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ORIGINAL ARTICLE

Multiple indications for everolimus after liver transplantation in current clinical practice

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Abstract

AIM: To assess our experience with the use and management of everolimus-based regimens post-liver transplantation and to redefine the potential role of this drug in current clinical practice.

METHODS: From October 1988 to December 2012, 1023 liver transplantations were performed in 955 patients in our Unit. Seventy-four patients (7.74%) received immunosuppression with everolimus at some time post-transplantation. Demographic characteristics, everolimus indication, time elapsed from transplantation to the introduction of everolimus, doses and levels administered, efficacy, side effects, discontinuation and post-conversion survival were analyzed.

RESULTS: Mean age at the time of conversion to everolimus was 57.7 ± 10 years. Indications for conversion were: refractory rejection 31.1%, extended hepatocellular carcinoma in explanted liver 19%, post-transplant hepatocellular carcinoma recurrence 8.1%, *de novo* tumour 17.6%, renal insufficiency 8.1%, severe neurotoxicity 10.8%, and others 5.4%. Median time from transplantation to introduction of everolimus was 6 mo (range: 0.10-192). Mean follow-up post-conversion was 22 ± 19 mo (range: 0.50-74). The event for which the drug was indicated was resolved in 60.8% of patients, with the best results in cases of refractory rejection, renal insufficiency and neurotoxicity. Results in patients with cancer were similar to those of a historical cohort treated with other immunosuppressants. The main side effects were dyslipidemia and infections. Post-conversion acute rejection occurred in 14.9% of cases. The drug was discontinued in 28.4% of patients.

CONCLUSION: Everolimus at low doses in combination with tacrolimus is a safe immunosuppressant with multiple early and late indications post-liver transplantation.

Key words: Everolimus; Liver transplantation; Indications; Off-protocol; Outcome

Core tip: Everolimus has a completely different mechanism of action to that of current basal calcineurine inhibitors used worldwide in liver transplantation. This immunosuppressant has a good profile for patients with pre- and post transplant renal dysfunction, one of the main concerns nowadays. It has also a promising role in cancer patients which is common in liver transplantation, either as an underlying disease (hepatocarcinoma in cirrhosis), or as de novo developing tumors. We

present our off-protocol experience with partial/total and early/late conversion to everolimus, highlighting its efficacy and safety in fitting with the different emerging scenarios after liver transplantation.

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INTRODUCTION

Over the last thirty years, immunosuppression protocols in liver transplant patients have been based on calcineurine inhibitors (CNI) - cyclosporine in the eighties and tacrolimus in the nineties. Both were administered in combination with steroids. In the late nineties, monoclonal antibodies and mycophenolate mofetil (MMF), an antimetabolite with a different mechanism of action, were widely used. In the year 2000, sirolimus was the first inhibitor of the mammalian target of rapamycin (mTORi) launched into clinical practice as a primary immunosuppressant to replace the widespread use of CNI. However, its use declined due to severe adverse events and the warning issued on the risk of arterial thrombosis[1]. A few years later, everolimus (EVER) another mTORi was approved for use after acute rejection in heart[2] and kidney[3] transplantation. In 2012, EVER was approved for liver transplantation[4] by the EMA. In Spain, EVER was also approved for liver transplantation and obtained full registration at the end of 2012. In non-transplant areas, it has been approved for the treatment of advanced renal cell carcinoma[5].

mTORi reduce the expression of vascular endothelial growth factor and transforming growth factor-B, which are associated with tumour angiogenesis[6,7]. In solid organ transplantation, efficacy and safety can be achieved by targeting EVER trough levels at 3-8 ng/mL in combination with CNI. EVER is dosed twice daily and yields a steady state by day four.

The use of EVER is gaining acceptance in adult[8-10] and paediatric[11] liver transplant recipients. It has been used as maintenance[12-14], in *de novo* liver transplant patients[15], in cases of renal dysfunction as a CNI-sparing regimen[16-18], and in recipients with cancer[19-21]. The most common adverse events are leucopoenia, hyperlipidemia, gastrointestinal disorders, delayed wound healing, stomatitis, angioneurotic oedema, proteinuria and interstitial lung disease[22-24].

EVER was introduced into clinical practice at our centre in 2005, when some of the medical community had lost confidence in mTORi and had relegated the drug to compassionate use and to sporadic and desperate cases when other drugs failed. However, experience with sirolimus, especially the weak points of the drug, prompted us to use EVER in order to optimise and redefine the true role of mTORi. The principal aim of this single-centre retrospective study was to study the current indications for total or partial conversion to EVER in liver transplant patients treated off-protocol.

MATERIALS AND METHODS

From October 1988 to October 2012, 1023 liver transplants were performed in 955 patients in our centre. We reviewed the prospectively collected data bases and medical records of these patients, focusing on the patients who received EVER for immunosuppression at some point post-transplantation. We recorded the demographic characteristics of these patients, the causes of conversion to EVER, the pre- and post-conversion immunosuppression regimens, the time elapsed between liver transplantation and the start of EVER treatment, doses and trough levels, efficacy, side effects, causes of discontinuation and mean follow-up post-conversion. Efficacy was assessed overall and according to the time elapsed from liver transplantation to the introduction of EVER. All patients receiving EVER gave their signed informed consent and met all the requirements for compassionate use of the drug.

Demographic characteristics

The following information on the demographic characteristics of the patients was obtained: age at time of transplantation and at time of conversion; gender; hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) status; indication for transplantation; Child and United Network for Organ Sharing classification status; body mass index (BMI) > 30; presence or absence of hypertension, diabetes mellitus (DM) and renal dysfunction at time of transplant; donor age; donor cause of death; donor time spent in the intensive care unit; presence or absence of graft steatosis > 20%; type of graft; presence or absence of portal thrombosis; type of biliary anastomosis; mean intraoperative red blood cells; and mean cold ischemia time. Renal dysfunction at time of transplant was defined as serum creatinine > 1.5 mg/dL or hepato-renal syndrome or need for dialysis.

Definition of the causes of conversion

Refractory rejection was defined as an incomplete response to treatment with steroid pulses with or without MMF. Patients outside the Milan criteria and/or with macro- or microvascular invasion in the explanted liver were considered advanced hepatocellular carcinoma (HCC). HCC recurrence was defined as tumour recurrence at any time during the follow-up period after liver transplantation. Diagnosis was based on radiologic images and assessed by a pathologist in either hepatic or extrahepatic specimens. *De novo* tumour was defined as the development of a malignant tumour (excluding HCC) during post-transplantation follow-up. Post-transplant neurological disorders were diagnosed by a neurologist

based on clinical symptoms, electroencephalograms, craneoencephalic computed tomography, cerebral magnetic resonance imaging, lumbar punctuation and viral serological testing. Renal dysfunction was defined as the presence of serum creatinine > 1.5 mg/dL. Amelioration of renal function was defined as a statistically significant ($P < 0.05$) difference between mean serum creatinine levels at two different points of follow-up.

Doses and trough levels

Doses and trough levels of EVER were assessed on the day of conversion and at 15 d and 1, 3, 6 and 12 mo post-conversion. Tacrolimus levels were also assessed at the same times.

Assessment of efficacy

The variables analysed at the time of conversion and thereafter were: total bilirubin and transaminases; serum creatinine; amelioration or resolution of neurotoxicity or other causes for which EVER was introduced. Serum creatinine was assessed on the day of conversion and at 3, 6 and 12 mo post-conversion. Acute rejection post-conversion was suspected based on enzymatic alteration of liver function, assessed by liver biopsy, and defined according to the Banff criteria.

Patients converted to prevent HCC recurrence were compared with a historical cohort not receiving EVER and matched for MELD status, year of transplantation ± 18 mo, presence or absence of vascular invasion, tumour type and size. We found appropriate matches for all the variables except vascular invasion due to a scarcity of receptors. Efficacy was assessed by comparing patient survival and the time elapsed from liver transplant to recurrence in the patients receiving EVER and those in the historical cohort.

Patients with HCC recurrence after transplantation were also compared with a historical cohort not receiving EVER and matched for the time elapsed from liver transplantation to tumour recurrence, site of recurrence, and Milan criteria. Efficacy was assessed by comparing patient survival post-recurrence for patients receiving EVER and those in the historical cohort.

Patients who developed *de novo* tumours were compared with a historical cohort of patients not receiving EVER and matched for tumour histology, time elapsed from liver transplantation to tumour, and type of treatment post-diagnosis. Efficacy was assessed by comparing patient survival post-recurrence for patients receiving EVER and those in the historical cohort.

Other efficacy variables were glucose levels and the need for anti-diabetic therapy post-conversion and blood pressure and the need for antihypertensive drugs. These variables were evaluated qualitatively as "amelioration or resolution", "worsening" and "no change".

Time elapsed from liver transplantation to conversion

Early conversion was defined as conversion during the first year post-transplantation, and late conversion as conversion after the first year post-transplantation.

Side effects and discontinuation

Possible side effects assessed were: hematologic toxicities; diarrhoea; proteinuria (though not assessed in all patients); levels of serum cholesterol and triglycerides and the need for hypolipidemic therapy; infections; and any other post-conversion adverse event.

Discontinuation was defined as stopping the drug when the patient was alive. The reason for EVER discontinuation was recorded.

Survival post-conversion

All patients were followed up until December 2012, death or drug withdrawal. Patient survival post-conversion and cause of death were analyzed according to the reason for conversion and EVER-related deaths.

Statistical analysis

The student's *t*-test or the Mann-Whitney *U* test were used for quantitative data and Pearson's χ^2 or Fisher's exact test for categorical data. Significance was set at $P < 0.05$. Data are expressed as mean ± SD, or as percentages. The Kaplan-Meier method was used for survival analysis. All analyses were performed with SPSS version 15.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

Data on the demographic characteristics of recipients, donors and surgery are shown in Table 1. Mean patient age at the time of conversion was 57.7 ± 10 years and median age was 60 years (range: 27-74); nine patients (12.2%)were over 70 years of age.

Reasons for conversion to EVER are shown in Table 2. Pre-conversion therapy was based on tacrolimus in 69 patients, neoral cyclosporine (CyA) in four, and MMF in one. Post-conversion therapy consisted of: tacrolimus in 54 patients, CyA in three, and a CNI-free regimen in 17. Pre- and post-conversion drug combinations are specified in Table 3.

Median time between transplantation and introduction of EVER was 6 mo (range: 0.10-192 mo). Forty-two patients (56.8%) were converted during the first year post-transplantation and the remaining 32 patients (43.2%) after the first year. Median time between event onset and conversion was 1 mo (range: 0.1-19) (Table 1).

Doses and trough levels

Conversion to EVER was managed differently according to the reason for conversion; however, loading doses were never used. In cases of refractory rejection, EVER was administered at an initial dose of 1 mg every 12 h, with subsequent doses adjusted to obtain trough levels between 3 and 5 ng/mL. At the same time, CNI was maintained at high doses. When the reason for conversion was CNI-related adverse events, EVER was started at 0.5 mg once or twice a day and the CNI dose was reduced to half or completely withdrawn, depending on the severity of the adverse events. When the reason for conversion was cancer (extended tumour in the explant, can-

Table 1 Characteristics of recipients, donors, surgery and post-transplant evolution in 74 patients receiving everolimus *n* (%)

Recipient	Mean age (yr)	55.5 ± 9 r (25-69)
	Patients > 65 yr	10 (13.5)
	Male/female	55 (74.3)/19 (25.7)
	Diagnosis	
	HCC with cirrhosis	35 (47.2)
	Alcoholic cirrhosis	18 (24.3)
	HCV cirrhosis	16 (21.6)
	Cholostatic cirrhosis	3 (4.1)
	Liver insufficiency	2 (2.8)
	HCV - HBV	40 (54)-3 (4)
	ETOH	38 (51.4)
	HIV	4 (5.4)
	Child-Pugh A/B/C (%)	35-30-35
	UNOS (home/Hosp/ICU (%)	90.5-6.8-2.7
	Pre-LT associated disease	
	Renal insufficiency	11 (14.9)
	Diabetes mellitus	18 (24.3)
	Arterial hypertension	14 (18.9)
	Cardiopathy	3 (4.1%)
	Previous surgery	15 (20.3)
Donor	Mean age (yr)	48 ± 19 r (14-81)
	Patients > 70 yr	14 (19)
	Male/female (%)	49 (66)/25 (34)
	Graft steatosis > 20%	11 (15)
	Death (CET, CVA, Other) (%)	43-43-14
Surgery	E-E/E-E + Kehr/C-Y (%)	84-8-8
	Previous portal thrombosis	10 (13.6)
	Median RBC units	4 (r: 0-40)
	Cold ischaemia time (min)	378 ± 97
Post-transplant evolution	Ischaemia-reperfusion injury (ALT > 1000 IU, Quick < 60%)	14 (19)
	Biliary complication	7 (9.5)
	Postoperative arterial complication	2 (2.7)
Median time from event to conversion		1 mo (r: 0.1-19)
Median time from LT to conversion		6 mo (r: 0.1-192)
Early/late conversion	< 1 yr/ ⩾ 1 yr	42 (56.8)/32 (43.2)
Mean follow-up post-conversion		22 ± 19 mo (r: 0.5-74)
Median follow-up post-conversion		17.5 mo

HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HBV: Hepatitis B virus; ETOH: Cirrhosis due to alcohol; HIV: Human immunodeficiency virus; UNOS: United Network for Organ Sharing classification; ICU: Intensive care unit; LT: Liver transplantation; CET: Cranioencephalic trauma; CVA: Cerebrovascular accident; E-E: End-to-end choledoco-choledostomy; E-E + K: End-to-end choledoco-choledostomy + kehr; C-Y: Choledoco-jejunostomy; RBC: Red blood cells; IU: International units; r: Range.

cer recurrence during follow-up, or *de novo* tumor), EVER was introduced at a dose of 0.5 mg/d, with trough levels adjusted to under 3 ng/mL, and CNI was drastically reduced to half or completely withdrawn. Doses and levels of EVER for the entire series of patients and tacrolimus levels for patients receiving this drug post-conversion are shown in Figure 1.

Efficacy

The cause of conversion to EVER was resolved in 60.8% of patients.

Table 2 Causes of conversion and other comorbidities at the time of conversion to everolimus in 74 liver transplant patients *n* (%)

Cause of conversion			
Refractory rejection	23 (31.1)	Resolution	17 (73.9)
Extended HCC in explanted liver	14 (19)	Prevention of recurrence	7 (50)
HCC recurrence during follow-up	6 (8.1)	Stabilization	0 (0)
De novo tumour	13 (17.6)	Prevention of recurrence	8 (61.5)
CNI-related neurotoxicity	8 (10.8)	Resolution or Stabilization	8 (100)
Renal dysfunction	6 (8.1)	Resolution or Amelioration	3 (50)
Other causes	4 (5.4)	Resolution	2 (50)
Comorbidity at time of conversion			
Chronic renal insufficiency	22 (29.8)	Resolution or Amelioration	15 (68.2)
Diabetes mellitus	21 (28.4)	Resolution or Amelioration	8 (38)
Arterial hypertension	25 (33.8)	Resolution or Amelioration	3 (12)
Dyslipidemia	30 (40.5)	Resolution or Amelioration	2 (6.7)

Outcome to everolimus shown as resolution, stabilization or amelioration of the cause or comorbidity. In 45 of 74 patients (60.8%), the cause was resolved, stabilized or ameliorated. HCC: Hepatocellular carcinoma; CNI: Calcineurin inhibitors. Other causes include: 1 chronic biliary cirrhosis recurrence plus chronic rejection, 1 sinusoidal hepatic fibrosis, 1 graft-versus-host disease, 1 chronic cholostatic liver dysfunction in the postoperative period.

Refractory rejection: When EVER was used to treat refractory rejection (n = 23), the event was resolved correctly in 17 patients (73.9%) (Table 2). The remaining six patients failed to respond: four progressed to severe chronic refractory rejection finally requiring retransplantation and two died, one due to sepsis and one from concomitant severe hepatitis C recurrence.

Prevention of HCC recurrence: When EVER was indicated for prevention of HCC recurrence (n = 14), seven patients (50%) remained recurrence-free for a mean post-conversion follow-up of 33.8 ± 27 mo (Table 2). Three patients suffered recurrence at a mean post-conversion follow-up of 33.7 ± 33 mo, and four patients died due to HCC recurrence at a mean post-conversion follow-up of 15.1 ± 11 mo. When these 14 patients were compared with the historical cohort matched for MELD status, year of transplantation, and some pathological characteristics of the explanted liver, no differences either in survival or in time to recurrence were observed between the two groups (Table 4).

Patients with HCC recurrence: Six patients were converted to EVER due to HCC recurrence after liver transplantation. Types of post-transplant recurrences were: intra-abdominal at 122 mo; pulmonary at 6 mo; bone metastasis at 42 mo; liver recurrence at 46 mo; brain metastasis at 10 mo, and peritoneum-pulmonary metastasis

Table 3 Type of immunosuppression pre- and post-conversion to everolimus

Pre-conversion	*n* = 74	Post-conversion	*n* = 74
FK + MMF + ST	16	FK + EVER	38
FK + MMF	20	FK + EVER + MMF	1
FK + ST	12	FK + EVER + ST	11
FK	21	FK + EVER + MMF + ST	4
CyA + MMF + ST	1	CyA + EVER	3
CyA + MMF	1		
CyA	2	EVER	2
		EVER + ST	5
MMF + ST	1	EVER + MMF	2
		EVER + MMF + ST	8

FK: Tacrolimus; MMF: Mycophenolate mofetil; ST: Steroids; CyA: Neoral cyclosporine; EVER: Everolimus.

at 3 mo. Two patients were within the Milan criteria and four outside. All died at a mean time post-conversion of 14 ± 10.9 mo (3-31). When these six patients were compared with the historical cohort matched for recurrence site (1 suprarenal, 2 lung, 1 liver, 1 brain, 1 bone), time to recurrence and Milan criteria, survival post-recurrence was similar in those receiving EVER and those receiving other, non-mTORi immunosuppressants (Table 4).

Patients with *de novo* tumour: In thirteen patients, the reason for conversion to EVER was the appearance of a *de novo* tumor: 4 colon, 2 prostate, 2 esophagus, 2 larynx, 1 lung, 1 anus, and 1 breast. After onco surgical treatment of the tumor, eight patients remained alive and tumor-free at a mean follow-up post-tumor treatment of 37.7 ± 14.5 mo, four died at a mean follow-up post-tumor treatment of 21.5 ± 12.3 mo, and one (with colon cancer) is alive but with liver metastasis at 40 mo post-tumor treatment. In patients undergoing surgery, EVER was introduced as soon as healing was completed - 2-4 wk post-surgery. When these 13 patients were compared with the historical cohort matched for tumor type, time to development of the *de novo* tumor, and type of treatment, survival post-tumor treatment was similar in those receiving EVER and in those receiving other, non-mTORi immunosuppressants (Table 5).

Neurotoxicity: EVER was indicated in three patients with seizures, two with akinetic mutism, one with a cerebrovascular stroke plus multifocal progressive leukoencephalopathy, one with Guillain-Barré syndrome, and one with generalized tremor. Acompanying symptoms were different levels of speech disorders, including dysarthria, expressive dysphasia and apraxia. In all patients, EVER-based immunosuppression allowed a CNI-free period of time to reverse or ameliorate neurotoxicity.

Renal dysfunction: In the six patients in whom EVER was indicated due to renal insufficiency, serum creatinine changed from 2.54 ± 1.11 mg/dL pre-conversion to 1.63 ± 0.86 mg/dL at 3 mo post-conversion, 1.69 ± 0.91 mg/dL at 6 mo post-conversion, and 2 ± 1.45 mg/dL at 12 mo post-conversion. In the three patients who converted within the first year post-transplantation, renal function ameliorated, while two patients with established chronic renal insufficiency for more than five years post-transplantation remained unchanged, and one patient with an episode of acute renal insufficiency in the immediate postoperative period failed to improve. If we consider all the patients suffering from renal insufficiency at the time of EVER introduction, whatever the reason for conversion, the improvement was statistically significant: serum creatinine was 2.5 ± 1.01 pre-conversion, 1.59 ± 0.62 at 3 mo post-conversion, 1.62 ± 0.56 at 6 mo post-conversion, and 1.74 ± 0.76 at 12 mo post-conversion.

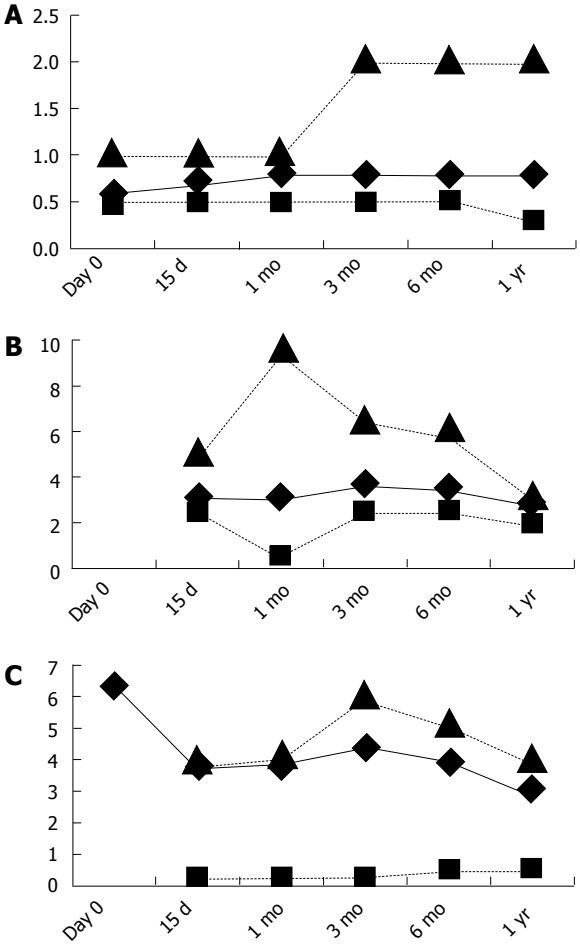

Figure 1 (A) Doses and (B) trough levels of everolimus for the entire series and (C) trough levels of tacrolimus for patients receiving this drug in the post-conversion regimen. Mean values and range (minimum-maximum). A: Doses of everolimus (ng/d); B: Trough levels of everolimus (ng/mL); C: Trough levels of tacrolimu (ng/mL).

Other causes: One patient was converted to EVER owing to liver dysfunction with cholostasis starting at 7 mo post-transplantation and progressing to severe cholostasis three months later (Table 2). Two liver biopsies at 8 and 10 mo post-transplantation revealed sinusoidal fibrosis and undetermined hepatitis. After conversion, liver function was completely restored within 1 mo. Another patient with a similar cholostatic syndrome one year af-

Table 4 Comparison between patients with hepatocellular carcinoma outside Milan criteria in the explanted liver receiving everolimus and a historical cohort not receiving mTOR inhibitors, and liver-transplanted patients with recurrence of hepatocellular carcinoma receiving everolimus and a historical cohort not receiving mammalian target of rapamycin inhibitors *n* (%)

HCC outside Milan criteria in explanted livers	Patients receiving everolimus *n* = 14	Historical controls without mTORi *n* = 14	*P*
Recipient age at transplant (yr)	55.5 ± 11.3	56.38 ± 7.1	NS
Recipient sex (male-female) (%)	86-14	79 - 21	NS
Child–Pugh status	6.7 ± 1.8	6.5 ± 1.4	NS
MELD score	13.6 ± 5	11.4 ± 3.4	NS
Size of largest tumour on pathologic exam	3.43 ± 1.50	3.152 ± 1.05	NS
Nº of tumours at pathologic exam	2.70 ± 1.7	2.74 ± 1.7	NS
Microvascular invasion	10 (78)	4 (29)	0.02
Macrovascular invasion	5 (39)	0	0.01
Satellitosis	7 (50)	3 (21.4)	NS
Well-moderately differentiated tumour (%)	31-69	50-50	NS
Mean alpha-fetoprotein	366 ± 771	55 ± 125	NS
Median alpha-fetoprotein	12 (3-2571)	8 (2-445)	NS
HCC treatment while on waiting list	9 (64.3)	8 (57)	NS
Mean donor age in years	59 ± 14.9	58 ± 12.6	NS
Mean and median patient survival post-LT (mo)	56 ± 8.5 (59)	67 ± 11 (54)	NS
HCC recurrence in post-LT follow-up	*n* = 6	*n* = 6	*P*
Recipient age at transplant (yr)	53.6 ± 10	46.5 ± 13	NS
Recipient sex (male-female) (%)	100-0	83-17	NS
Milan criteria in explanted liver (yes-no) (%)	33-67	33-67	NS
Mean donor age (yr)	52.1 ± 16	41 ± 12.8	NS
Months from LT to recurrence	37.9 ± 45	28.5 ± 30	NS
Immunosuppression at recurrence (CyA-FK) (%)	17-83	17-83	NS
Type of recurrence (intra–extrahepatic) (%)	17-83	17-83	NS
Survival after recurrence (mo)	14.1 ± 11	16.6 ± 12.5	NS

HCC: Hepatocellular carcinoma; mTORi: Mammalian target of rapamycin inhibitors; LT: Liver transplantation; CyA: Neoral cyclosporine; FK: Tacrolimus; NS: No significant.

ter transplantation did not improve and finally died. A third patient converted to EVER due to graft-versus-host disease one month post-transplantation. Immunosuppression was changed from tacrolimus to low doses of EVER to reduce any hypersensitivity to tacrolimus and counterbalance the steroid bolus administered. This patient was maintained on EVER monotherapy at 2-3 ng/mL and did well for two months but finally died from sepsis due to bone marrow aplasia as progression of his graft-versus-host disease. A fourth patient converted to EVER suffered long-lasting primary dysfunction of the liver. Two liver biopsies confirmed cholostatic preservation injury. Total bilirubin was normalized after introduction of EVER in combination with tacrolimus and steroids.

Efficacy for other comorbidities: Although EVER was never indicated for arterial hypertension and diabetes mellitus, 25 patients had high blood pressure at the time of conversion and 21 had insulin-dependent diabetes mellitus (Table 2). Blood pressure improved in three patients (12%), as shown by lower blood pressure or by a reduced need for antihypertensive drugs. One of them was converted to CNI free regimen (EVER and steroids). Glucose values or insulin doses improved in eight patients (38%). Three of them were converted to CNI free regimen (EVER and mycophenolate mofetil) . Dyslipidemia was present in 30 patients and serum values improved in only two (6.7%), whose regimen were CNI low dose and EVER.

Table 5 Comparison between liver-transplanted patients with *de novo* tumour receiving everolimus and a historical cohort not receiving mammalian target of rapamycin inhibitors

	Patients receiving everolimus *n* = 13	Historical controls without mTORi *n* = 13	*P*
Recipient age at transplant (yr)	60.8 ± 5.8	59.5 ± 6.6	NS
Recipient sex (male-female) (%)	77-23	75-25	NS
Indication for LT (%)			NS
Postnecrotic-HCC in cirrhosis	68%	70%	NS
Mean time from LT to diagnosis of *de novo* tumour (mo)	67 ± 50	65.9 ± 37	NS
Tumour site and histology			NS
Colon ADK	4	4	
Prostate ADK	2	2	
Lung SCC	1	1	
Larynx SCC (4)	2	2	
Esophagus SCC(3) + ADK(1)	2	2	
Anus SCC	1	1	
Breast IDC	1	1	
Type of treatment			NS
Surgery ± QT ± RT	10	10	
QT ± RT	3	3	NS
Immunosuppression at diagnosis			
Cyclosporine-tacrolimus (%)	8-92	24-76	NS
Mean patient survival from diagnosis of tumour (mo)	32.9 ± 15	30.7 ± 20.6	NS

mTORi: Mammalian target of rapamycin inhibitors; LT: Liver transplantation; HCC: Hepatocellular carcinoma; ADK: Adenocarcinoma; SCC: Squamous cell carcinoma; IDC: Infiltrative ductal carcinoma; QT: Chemotherapy; RT: Radiotherapy; NS: No significant.

Efficacy according to the time elapsed between transplantation and conversion to EVER

In general, conversion to EVER was successful in a greater percentage of patients when the conversion occurred during the first year post-transplantation (Table 6). Success rates in cases of early conversion were higher than in those of late conversion, especially in cases of refractory rejection (84.6% *vs* 60%), neurotoxicity (100%) and renal dysfunction (75% *vs* 0%).

Side effects and discontinuation

Liver graft function after conversion was well preserved in all cases except in 11 patients (14.9%) who presented acute cellular rejection (4 moderate and 7 mild) requiring the reintroduction of CNI (Table 7). Ten of these

Table 6 Efficacy in cases of early (within one year post-transplantation) and late (after one year post-transplantation) conversion to everolimus *n* (%)

Early conversion		
Cause of conversion	42 (56.8)	Resolution/stabilization or prevention of recurrence in 29 patients (69)
Refractory rejection	13 (17.6)	Resolution in 11 (84.6)
Advanced HCC in explanted liver	12 (16.3)	Prevention of recurrence in 6 (50)
HCC recurrence during follow-up	3 (4.1)	-
De novo tumour	0	-
CNI-related neurotoxicity	8 (10.8)	Resolution or amelioration in 8 (100)
Renal dysfunction	4 (5.4)	Resolution in 3 (75)
Other causes	2 (2.6)	Resolution in 1 (50)
Late conversion		
Cause of conversion	32 (43.2)	Resolution/stabilization or prevention of recurrence in 16 patients (50)
Refractory rejection	10 (13.5)	Resolution in 6 (60)
Advanced HCC in ex planted liver	2 (2.7)	Prevention of recurrence in 1 (50)
HCC recurrence during follow-up	3 (4.1)	-
De novo tumour	13 (17.6)	Prevention of recurrence in 8 (61.5)
CNI-related neurotoxicity	0	-
Renal dysfunction	2 (2.7)	Resolution in none (0)
Other causes	2 (2.7)	Resolution in 1 (50)

HCC: Hepatocellular carcinoma; CNI: Calcineurine inhibitors.

patients experiencing acute rejection had converted to EVER without CNI within the first year post-transplant.

EVER-related side effects occurred in 27 patients (36.5%), some of whom experienced more than one (Table 7). Dyslipidemia was managed with the introduction of hypolipemic drugs. Infections included severe hepatitis C recurrence in four cases, bacterial pneumonia in two, pulmonary tuberculosis in one, CMV infection, pulmonary aspergillosis and sepsis in graft-versus-host disease in one, and bacteriemia in one. Infections were treated according to the cause and by reducing the total amount of immunosuppression. Twenty-one patients (28.4%) stopped taking EVER (Table 7): six owing to resolution of the cause (acute rejection in four, convulsions in one, renal dysfunction in one); six because of inefficacy in resolving chronic rejection; five due to adverse events (infections in four, proteinuria in one); and four due to intercurrent surgery, with reintroduction of EVER two to three weeks after surgery.

Patient survival and follow-up

Mean follow-up post-conversion for the entire series was 22 ± 19.33 mo (range: 0.5-74), with a median of 17.5 mo. Actuarial patient survival post-conversion was 54%, 46% and 23% at 1, 3 and 5 years, respectively. Mean and median follow-up differed according to the reason for conversion: refractory rejection, 15.10 ± 15.96 mo (range: 0.5-54) and 9 mo; HCC outside Milan criteria, 29.10 ± 24.72 mo (range: 6-74) and 21.50 mo; post-transplant HCC recurrence, 14.16 ± 10.94 mo (range: 3-31) and 13 mo; *de novo* tumor, 32.92 ± 15 mo (range: 5-54) and 32 mo; renal dysfunction, 14.85 ± 13.58 (range: 0.5-41) and 18 mo; and other CNI-related adverse events, 25.87 ± 21.53 mo. Causes of death are shown in Table 7. There were no EVER-related deaths.

Table 7 Adverse events, causes of discontinuation and mortality *n* (%)

	Patients receiving everolimus (*n* = 74)
Adverse events	27 (36.5)
Dyslipidemia	27 (36.5)
Infections	9 (12.2)
Mucositis	3 (4.1)
Diarrhoea	1 (1.4)
Proteinuria	1 (1.4)
Acute rejections post-conversion	11 (14.9)
Causes of discontinuation	21 (28.4)
Resolution of the cause of conversion	6 (8.1)
Non-responding rejection and retransplantation	6 (8.1)
Drug-related adverse events	5 (6.7)
Intercurrent surgery	4 (5.5)
Causes of mortality	25 (33)
HCC recurrence during follow-up	10
De novo tumour	4
HCV recurrence	4
Chronic rejection	4
Sepsis	1
Graft-*vs*-host disease	1
Other causes	1

HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.

DISCUSSION

The principal aim of this retrospective study was to study the real use and management of EVER in patients treated off-protocol and help redefine the true role of mTORi in clinical practice. In the field of liver transplantation, we are faced with clear challenges for the 21st century, one of which is establishing patient profiles for individualising immunosuppression strategies. Sirolimus, the first mTORi introduced into clinical practice some years ago, was largely unsuccessful[1], but it has provided sufficient experience to help improve the use and management of EVER, another mTORi.

Reasons for introducing EVER

The most frequent indication for introducing EVER in our series was a high risk of tumour recurrence. So, our first experience with EVER was at low doses within a dual regimen while minimizing CNI. This experience provided evidence of the safety and efficacy of EVER, and we were able to avoid the adverse events associated with high doses of sirolimus. Furthermore, the pharmacokinetic differences between EVER and sirolimus permitted a 12-h administration and offered the possibility of providing much greater accuracy in trough levels and dose calculation.

We have used EVER in all types of transplant patients, regardless of age, sex, cause, the severity of liver disease, or concomitant diseases. Advanced age, co-infection with HCV and HIV viruses, diabetes mellitus, arterial hypertension, obesity, renal insufficiency, or even dyslipidemia did not constitute a contraindication for the use of EVER.

Cancer patients

Cancer patients deserve special mention, since the mTOR pathway is necessary for tumour cells to grow[25]. There are three potential profiles of cancer patients. Firstly, in patients transplanted for HCC outside the Milan criteria and/or with macro- or microvascular invasion in the explanted liver, EVER would be used as prophylaxis and would be introduced in the early post-transplantation period[10]. Secondly, in patients transplanted for HCC with recurrence of the original tumour during follow-up, EVER would be used as treatment[26]. Finally, in transplanted patients who develop a *de novo* tumour during follow-up, EVER would also be used as treatment[27,28].

In our study, in patients whose tumours were outside the Milan criteria in the explanted liver, either EVER or CNI was administered at low doses between six and twelve weeks post-transplant. We had difficulty finding appropriate historical matches for this subgroup of patients. Although macro- and microvascular invasion was greater in the EVER group, there was also a trend towards longer survival. This trend did not, however, reach statistical significance - probably due to the low number of patients. To date, no published randomized study has demonstrated the beneficial effect of the use of mTORi as prophylaxis, but we believe that EVER provides a benefit since it is the least pro-carcinogenic immunosuppressant and allows doses of known pro-carcinogenic immunosuppressants to be reduced. We await the results of a future randomized prospective study[29].

One of the main late indications in our study was the development of a *de novo* tumour or recurrence of the original HCC. Again, survival in the EVER group was longer but did not reach statistical significance compared to our historical cohort, probably due to the low number of patients included. Taking into account that the anti-tumour properties of mTORi are at doses much higher than those used in clinical practice[30], we agree with other authors[20,21] that EVER appears to be effective at reducing tumour recurrence.

Patients with acute rejection

The second most frequent indication for the introduction of EVER was to reinforce the immunosuppressive regimen in cases of severe or refractory acute rejection. In this situation, EVER could be safely administered together with CNI and steroids as triple therapy or with the addition of MMF as quadruple therapy as early as 10 d post-transplant, once healing was complete. The initial doses and trough levels reached were the highest. The phase Ⅱ trial[31] comparing three doses of EVER showed that freedom from rejection correlated with trough blood levels of 3 ng/mL or more. Six patients with chronic rejection did not benefit from the introduction of EVER and were finally retransplanted, suggesting that the drug has the greatest effect during the early post-transplantation period and that there is little or no benefit from EVER in the case of chronic rejection.

Neurotoxicity and other CNI-induced toxicities

Our experience with EVER without CNI was in patients with severe neurotoxicity or other severe adverse effects triggered by CNI, especially in the early post-transplantation period, although some cases were observed during the late post-transplantation period. Initial doses and trough levels were high, in the same range as for patients with refractory rejection. Our findings, consistent with other authors[32], indicate that EVER-based immunosuppression - either with or without other non-CNI drugs - is a feasible and effective option, at least for the time required for CNI-induced neurological complications to disappear. However, the risk of acute rejection during the first year post-transplantation indicates a need for caution. Therefore, we do not believe that regimens based on EVER without CNI should be the principal use of this drug, at least during the first year post-transplantation.

The improvement achieved in some patients with diabetes and arterial hypertension was probably due to the parallel decrease in CNI levels and/or steroids. None of these co-morbidities were indications for conversion and they were evaluated in a qualitative and global way that makes difficult to explain the real cause of improvement. However, we believe that regimens based on EVER and low levels of CNI could play a role in patients with metabolic syndrome[33], although further studies are required to ascertain their ability to modify the risk of cardiovascular disease[34].

Early and late renal dysfunction

In our study, an overall improvement in serum creatinine levels was observed in patients whose indication for receiving EVER was renal dysfunction. However, when we specifically analyzed the six patients converted for renal insufficiency, the maximum benefit was attained in those converted within the first year post-transplantation. Several liver studies and multicentre randomised trials[35,36] introducing EVER at one month post-transplantation have reported an amelioration in the glomerular filtration rate at 12 and 24 mo post-transplantation in patients receiving tacrolimus plus EVER and minimizing CNI compared to those receiving standard tacrolimus and steroids.

Adverse events and discontinuation at low doses

No life-threatening adverse events were observed. The main adverse event was dyslipidemia, which was easily controlled by reducing the EVER dose and adding a statin. None of our patients presented EVER-associated interstitial pneumonitis or severe sepsis, as had previously been reported in other studies[37], and drug-related deaths

Table 8 Future challenges in liver transplantation and the potential role of everolimus

Future challenges	Potential role of everolimus
More marginal donors	Renal function protection
Recipients with more serious disease, selected by MELD	Renal function protection
Recipients with more serious disease, with metabolic syndrome	Prevention of cardiovascular events
Less HCV cirrhosis but more aggressive strains	Antifibrotic effect
More NASH	Prevention of cardiovascular events
More metabolic syndrome during follow-up	Prevention of cardiovascular events
More HCC recurrence	Antiproliferative effect
More *de novo* tumours	Antiproliferative effect
CNIe-related neurotoxicity	Good neurological profile

MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; NASH: Non-alcoholic steato hepatitis; HCC: Hepatocellular carcinoma; CNI: Calcineurine inhibitors.

did not occur. This was probably due to the low doses of EVER (Figure 1) and the lessons learned from our previous experience with sirolimus[38].

A good percentage of failure or discontinuation of the drug is probably related to the timing of the introduction of EVER in critical and irreversible situations where other immunosuppressants have failed. A real problem in the long-term management of mTORi is wound complications, which would render EVER inadvisable in stable patients with good liver function who must undergo some type of intercurrent surgery. In such cases, we would recommend withdrawal of the drug and its reintroduction, if necessary, four weeks after surgery.

Challenges in the 21st century and the potential role of EVER

According to our study, there are several potential indications for the use of EVER after liver transplantation. During the early post-transplantation period, EVER can be used as one component of a triple therapy for refractory rejection, as one component of a double therapy with CNI (both at half the normal dose) in cases of extended tumours in the explanted liver, and at low doses without CNI in cases of severe CNI-related adverse effects. During the late post-transplantation period, EVER can be used at low doses in patients with CNI-related adverse effects and in those with HCC recurrence or *de novo* tumours. In general, we recommend EVER at low doses and as a support immunosuppressant. In this scenario, the rate of adverse events, discontinuations and drug-related deaths will be acceptable.

The future of liver transplantation presents the following scenario (Table 8): (1) increasing acceptance of marginal donors to increase the pool of grafts; (2) recipients with more severe liver disease according to the MELD criteria[39]; (3) a higher frequency of recipients with metabolic syndrome as a comorbidity; (4) less HCV cirrhosis and more NASH as the reason for transplantation[40]; and (5) longer patient survival but with increased HCV and HCC recurrence, *de novo* tumours and cardiovascular events. Looking at this scenario, we can imagine more renal dysfunction, more metabolic syndrome and cardiovascular events, and more cases of cancer. Marginal donors would increase the incidence of primary liver dysfunction and resultant renal dysfunction. The use of the MELD score to select patients for transplantation would increase the incidence of post-transplant renal dysfunction. The incidence of metabolic syndrome is increasing both in candidates for liver transplantation and in recipients during the post-transplantation period, as well as in the general non-transplanted population, which in turn would increase the risk of cardiovascular events in the long term. The new antiviral therapies for hepatitis C may affect the need for liver transplantation; however, the HCV in the small number of patients not responding to the new drugs will be more selected and perhaps more aggressive. The incidence of HCC secondary to HCV cirrhosis would decrease, but HCC secondary to NASH would increase. Improved post-transplantation management of patients would mean longer patient survival and thus a greater probability of tumour recurrence or a *de novo* tumour (Table 8). We urgently need an immunosuppressant that will meet all the requirements. EVER is a drug with a good profile for renal dysfunction, a certain antifibrotic effect, an ability to inhibit the mTOR pathway used by cancer cells, and a good degree of effectiveness in reducing cardiovascular risk events. Future trials will demonstrate if EVER is the immunosuppressant we need.

COMMENTS

Background

Calcineurine inhibitors (CNI) are the most powerful immunosuppressants used in liver transplantation, however the long-term survival and quality of life are partly overshadowed by the appearance of adverse effects of its chronic use, such as renal dysfunction, metabolic syndrome, cardiovascular complications, *de novo* tumor and recurrence of underlying disease. Previous attempts to overcome these complications with the use of mammalian target of rapamycin (mTOR) inhibitors (an immunosuppressant with a different way of action), did not succeed. However, everolimus seems to cope with them and to partially contribute to search their role.

Research frontiers

New emerging immunosuppressants must be powerful enough to avoid rejection in the same way as calcineurine inhibitors, but at the same time must avoid calcineurine inhibitors-related adverse events. The association of tacrolimus and everolimus could represent the best regimen to cope with the different profiles of patients after liver transplantation.

Innovations and breakthroughs

Recent multicentre trials have highlighted the important of everolimus introduction at one month post-transplant together with low dose tacrolimus to protect early and long term renal function after liver transplantation. In this single-centre study, the authors report other off-protocol indications for everolimus, that could fit into the various profiles of patients that most concern to medical teams, cancer patients and patients with co-morbidities derived from calcineurine inhibitors.

Applications

Due to the lack of new immunosuppressants, optimization of treatment regimens is of great value to increase patient and graft survival after liver transplantation. In the near future two facts will be relevant. First, survival will continue to increase over time, to the same extent that the need for calcineurine inhibitors sparing protocols. Second, the authors probably will see a change in the indications for liver transplantation, from hepatitis C liver cirrhosis toward cancer

patients. This article could serve as a starting point to be explored with further studies.

Terminology

Everolimus is an orally administered mTOR inhibitor, a proliferation signal employed by many mammalian cells, especially those with a high level of turnover (skin, intestinal and hematological cells), but also many cancer cells and T-cells implied in the second phase of the alloantigenic response. The same pathway used by different cells of the human body, explains the dual characteristic of this mTOR inhibitor as immunosuppressant and as antineoplastic.

Peer review

This an interesting review, it can be usefull for the readers.

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