maximally reduce its nephrotoxic impact over the 24-month follow-up period. Mean serum tacrolimus level was 10.25 ng/mL at baseline/randomization, then 9.98, 7.22, and 5.98 ng/mL at 6, 12, and 24 months, respectively, post–liver transplantation. EVR levels were maintained stable at around 6 ng/mL throughout the study period. Patients were maintained at baseline on the MPA dose they were on at the time of randomization. The median MPA dose in the EVR arm at the start of the study was 360 mg and in the SOC arm was 540 mg ($P = 0.244$). At the end of the study at 2 years, the median dose of MPA for both arms was 180 mg ($P = 0.444$). The decrease in MPA dose from baseline to 24 months in both the EVR and the SOC arms was not significant ($P = 0.123$ and $P = 0.0718$, respectively).

There was no significant difference in white cell count and absolute neutrophil count on study visit follow-up (Table 3). There was also no significant difference in the overall incidence of leukopenia (SOC n = 7; EV n = 9; $P = 0.386$) and number of filgrastim doses between the 2 cohorts (SOC mean 4.17; EV mean 2.42; $P = 0.356$) (Table 4). Serum testosterone levels were significantly lower in the EVR arm at 6 and 12 months ($P = 0.041$ and $P = 0.036$, respectively); the trend was maintained but was not significant by 24 months ($P = 0.083$).

Adverse events are summarized in Table 4. There was a higher incidence of cytomegalovirus infections in the SOC arm (SOC n = 6; EV n = 2), although this did not reach statistical significance ($P = 0.083$). One case of HAT occurred in the EVR arm. There was no significant difference between the 2 arms in cellular rejection episodes (SOC n = 1; EV n = 3; $P = 0.273$) or in donor-specific antibody (DSA)-positive antibody-mediated rejection (SOC n = 1; EV n = 2; $P = 0.537$). All rejection episodes in the EVR arm occurred within the first 4 months after randomization and conversion to EVR.

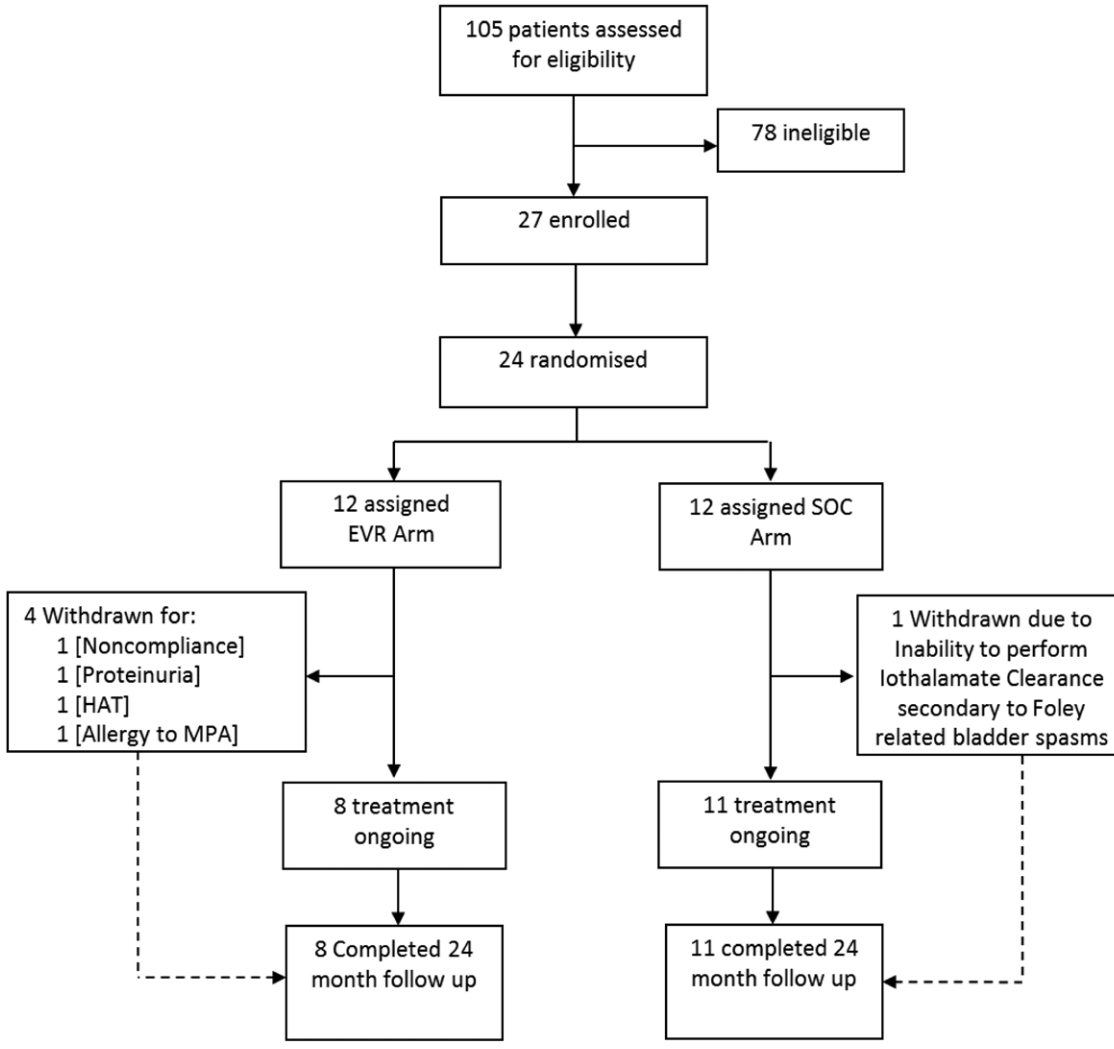

FIGURE 1. Study trial profile summary. EVR, everolimus; HAT, hepatic artery thrombosis; MPA, mycophenolic acid; SOC, standard of care.

In terms of the HRQoL, there were no significant differences between the EVR and SOC patients at baseline, or at 6 or 12 months of follow-up. However, at 2 years of follow-up, patients in the EVR arm reported significantly better physical well-being ($P = 0.006$) and functional well-being ($P = 0.03$) (Figure 3). The hepatobiliary cancer scale, which focuses on pain, gastrointestinal symptoms, weight loss, and jaundice, showed no significant difference between EVR and SOC patients at any follow-up (Figure 4). However, the trial outcome index, which combines the hepatobiliary cancer scale with the physical and functional well-being scales, also seen in Figure 4, showed that EVR arm patients reported significantly better quality of life on this scale at 24 months of follow-up. This was mostly driven by the improvements observed in physical and functional well-being. By 24 months of follow-up, on the FACT-Hep total score (Figure 4), which combines all 4 well-being scales with the hepatobiliary cancer scale, the EVR group had significantly better overall self-reported quality of life ($P = 0.0143$). These data are also summarized in Table 5.

DISCUSSION

CKD and renal failure are known to be associated with worse long-term outcomes after liver transplantation.[1-3] Reasons for renal dysfunction are multifactorial. In the pretransplant phase, the MELD-based liver allocation system is heavily weighted in terms of renal function, and with the ongoing liver organ shortage, more patients are arriving at liver transplantation with underlying kidney dysfunction. Posttransplant risk factors for chronic renal failure include hypertension, diabetes mellitus, and hepatitis C infection.[1] While CNIs have become the mainstay of post–liver transplant immunosuppression given their positive impact on posttransplant survival,[1,11] they are associated with nephrotoxicity leading to progressive renal function deterioration.[1,4,12] The negative impact of renal dysfunction on liver transplant outcomes has led to examination of strategies that protect and possibly improve long-term renal function. To our knowledge, this is the only study that estimates the potential renal sparing effect of EVR with MPA by performing direct measurements of renal function through scheduled study visits during a 24-month follow-up, compared with a standard CNI immunosuppression arm. To date, published single-center and multicenter trials examining the effect of EVR on renal function have relied solely on calculated estimates, which tend to underestimate the degree of renal dysfunction.[13]

Additionally, there are little data published on the immunosuppressive combination of EVR with MPA, with the

TABLE 1.

Subject demographics

Variable	Everolimus (N = 12)	Standard of care (N = 12)	Cohen *d*	*P*
Age at transplant	56.5	55.1	–0.171	0.488
Age at randomization (y)	56.7	55.1	–0.195	0.142
18–49	25.0%	16.7%		
50–54	8.3%	33.3%		
55–59	16.7%	25.0%		
60+	50.0%	25.0%		
Sex			0.251	0.217
Female	8.3%	16.7%		
Male	91.7%	83.3%		
MELD score				
Mean	24.1	29.5	0.488	0.308
Median	27.0	30.0		
Dialysis			0.242	0.178
Yes	16.7%	41.7%		
No	83.3%	58.3%		
Diabetes mellitus			–0.254	0.537
Yes	16.7%	8.3%		
No	83.3%	91.7%		
Hypertension			0.173	0.673
Yes	33.3%	41.7%		
No	66.7%	58.3%		
Dyslipidemia				
Yes	8.3%	8.3%	0.000	1.000
No	91.7%	91.7%		
Primary diagnosis			0.146	0.312
Alcohol	33.3%	25.0%		
Fulminant liver failure	0.0%	8.3%		
Hepatocellular carcinoma	50.0%	25.0%		
Hepatitis C	8.3%	33.3%		
NASH	0.0%	8.3%		
Alpha 1 antitrypsin deficiency	8.3%	0.0%		

MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis.

majority of studies comparing EVR alone or EVR with low-dose tacrolimus to standard CNI-based immunosuppression. In the United States, EVR has only been approved for use in combination with low-dose tacrolimus, thus requiring an IND submission and authorization (IND # 113882) from the FDA before we could proceed with our study.

Published single-center and multicenter trials examining the renal protective effect of EVR have also been limited by their retrospective or observational study design, variations in immunosuppressive protocols, and limited long-term follow-up.[6,12,14-21] The early H2304 trial in 2012 discontinued the tacrolimus elimination arm due to an observed higher incidence of rejection with EVR, and some studies have only examined EVR combined with low-dose tacrolimus.[14,15,21,22]

To our knowledge, there are only 2 large studies that have looked the immunosuppressive combination of EVR with MPA: the SIMCER and CERTITUDE trials.[12,19] The French multicenter SIMCER trial, published in 2017, examined the combination of EVR with MPA, but patients were also given basiliximab induction for additional renal protective effect, subjects were highly selected with low MELD scores, and unlike our study, follow-up was limited to 6 months.[12] The CERTITUDE trial was an extension of the SIMCER study with 2-year follow-up of subjects, but it was observational in design, and physicians were allowed to modify immunosuppressive treatment at any time during the follow-up. This resulted in up to 47.7% of EVR arm subjects receiving tacrolimus at some time point during the 6- to 24-month follow-up, and in the CERTITUDE study, the observed eGFR was only significantly higher at 3–12 months in the EVR group, with a nonsignificant trend to higher GFR thereafter. This was attributed to the extensive changes in maintenance immunosuppression with the utilization of tacrolimus in some of the subjects in the EVR arm. Proteinuria was also only checked in one-third of the subjects in the CERTITUDE observational study, using a urine protein-to-creatinine ratio.

In our study, no induction agents were administered, and subject demographics reflected a patient population more typically seen in transplant practice, with a mean MELD score of 24 in the EVR arm and 29 in the SOC arm. Patients were maintained on the same immunosuppressive protocol with prospective long-term 2-year follow-up, and direct urinary protein measurement using 24-hour urine collection was performed at 4 separate study visits. Finally, unlike the CERTITUDE study, our results showed a significant improvement in renal function at 24 months in the EVR arm by 24-hour urine CrCl ($P = 0.032$), as well by calculated MDRD eGFR ($P = 0.013$) and serum creatinine ($P = 0.015$).

The mean serum creatinine level at baseline was not significantly different between the 2 groups (EVR 1.23; SOC 1.3;

TABLE 2.

Summary of renal function results

		Everolimus		Control			
	Measure	N	Mean	N	Mean	Cohen *d*	*P*
Baseline	Creatinine (mg/dL)	12	1.23	12	1.30	0.207	0.686
	Cockroft clearance (mL/min)	12	85.15	12	78.11	−0.199	0.773
	MDRD clearance	12	69.91	12	61.24	−0.310	0.419
	24-h urine clearance (mL/min)	12	59.25	12	64.00	0.132	0.488
	Iothalamate clearance (mL/min)	12	58.10	12	60.60	0.066	0.954
	24-h urinary protein (g/24 h)	12	0.19	12	0.20	0.041	0.954
6 mo	Creatinine (mg/dL)	12	1.02	12	1.29	0.816	0.088
	Cockroft clearance (mL/min)	12	100.17	12	79.84	−0.556	0.387
	MDRD clearance	12	81.27	12	62.18	−0.731	0.069
	24-h urine clearance (mL/min)	12	70.75	11	68.09	−0.094	0.442
	Iothalamate clearance (mL/min)	12	74.23	12	67.99	−0.184	0.299
	24-h urinary protein (g/24 h)	12	0.88	12	0.26	−0.495	0.030
12 mo	Creatinine (mg/dL)	10	0.95	11	1.29	1.069	0.024
	Cockroft clearance (mL/min)	10	113.47	11	86.84	−0.562	0.360
	MDRD clearance	10	88.01	11	60.63	−1.047	0.020
	24-h urine clearance (mL/min)	10	86.80	11	68.09	−0.675	0.181
	Iothalamate clearance (mL/min)	10	104.01	11	66.65	−1.034	0.049
	24-h urinary protein (g/24 h)	10	0.35	11	0.58	0.292	0.067
24 mo	Creatinine (mg/dL)	8	0.95	11	1.51	1.030	0.015
	Cockroft clearance (mL/min)	8	108.16	11	80.85	−0.643	0.283
	MDRD clearance	8	87.37	11	53.29	−1.445	0.013
	24-h urine clearance (mL/min)	8	90.63	11	61.54	−1.062	0.032
	Iothalamate clearance (mL/min)	8	79.41	11	57.19	−0.892	0.099
	24-h urinary protein (g/24 h)	8	0.32	11	0.47	0.255	0.364

MDRD, Modification of Diet in Renal Disease.

$P = 0.686$), and although the SOC arm had a higher prevalence of dialysis at the time of transplant (41.7% versus 16.7% EVR arm), this was not statistically significant ($P = 0.178$). The incidence of pretransplant hypertension, diabetes mellitus, and dyslipidemia was also not significantly different in the 2 arms (Table 1). Unlike other studies, renal function was assessed by both gold-standard tests (24-h urine CrCl and iothalamate clearance) and also calculated by Cockcroft-Gault CrCl and MDRD eGFR.

As mentioned, our findings showed a significant improvement in renal function at 24 months in the EVR arm by 24-hour urine CrCl ($P = 0.032$), MDRD eGFR ($P = 0.013$), and serum creatinine ($P = 0.015$). The difference in Cockcroft-Gault eGFR was not statistically significant at any follow-up; however, the MDRD equation has been reported to be a more precise calculated estimate of renal function in liver transplant recipients.[13] The iothalamate clearance in the EVR arm showed a strong trend toward renal function improvement but did not reach significance by 24 months ($P = 0.099$), which may have been due to lower subject numbers by month 24 (n = 8). The mean tacrolimus level was 5.98 ng/mL at 24 months in the SOC arm, and it was particularly important to wean CNIs, per the standard practice of transplant programs, to minimize their nephrotoxic effect, and to provide an appropriate comparison for the renal protective effect of the EVR arm on follow-up. These results provide a clear confirmation of the long-term renal protective effect of EVR.

The combination of EVR and MPA was tolerated in our study and adverse events were not significantly different between the 2 cohorts, including leukopenia (SOC n = 7; EV n = 9; $P = 0.386$; Table 4), despite the known combined bone marrow suppressive effect of EVR and MPA. Acute cellular rejection episodes (EV n = 3; SOC n = 1; $P = 0.273$) and DSA-positive antibody-mediated rejection (SOC n = 1; EV n = 2; $P = 0.537$) were also not significantly different between the 2 cohorts but were higher in the EVR arm. All were treated with steroid bolus and an increase in the maintenance immunosuppression; 1 case in the EVR arm also developed a concomitant DSA that was complement fixing (C1q assay positive) requiring administration of IVIG; all patients responded well to treatment and completed the study with excellent allograft function. Of the 3 cases of antibody-mediated rejection, only 1 required IVIG therapy as described for the 1 case in the EVR arm, while the remaining patients in both the SOC and EVR arm had their baseline immunosuppression increased and maintained normal liver function tests and excellent allograft function throughout the study. No subject withdrawal was undertaken for rejection. All rejection episodes in the EVR arm occurred within the first 4 months after randomization and conversion to EVR. This was consistent with the findings of the SIMCER study, which also showed that biopsy-proven acute rejection was more frequent in the EVR arm between randomization and month 6 (10.0% versus 2.2%; $P = 0.026$).

EVR has been reported to be protective of cytomegalovirus infections although the exact mechanism is not clear.[23,24] In our study, there was a higher incidence of cytomegalovirus infections in the SOC arm (SOC n = 6; EV n = 2), although this did not reach statistical significance ($P = 0.083$). Low serum testosterone levels have been reported in association with EVR.[25] In our study, serum testosterone levels were significantly lower

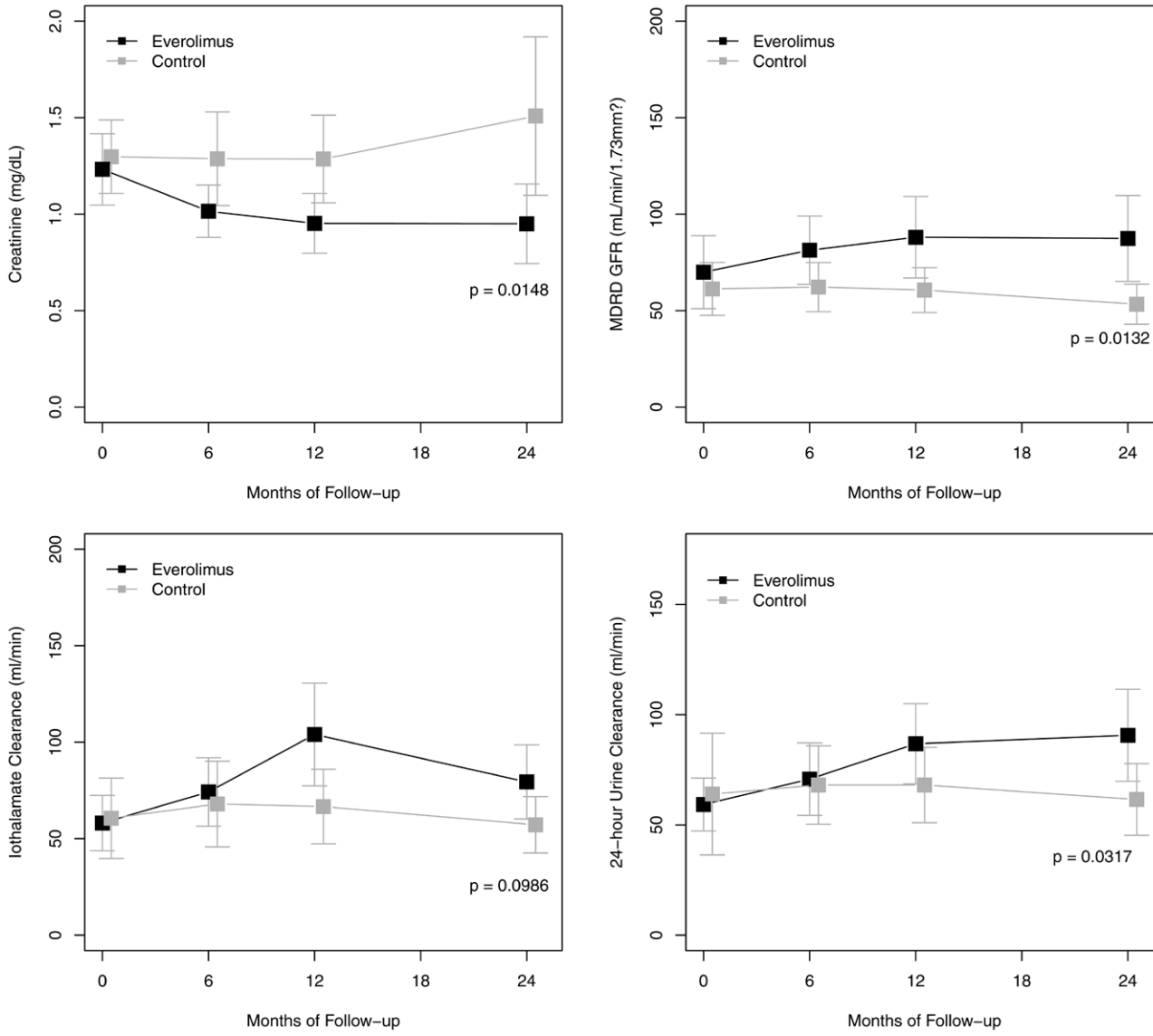

FIGURE 2. Renal function at 24 mo. GFR, glomerular filtration rate; MDRD, modification of diet in renal disease.

in the EVR arm at 6 and 12 months ($P = 0.041$ and $P = 0.036$, respectively; Table 3), and at 24 months, the trend was maintained but was not significant ($P = 0.083$; Table 3).

A greater number of patients dropped out of the EVR arm (EVR arm n = 4) compared with the SOC arm (SOC arm n = 1) at 24 months. Similar findings were described in other reports looking at EVR immunosuppression, reflecting that not all patients can be maintained long term on EVR. However, in this study, at least 50% of the causes of patient removal in the EVR arm were not directly related to EVR (patient noncompliance n = 1 and allergy to MPA n = 1). There were still 2 patients who dropped out of the EVR arm due to the development of adverse events, and this was higher compared with 1 patient drop out at 12 months on the SOC arm.

One patient in the EVR developed refractory proteinuria as the reason for study removal (n = 1). Proteinuria is an important limiting factor: 3 patients were excluded from enrollment in this study due to the presence of proteinuria at screening, and as mentioned, 1 subject in the EVR arm was removed from the study after 6 months due to the development of significant refractory proteinuria; however, the overall incidence of proteinuria was not significantly different between the 2 arms at 24 months ($P = 0.364$; Table 2).

HAT did occur in 1 patient on the EVR arm. Although thrombotic complications have led to a black box warning with sirolimus, an earlier mTOR inhibitor, the data behind the perceived increased incidence of HAT with sirolimus remain controversial, with conflicting data, as multiple studies have shown either no statistical difference in the incidence of HAT or an actual reduced incidence of this complication.[26] Unlike sirolimus, EVR has not been associated with HAT in liver transplantation in multiple large studies.[18,27] However, the occurrence of HAT in our study in 1 patient in the EVR arm at 6 months should be interpreted with caution as the cause was unclear.

The reduced number of subjects by month 24 in the EVR arm does represent a weakness in the study, and to address this, the missing values were further analyzed by imputation (see Table S1, SDC, http://links.lww.com/TXD/A328). Results from imputation showed the same outcome in terms of effect

TABLE 3.

White cell counts and serum testosterone levels

		Everolimus		Control			
		N	Mean	N	Mean	Cohen *d*	*P*
Baseline	White blood cell count (cells/mm^3)	12	3.78	12	4.28	0.302	0.795
	Absolute neutrophil count (cells/mm^3)	11	2.32	12	3.05	0.495	0.460
	Serum testosterone (ng/dL)	11	280.36	10	293.50	0.087	0.972
6 mo	White blood cell count (cells/mm^3)	12	3.71	12	3.76	0.025	0.795
	Absolute neutrophil count (cells/mm^3)	10	1.91	8	2.30	0.263	0.306
	Serum testosterone (ng/dL)	11	169.73	10	283.30	1.223	0.041
12 mo	White blood cell count (cells/mm^3)	10	4.05	10	4.34	0.233	0.762
	Absolute neutrophil count (cells/mm^3)	9	2.35	9	2.35	–0.001	0.757
	Serum testosterone (ng/dL)	8	209.75	8	319.25	1.111	0.036
24 mo	White blood cell count (cells/mm^3)	8	4.91	11	5.77	0.626	0.283
	Absolute neutrophil count (cells/mm^3)	6	2.57	10	3.10	0.671	0.278
	Serum testosterone (ng/dL)	7	222.57	8	345.50	1.046	0.083

TABLE 4.

Summary of adverse events

Adverse event	EVR (N = 12)	SOC (N = 12)	Cohen *d*	*P*
Medication side effects				
Mean filgrastim doses	2.42	4.17	0.402	0.356
Leucopenia	75.0%	58.3%	–0.359	0.386
Hyperlipidemia	75.0%	58.3%	–0.359	0.386
Rejection and infection				
Cellular rejection	25.0%	8.3%	–0.459	0.273
DSA positive	16.7%	8.3%	–0.254	0.537
CMV	16.7%	50.0%	0.756	0.083
HCV recurrence	8.3%	0.0%	–0.426	0.307
Minor Infection	41.7%	25.0%	–0.359	0.386
Dental infection	8.3%	0.0%	–0.426	0.307
C diff	0.0%	8.3%	0.426	0.307
HSV/HZV	16.7%	8.3%	–0.254	0.537
GI				
GI symptoms	33.3%	58.3%	0.518	0.219
Constipation	8.3%	16.7%	0.254	0.537
Cardiovascular				
Supraventricular tachycardia	25.0%	8.3%	–0.459	0.273
Hypertension	25.0%	33.3%	0.184	0.653
Pulmonary hypertension	8.3%	0.0%	–0.426	0.307
Neurological				
Headache	0.0%	8.3%	0.426	0.307
Depression	8.3%	8.3%	0.000	1.000
Tremor	0.0%	8.3%	0.426	0.307
Insomnia	8.3%	0.0%	–0.426	0.307
Mental state changes	0.0%	8.3%	0.426	0.307
Syncope	8.3%	0.0%	–0.426	0.307
Metabolic				
Anemia	16.7%	8.3%	–0.254	0.537
Gout	8.3%	8.3%	0.000	1.000
Hypomagnesemia	8.3%	41.7%	0.834	0.059
Hypocalcemia	8.3%	8.3%	0.000	1.000
Hypokalemia	8.3%	8.3%	0.000	1.000
Hyperglycemia	0.0%	16.7%	0.632	0.140
Hyperkalemia	16.7%	25.0%	0.206	0.615
Fluid retention	16.7%	8.3%	–0.254	0.537
Other				
Skin cancer	0.0%	16.7%	0.632	0.140
Hepatic artery thrombosis	8.3%	0.0%	–0.426	0.307
Hepatic steatosis	0.0%	8.3%	0.426	0.307

C diff, *Clostridioides difficile*; CMV, cytomegalovirus; DSA, donor-specific antibody; EVR, everolimus; GI, gastrointestinal; HCV, hepatitis C virus; HSV, herpes simplex virus; HZV, herpes zoster virus; SOC, standard of care.

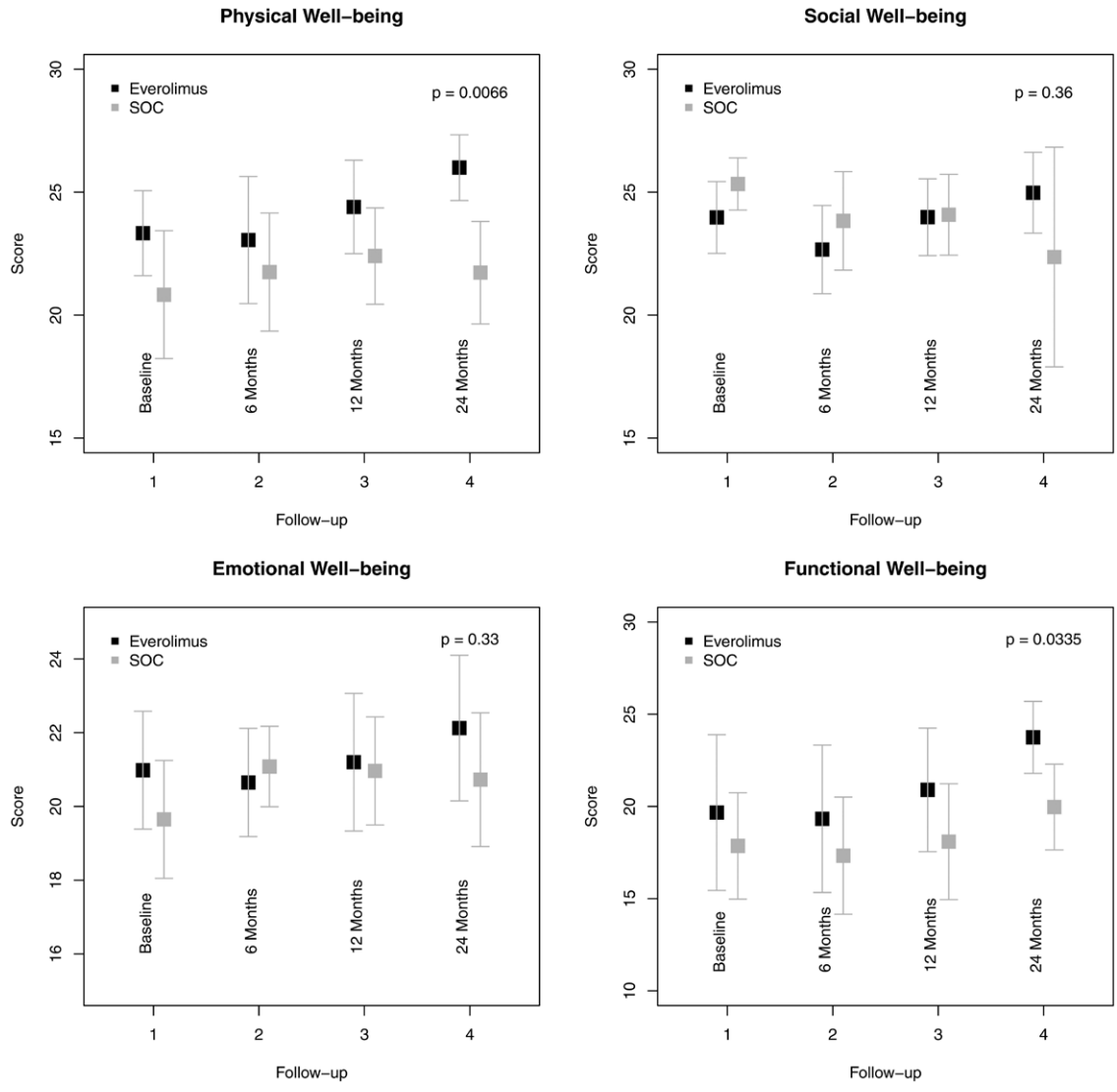

FIGURE 3. Summaries of physical, social, emotional, and functional well-being domains, stratified by treatment arm. SOC, standard of care.

and significance of the renal protective effect of EVR, leading us to report data from the full sample (Table 2).

Although a greater number of patients dropped out of the EVR arm, those who remained on the combination of EVR with MPA showed significantly better physical and functional well-being (Figure 3; $P = 0.006$ and $P = 0.03$, respectively), which also translated into significantly better overall self-reported quality of life (Figure 4; $P = 0.0143$) at

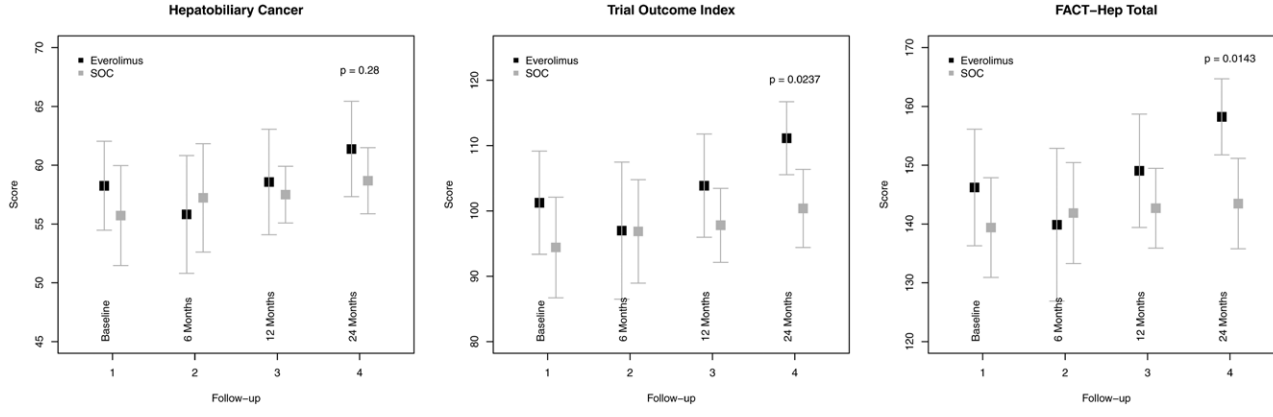

FIGURE 4. Summaries of disease specific and summary subscales, stratified by treatment arm. FACT-Hep, Functional Assessment of Cancer Therapy-Hepatobiliary cancer; SOC, standard of care.

TABLE 5.

Summary of health-related quality of life subscales, stratified by treatment group and follow-up

		EVR		SOC		
	Measure	**N**	**Mean**	**N**	**Mean**	***P***
Baseline	Physical well-being	12	23.3	12	20.8	0.1305
	Social well-being	12	24.0	12	25.3	0.1539
	Emotional well-being	12	21.0	12	19.7	0.2598
	Functional well-being	12	19.7	12	17.9	0.4964
	FACT-Hep trial outcome index	12	58.3	12	55.7	0.2364
	Hepatobiliary cancer	12	88.0	12	83.7	0.3915
	FACT-Hep total	12	146.2	12	139.4	0.3169
6 mo	Physical well-being	12	23.1	12	21.8	0.4757
	Social well-being	12	22.7	12	23.8	0.4042
	Emotional well-being	12	20.7	12	21.1	0.6471
	Functional well-being	12	19.3	12	17.3	0.4507
	FACT-Hep trial outcome index	11	55.8	11	57.2	0.9866
	Hepatobiliary cancer	12	85.7	12	84.0	0.6909
	FACT-Hep total	11	139.9	11	141.9	0.8044
12 mo	Physical well-being	10	24.4	12	22.4	0.1713
	Social well-being	10	24.0	12	24.1	0.9329
	Emotional well-being	10	21.2	11	21.0	0.8457
	Functional well-being	10	20.9	11	18.1	0.2451
	FACT-Hep trial outcome index	10	58.6	12	57.5	0.2304
	Hepatobiliary cancer	10	90.5	11	85.3	0.6721
	FACT-Hep total	10	149.0	11	142.7	0.296
24 mo	Physical well-being	8	26.0	11	21.7	0.0066
	Social well-being	8	25.0	11	22.4	0.3613
	Emotional well-being	8	22.1	11	20.7	0.3271
	Functional well-being	8	23.8	11	20.0	0.0335
	FACT-Hep trial outcome index	8	61.4	11	58.7	0.0237
	Hepatobiliary cancer	8	96.9	11	84.8	0.2832
	FACT-Hep total	8	158.2	11	143.5	0.0143

EVR, everolimus; FACT-Hep, Functional Assessment of Cancer Therapy-Hepatobiliary cancer; SOC, standard of care.

24 months. The impact of the side-effect profile of immunosuppressant drugs on quality of life has been examined in the past.[28-30] Benzing et al[30] compared mTOR-based immunosuppression with standard CNI regimens and concluded that only early (<2 mo) conversion to an mTOR inhibitor combined with another agent, such as mycophenolate mofetil or steroids, produced a statistically significant improvement in patient quality of life.[30] Their study, however, was hampered by its retrospective cross-sectional design and the fact that it was based on responses to a single survey that was sent to patients who were at variable time points post–liver transplantation. Our patient population was randomized at 90 days after liver transplantation, and subjects were prospectively followed at the same specific follow-up time points for completion of HRQoL surveys over a 2-year period. Interestingly, our results showed no significant difference between the EVR and SOC study cohorts at 6 and 12 months after randomization, but at 24 months, patients in the EVR group reported significantly better physical and functional well-being and better overall quality of life. This suggests that the combination of EVR with MPA is well tolerated in the long term if patients can be maintained on the combination of EVR and MPA. The renal protective effect observed in the EVR group may also have contributed to the significantly better physical and functional well-being of the EVR cohort, but larger prospective long-term studies are needed to confirm this finding.

In conclusion, there was a significant long-term improvement in renal function in patients receiving EVR with MPA compared with those receiving CNI with MPA; however, patients should be carefully selected. The combination of EVR with MPA appears to be tolerated, with comparable safety and immunosuppressive effect to CNI-based immunosuppression; however, the complication of HAT in 1 subject should be viewed with caution. These results suggest that EVR with MPA, if tolerated, represents a potentially good alternative immunosuppressive regimen to standard CNI-based therapy in liver transplant recipients with underlying renal dysfunction.

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