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ORIGINAL ARTICLE

Conversion to everolimus monotherapy in maintenance liver transplantation: feasibility, safety, and impact on renal function

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Summary

We present the 12-month results of a prospective trial of conversion from calcineurin inhibitors (CNI) to everolimus (EVL) in maintenance liver transplant (LT) recipients. Forty (M:F = 28:12; 54.9 ± 11 years) patients were enrolled at a mean interval of 45.5 ± 31.2 months from transplantation. Conversion was with EVL at a dosage of 0.75 mg b.i.d., withdrawal of antimetabolites, and a 50%-per-week reduction of CNI to a complete stop within 4 weeks. The treatment success was conversion to EVL monotherapy at 12 months while failure was presence of CNI, death, and graft loss. Indication to conversion was deteriorating renal function in 36 (90%). At 12 months, patient- and graft survival were 100% and the success rate was 75% (30/40). Ten patients (25%) were failures: four (10%) for acute rejection; three hepatitis C virus-RNA positive patients (7.5%) for hypertransaminasemia; one (2.5%) for acute cholangitis; and two (5%) due to persistent pruritus and oral ulcers. In patients on EVL monotherapy, at 12 months the mean change of calculated creatinine clearance (cCrCl) was 4.03 ± 12.6 mL/min and the only variable correlated with the probability of improvement was baseline cCrCl ($P < 0.0001$). Conversion from CNI to EVL is feasible in 75% of the cases and associated with improvement in renal function for patients with higher baseline cCrCl.

Introduction

Use of calcineurin inhibitors (CNI) as main immunosuppressants in solid organ transplantation is associated with reduced risk of rejection but early and long-term related side-effects [1]. CNI can contribute to reduced levels of renal function [2], neurotoxicity [3], increased cardiovascular risk [4] and incidence of post-transplant diabetes mellitus [5]. However, elimination of cyclosporine (CsA) or tacrolimus (TAC) in the early post-transplant course has so far not been achieved by immunosuppressive strategies without increasing the risk for acute rejection [6–8]. Everolimus (EVL) belongs to a novel class of immunosuppressants – the proliferation signal inhibitors (PSI) [9] – whose renal sparing profile make them a promising alternative in solid organ transplantation. *In vitro* and clinical studies have demonstrated that EVL enhances immunosuppression in CsA-based regimens [10,11]. A phase I study in liver transplantation (LT) demonstrated that combined administration of EVL, micrcoemulsion CsA (CsA-ME) and steroids (S) was not associated with significant changes in clinical parameters or increased rates of infections [12]. A recent, phase II trial on fixed doses of

EVL and CsA-ME in *de novo* LT recipients provided further evidence supporting the efficacy and safety of EVL in this category of patients [13]. Furthermore, *in vitro* and *in vivo* studies have demonstrated that EVL inhibits cell growth and proliferation by blockage of the mammalian target of rapamycin [14–16], attracting interest in the use of this drug for treatment and prevention of post-transplant malignancies [17].

Preliminary experiences on the use of PSI in maintenance LT have mainly focused on sirolimus (SRL) in patients affected with CNI-related renal impairment [18–27]. These studies have demonstrated that CNI minimization with SRL or conversion from CNI-based to SRL-based immunosuppression is feasible, and associated with a 5–15% risk for acute rejection, and a variable degree of improvement in renal function depending on the baseline creatinine clearance (CrCl) [23,27], concurrent non-CNI-related renal disease [25], and interval from transplantation [25,27]. However, two small, prospective, randomized, single-center trials on conversion to SRL versus CNI continuation in patients with impaired renal function have recently demonstrated that CNI withdrawal is associated with a significant improvement in CrCl 3 months after switch, but not at 12 months, suggesting that earlier CNI minimization be the key to prevention of the post-transplant decline in renal function in LT patients [25,26]. This study was performed to explore the feasibility of conversion to EVL with discontinuation of CNI in adult, maintenance LT recipients with a minimum follow-up of 12 months, CNI-related renal impairment or at risk of neurological and renal complications. The secondary objectives were to assess the impact of conversion to EVL on renal function and derive information for refinement of the mode and timing of introduction of EVL.

Materials and methods

The study was designed to evaluate the feasibility of conversion to EVL (Certican®, Novartis, Basel, Switzerland) with discontinuation of CNI within 4 weeks of Certican® initiation in adult, maintenance LT recipients with a minimum follow-up of 12 months and CNI-related renal impairment or at risk of neurological and renal complications, while maintaining efficacy. This was evaluated by efficacy and safety parameters within 12 months of Certican® initiation by: incidence of efficacy failure [biopsy-proven acute rejection (BPAR), graft loss or death]; incidence of treated BPAR; incidence and reasons for failure; patient- and graft survival; safety parameters, including hypertension; diabetes mellitus; hyperlipidemia; anemia; infections; hepatitis C virus (HCV) load, and malignancies; incidence of discontinuation of study medication; incidence of premature study withdrawal; and incidence of serious adverse events. Treatment success was defined as percent of patients at 12 months alive, with a functioning graft and on EVL monotherapy, while the treatment failure was persistence of CNI four weeks of EVL initiation, needed for dialysis, CNI reintroduction, graft loss and death. The study secondary objective was to assess whether conversion to Certican® monotherapy improved renal function by assessment of the change in the calculated CrCl from baseline (−day 1 before EVL initiation) to month 12. The study was carried out at the Unità Operativa Chirurgia Generale e Trapianti di Fegato of the Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy in cooperation with the Laboratory Department. Enrollment aimed at maintenance, LT patients with a minimum follow-up of 12 months and: a calculated CrCl (cCrCl) according to Cockroft and Gault [28] between 80 and 20 ml/min (stage II–IV of the National Kidney Foundation-Dialysis Outcome Quality Initiative [29]); a cCrCl ≥ 81 ml/min in the presence of anti-hypertensive medication, chronic ischemic cardiomyopathy, insulin-dependent diabetes mellitus, either pre-existing or *de novo* post-transplantation (fasting blood glucose ≥ 7 mmol/l); noninsulin-dependent diabetes mellitus, either pre-existing or *de novo* post-transplantation (fasting blood glucose ≥ 7 mmol/l); peripheral arteriopathy (≥grade II); prior or active neurological disease (as for epilepsy, prior stroke, prior neurosurgery, etc.), for whom conversion to EVL was perceived to be protective against the decline in renal and/or neurological functions. Eligible patients had to be on CNI-based immunosuppression, either with TAC with C0-h level between 3 and 8 ng/ml or CsA-ME with C0-h level ≤ 150 ng/ml and/or C2-h level within 650 ng/ml with or without any of the following: mycophenolate mofetil (MMF), mycophenolic acid (MPA), azathioprine (AZA) or S. Only patients willing and capable of giving written informed consent for study participation and able to participate in the study for at least 12 months were enrolled. Exclusion criteria called for: recipients of multiple solid organ transplants; patients with a cCrCl ≥ 81 ml/min in the absence of coexisting morbidities and/or risk factors indicated above; patients on dialysis; patients with proteinuria ≥ 1.0 g/24 h; patients with any acute rejection within 6 months prior to randomization; a platelet count of ≤50 000/mm^3 or white blood cell count of ≤2000/mm^3 or hemoglobin ≤ 8 g/dl; patient with graft dysfunction as clinically indicated or associated with bilirubin >2 mg/dl, or albumin <35 g/l (in the absence of proteinuria) or prothrombin time >1.3 INR (in the absence of anticoagulant medication); HCV positive patients requiring an active anti-viral treatment; HIV positive patients; breast feeding females; patients with concurrent severe systemic infection; presence of any hypersensitivity to drugs similar to Certican® (e.g. macrolides); and use of any

immunosuppressive drugs other than Prograf®/Neoral®, S, MMF, MPA, and AZA. Once written informed consent was signed, patients were screened to evaluate for eligibility into the study. Screening assessments occurred at least 12 months after LT. Patients who satisfied the inclusion and exclusion criteria were administered EVL at a dosage of 0.75 mg b.i.d. (1.5 mg/daily) starting on day 1 and CNI were decreased by 50% per week when EVL was initiated. MMF, MPA and AZA were discontinued upon EVL initiation. Steroids were maintained at preswitch levels. EVL dosage was adjusted throughout the study in order to achieve a recommended trough level between 3 and 8 ng/ml (FPIA) according to liver function tests and incidence of adverse events. Levels below 3 ng/ml and higher than 8 ng/ml were based on proven clinical indications, occurrence of adverse event(s), and/or lack of efficacy. CNI were decreased each week by 50% of the current dose or so as to allow CNI discontinuation within 4 weeks of EVL initiation, unless otherwise clinically indicated. The study duration was 12 months after EVL initiation. Patients visits were scheduled within 4 weeks prior to day −1, at day −1, week 1, week 2, week 3, week 4 and months 2, 3, 4, 5, 6 and 12. Once suspected, acute rejection was to be confirmed by biopsy and graded according to the 1997 Banff classification (RAI scoring system) [30]. We decided to treat rejection episodes with a RAI score < 7 with EVL dose adjustments and those with a RAI score ≥ 7 with either pulse steroids, CNI reintroduction/dose adjustments or a combination thereof. Data management was according to the Italian protection code law.

Results

Between October 1st 2006 and May 31st 2007 a total of 40 patients (mean age 54.9 ± 11 years; M/F = 28/12) were enrolled at a mean of 45.5 ± 31.2 months post-LT. Native disease was HCV-related chronic failure in 15 (37.5%); HBV-related in 10 (25%); alcohol-related in eight (20%); primary biliary cirrhosis in four (5%); Wilson's disease, polycystic liver and cholangiocarcinoma in one patient (2.5%) each (Table 1). All of the 15 HCV patients were PCR-RNA positive at the time of switching and viral genotype was one in all such cases. Eleven patients (27.5%) were concomitantly affected with hepatocellular carcinoma (nine within Milan and two beyond Milan on explant histology) (Table 1). Indications for conversion were deteriorating renal function in 36 (90%), CNI-related peripheral neuropathy in three (7.5%), and CNI-related microangiopathy in one (2.5%). At baseline (1 day before EVL initiation), patients' immunosuppression was: CsA-ME alone in 16 cases (40%); CsA-ME in association with antimetabolites in 12 (30%); TAC alone in nine (22.5%); TAC with antimetabolites, TAC with S, and TAC with S and antimetabolites in one (2.5%) each. At baseline, mean CsA-ME daily dosage was 131.8 ± 39.8 vs. 3.1 ± 1.7 mg for TAC; mean CsA trough level was 92.1 ± 43.8 ng/ml; mean CsA C2-h level was 435.4 ± 154.8 ng/ml; and mean TAC trough level was 6.7 ± 1.3 ng/ml. As for renal function, mean cCrCl was 60.6 ± 23 ml/min and mean proteinuria (spot sample) was 265 ± 72.3 mg/24 h (Table 1).

Table 1. Demographic characteristics of the study population at baseline*.

Variable	
Mean age	54.9 ± 11 years
Gender (M:F)	28:12
Mean interval from LT	45.5 ± 31.2 months
Native disease (%)	
HCV	15 (37.5)
HBV	10 (25)
Alcohol	8 (20)
PBC	4 (5)
Wilson's disease	1 (2.5)
CHC	1 (2.5)
Polycystic liver	1 (2.5)
HCC (%)	11 (27.5)
Milan	9 (22.5)
Non-Milan	2 (5)
Indication to conversion (%)	
cCrCl ≤ 80 ml/min	36 (90)
CNI-related peripheral neuropathy	3 (7.5)
CNI-related microangiopathy	1 (2.5)
Mean cCrCl (range)	60.6 ± 23 ml/min (28.2–154.6)
Mean proteinuria†	265 ± 72.3 mg/24 h
Immunosuppression (%)	
CsA-ME	16 (40)
CsA-ME + MMF/MPA/AZA	12 (30)
TAC	9 (22.5)
TAC + MMF/MPA/AZA	1 (2.5)
TAC + S	1 (2.5)
TAC + AZA + S	1 (2.5)
Mean CsA-ME daily dosage	131.8 ± 39.8 mg
Mean TAC daily dosage	3.1 ± 1.7 mg
Mean CsA C0-h blood level	92.1 ± 43.8 ng/ml
Mean CsA C2-h blood level	435.4 ± 154.8 ng/ml
Mean TAC C0-h blood level	6.7 ± 1.3 ng/ml

PBC, primary biliary cirrhosis; CHC, cholangiocellular carcinoma; cCrCl, calculated creatinine clearance according to Cockroft–Gault.
*Baseline is –Day 1 before EVL initiation.
†Spot sample analysis.

Twelve months after conversion, patient and graft survival was 100%. The treatment success rate (i.e. CNI elimination) was 75% (30/40). Ten patients (25%) were treatment failures: namely, six (15%) patients discontinued EVL because of adverse effects, while four (10%) for BPAR (Table 2). Incidence of treated BPAR was 15%

(6/40). Rejection episodes occurred at a mean of 70 ± 14.1 days after EVL initiation with a mean RAI score of 7 ± 0.6. BPAR was treated with CNI reintroduction in three (7.5%) cases; EVL dose adjustment in two (5%); and steroid boluses and resumption of previous CNI-based therapy (TAC, S, AZA) in one case (2.5%) (Table 2). In patients with BPAR, mean EVL blood level until the time of diagnosis was 4.9 ± 2.4 vs. 5.03 ± 2.32 ng/ml in patients with no BPAR ($p = 0.8562$). The complications observed in the entire population of patients who received at least one dose of the study drug (40/40) are listed in Table 2 and included: hyperlipemia (as per cholesterol > 220 mg/dl and/or triglycerides > 350 mg/dl) in 17 patients (42.5%); mouth sores in nine (22.5%); hypertransaminasemia ≥ 3 times the upper limit of the range in three HCV-RNA positive patients (7.5%); pruritus in three (7.5%); acneic dermatitis in three (7.5%); low tract urinary infection in two (5%); pharyngitis in one (2.5%); urticaria in one (2.5%); persistent headache in one (2.5%); eczema in one (2.5%); psoriasis in one (2.5%); erythema in one (2.5%); oral abscess in one (2.5%); acute cholangitis in one (2.5%), and shingles in one (2.5%) (Table 2). EVL discontinuation occurred at a mean of 34 ± 19.2 days since drug initiation (Table 2).

Table 2. Twelve month results.

Variable	
Graft survival (%)	40 (100)
Patient survival (%)	40 (100)
Treatment success* (%)	30 (75)
Treatment failure† (%)	10 (25)
BPAR	4 (10)
Hypertransaminasemia (flare) ≥ 3 ULR	3 (7.5)
Acute cholangitis	1 (2.5)
Oral ulcers (consent withdrawal)	1 (2.5)
Intractable pruritus (consent withdrawal)	1 (2.5)
BPAR (%)	6 (15)
CNI reintroduction	3 (7.5)
EVL dose adjustment	2 (5)
Steroid boluses and resumption of preswitch regimen	1 (2.5)
Mean RAI score (±SD)	7 (0.6)
Mean days after EVL introduction (±SD)	70 (14.1)
Complications (%)	
Hyperlipemia‡	17 (42.5)
Oral ulcers/stomatitis	9 (22.5)
Hypertransaminasemia	3 (7.5)
Pruritus	3 (7.5)
Acne	3 (7.5)
Low tract urinary infection	2 (5)
Pharyngitis	1 (2.5)
Urticaria	1 (2.5)
Persistent headache	1 (2.5)
Eczema	1 (2.5)
Psoriasis	1 (2.5)
Erythema	1 (2.5)
Oral abscess	1 (2.5)
Shingles	1 (2.5)
Cholangitis§	1 (2.5)
mean 12-month proteinuria¶ (±SD)	246 ± 54.3 mg/24 h
mean 12-month cCrCl** (range)	67.7 ± 35.9 ml/min (34.3÷207.1)
ΔcCrCl** (range)	4.03 ± 12.6 ml/min (−10.6÷52.5)

BPAR, biopsy-proven acute rejection; cCrCl, calculated creatinine clearance according to Cockroft-Gault; ULR, upper limit of the range.
*CNI elimination 6 months postswitch.
†Persistence of CNI (±EVL) 6 months postswitch.
‡Cholesterol >220 mg/dl and/or triglycerides >350 mg/dl.
§In the absence of signs of biliary obstruction.
¶Spot sample analysis; 30 evaluable patients with no CNI.
**Thirty evaluable patients with no CNI.

Twelve months after switch, in patients successfully converted to EVL monotherapy (30/40) proteinuria (spot sample analysis) increased from a mean of 203 ± 95.8 mg/24 h at baseline to a mean of 246 ± 54.3 mg/24 h, while the mean change of cCrCl was 4.03 ± 12.6 ml/min (range −10.6÷52.5 ml/min; 95% CI −1.8÷9.1 ml/min), i.e. from a mean of 62.3 ± 24.6 mL/min (range 38.3–154.6) at baseline to a mean of 67.7 ± 35.9 ml/min (range 34.3−207.1 ml/min) (Table 2). Namely, 17 patients (56.7%) improved their baseline cCrCl (Table 3); 4 (13.3%) presented no improvement (as per baseline cCrCl ± 0.9 ml/min), and 9 (30%) presented deterioration of their baseline renal function despite EVL introduction (Table 3). Based on data from the international literature [2], we tested whether there was any correlation between the ΔcCrCl and some selected clinical variables, such as baseline cCrCl, patient's age, time from transplantation, gender, and HCV status (Table 4). On univariate and multivariate analysis the only clinical variable correlated with the probability of cCrCl improvement 12 months after switching to EVL was the baseline cCrCl ($P < 0.0001$) (Table 4).

Starting on day 1, all patients were administered 1.5 mg EVL in two split doses with overnight withdrawal

Table 3. Change in cCrCl at 12 months in 30 patients successfully converted to EVL monotherapy.

ΔcCrCl	#	%
≥−5.1 ml/min	3	10
From −1 to −5 ml/min	6	20
Baseline ± 0.9 ml/min	4	13.3
1–5 ml/min	3	10
5.1–10 ml/min	5	16.7
≥10.1ml/min	9	30

Table 4. Correlation analysis between the change in cCrCl at 12 months and selected patients clinical variables.

	Univariate	Multivariate
Baseline CrCl	<0.0001	<0.0001
Age	0.3822	0.6448
Interval from transplantation	0.45	0.3373
Gender	0.6511	0.3218
HCV-status	0.2235	0.5471

of antimetabolites and a 50% per week reduction of CNI with complete stoppage within 4 weeks. No patient dropped out of EVL within 7 days after drug initiation because of any adverse effects. Seven days after conversion, the mean EVL trough blood level was 6.4 ± 3.9 ng/ml in the overall population (range 1.3–15.5 ng/ml); 7.3 ± 4.2 ng/ml among patients on CsA-ME (range 2.2–15.5 ng/ml) vs. 4.1 ± 1.6 ng/ml for patients on TAC (range 1.3 ± 6.8 ng/ml) ($P = 0.0438$). Namely, six patients (15%) were below the target range (mean 2.2 ± 0.6 ng/ml); eight (20%) were above the target range (mean 12.5 ± 2.6 ng/ml); and 26 (65%) were within the target range (mean 5.3 ± 1.3 ng/ml) (Table 5). According to their baseline immunosuppression, 7 days after EVL initiation 7.1% (2/28) patients on CsA-ME were below the target range as opposed to 33.3% (4/12) on TAC ($P = 0.0548$); 67.8% (19/28) patients on CsA-ME were within the target range vs. 58.3% (7/12) on TAC ($P = 0.7201$); and 25% (7/28) patients on CsA-ME were above the target range vs. 8.3% (1/12) on TAC ($P = 0.3955$). Seven days after EVL introduction, patients on CsA-ME had a higher probability of being above >8 ng/ml when compared with patients on TAC (OR 3.6; 95% CI 0.37–180.02), while patients on TAC had a higher probability of EVL trough blood levels <3 ng/ml (OR 6.5; 95% CI 0.72–79.82) (Table 5). Mean EVL daily dosage and mean EVL trough blood level per study visit are illustrated in Table 6. These data are to be interpreted taking into account that CNI were being tapered within the first 4 weeks, according to the study protocol. There was a tendency towards EVL dose reduction within the first month, on account of the pharmacokinetic interaction between EVL and CNI, and the incidence of adverse events. From the second month onward, EVL trough blood levels remained quite stable by means of a slight increase in total daily dosage, attributable to CNI elimination (Table 6). At 12 months, mean EVL daily dosage in patients successfully converted to EVL (30/40) was 2.1 ± 1.09 mg and mean EVL blood trough level was 4.1 ± 1.9 ng/ml (Table 6).

Table 7 illustrates the HCV viral load by follow-up visit. No significant change in baseline log viral load was observed throughout the study period, with a mean change from baseline of 0.2 (Table 7). With respect to the

Table 6. EVL dosage and trough blood levels* by follow-up visit.

	Evaluable patients†	Mean daily dosage (mg)	Mean trough blood level (ng/ml)
Week 1	40	1.4 ± 0.3	6.4 ± 3.9
Week 2	38	1.4 ± 0.6	5.5 ± 2.9
Week 3	38	1.5 ± 0.6	4.9 ± 1.6
Week 4	36	1.5 ± 0.5	5.2 ± 2.3
Month 2	32	1.6 ± 0.7	4.8 ± 2.1
Month 3	30	1.7 ± 0.8	4.8 ± 1.5
Month 4	30	1.7 ± 0.8	4.7 ± 1.7
Month 5	30	1.8 ± 0.8	5 ± 1.5
Month 6	30	1.7 ± 0.9	4.6 ± 1.9
Month 12	30	2.1 ± 1.09	4.1 ± 1.9

*FPIA; target range 3–8 ng/ml.
†Treatment failures excluded.

Table 7. HCV-RNA* by follow-up visit.

	Evaluable patients†	Mean log	Mean change from baseline
Baseline	13	6.2 ± 0.7	–
Week 1	13	6.3 ± 0.6	0.1
Week 2	12	6.4 ± 0.6	0.2
Week 3	12	6.3 ± 0.6	0.1
Week 4	11	6.1 ± 0.6	−0.1
Month 2	9	6.3 ± 0.6	0.1
Month 3	8	6 ± 0	−0.2
Month 4	8	6.2 ± 0.4	0
Month 5	8	6.3 ± 0.4	0.1
Month 6	8	6.4 ± 0.5	0.2
Month 12	8	6.4 ± 0.5	0.2

*Amplicore assay. All patients were genotype 1.
†Treatment failures excluded.

Table 5. EVL blood trough levels* 7 days after drug introduction.

	Overall (40)	CsA-ME (28)	TAC (12)	*P*-value
Mean trough level	6.4 ± 3.9 ng/ml	7.3 ± 4.2 ng/ml	4.1 ± 1.6 ng/ml	0.0438
Patients within target (%)	26 (65)	19 (67.8)	7 (58.3)	0.7201
Patients below target (%)	6 (15)	2 (7.1)	4 (33.3)	0.0548
Patients above target (%)	8 (20)	7 (25)	1 (8.3)	0.3955

*FPIA; target range 3–8 ng/ml.

Table 8. Main clinical characteristics of the three HCV-RNA positive patients with hypertransaminasemia after EVL introduction.

	1	2	3
Gender	M	M	M
Age	65	68	64
Interval from LT (months)	25	16	15
Baseline immunosuppression	CsA-ME, MMF	CsA-ME	CsA-ME
Baseline CsA-ME daily dosage (mg/kg)	110 mg (1.5)	100 (1.7)	150 (2)
Baseline log HCV-RNA*	7	7	6
Viral genotype	1	1	1
EVL trough level at 7 days (ng/ml)†	12	15.1	15.5
7-Day log viral load	7	7	6
Baseline histology (grade/stage)‡	11/3	10/1	11/3
Previous attempt at antiviral treatment post-LT	Yes/failed	Yes/failed	Yes/failed
Postadverse event histology (grade/stage)§	12/3	12/1	12/3
Δgrade/Δstage¶	1/0	2/0	1/0
Endpoint liver function tests (AST/ALT after EVL withdrawal)	Within range	Within range	>2 upper limit

*Amplicore assay.
†FPIA.
‡Retrospective, Ishak-Knodell.
§Performed after occurrence of hypertransaminasemia, Ishak-Knodell.
¶Retrospective comparison of histology after adverse event and baseline, Ishak-Knodell.

three HCV-RNA positive patients with hypertransaminasemia (>3 ULR), Table 8 illustrates their main clinical and demographic features. All patients were on CsA as baseline primary immunosuppressant, and 7 days after drug administration their mean EVL trough level was 14.2 ± 1.6 ng/ml, with no increase in their log viral load. On a retrospective basis, HCV genotype was 1 in all cases and all patients had failed to respond to previous antiviral treatment in the post-transplant course for histology-proven recurrent HCV-related graft hepatitis. On occurrence of hypertransaminasemia, EVL was stopped, a liver biopsy was performed, and all patients resumed their baseline immunosuppressive regimen with endpoint normalization of liver function tests in two of them. The retrospective comparison of the baseline and post-adverse event histology revealed minimal deterioration in the grading score (Table 8).

Discussion

Two major challenges are posed to the LT community, i.e. developing immunosuppressive regimens that maintain high rates of transplantation success while reducing adverse side-effects, and improving the quality of life in maintenance patients. In that regard, EVL may offer advantages for both the objectives. The assumption of this study was to test the equivalence of EVL versus CNI either with or without antimetabolites in adult, maintenance LT patients with a minimum follow-up of 1 year, and a baseline CsA trough level ≤ 150 ng/ml (and/or C2-h ≤ 650 ng/ml) or baseline TAC trough level ≤ 8 ng/ml. Previous clinical studies have already demonstrated equivalent clinical efficacy of EVL and MMF when used in combination with CsA-ME in renal transplantation, but to the best of our knowledge, no study has ever attempted at testing equivalence of EVL versus CNI either with or without antimetabolites in LT. Collaterally, we also aimed at testing the impact of conversion to EVL on renal function at 12 months, as well as the mode and timing of switching from CNI to EVL because of the reported pharmacokinetic interaction between these two categories of drugs and the risk for EVL overexposure when administered in combination with CNI. A further secondary, exploratory objective was to assess the impact of conversion on HCV viral load and HCV-related recurrent graft hepatitis by observation of the course of the disease, viremia, and liver function tests in this subset of patients.

Conversion from CNI either with or without antimetabolites to EVL proved feasible in 75% of patients, matching favorably data of preliminary experiences with SRL [18–27]. Incidence of BPAR (15%) and of adverse effects was in agreement with data for SRL with hyperlipemia being the most frequent complication (42.5% of LT patients of the current experience). However, we are convinced that prevention of EVL overexposure might further increase the feasibility and safety rates of conversion. Upon introduction of EVL in combination with a 50%-per-week reduction of baseline CNI, the likelihood of a drug trough level ≥ 8 ng/ml 7 days thereafter was three times higher for patients on CsA-ME than for those on TAC (Table 4). In order to reduce inadvertent EVL overexposure while switching from CNI to EVL, a policy of CsA-ME reduction >50% may be anticipated. In contrast, patients on TAC as baseline immunosuppressant had a sixfold increase in the risk for EVL trough level ≤ 3 ng/ml 7 days after switching. For these patients an initial daily dose of 2 mg or a policy of waiting for achievement of EVL target range before TAC withdrawal might be a reasonable alternative to what is reported in the current experience. We were also convinced that EVL overexposure was responsible for the hypertransaminasemia observed in three HCV-RNA positive patients. Because of both interaction with CsA-ME and impaired liver metabolism secondary to the underlying recurrent graft hepatitis, EVL trough levels were >10 ng/mL in all three patients 7 days after drug introduction, despite a 50% reduction of baseline CsA-ME daily dosage

(Table 8). The fact that all these patients were genotype 1, were affected with advanced HCV-related graft hepatitis, and had failed to respond to previous antiviral treatment may well underscore that this category of patients might not benefit from a sudden change in their net immunosuppressive status. A policy of reduction of baseline CsA-ME or temporary switching to MMF monotherapy before EVL introduction should be tested in eventual clinical trials to help select the best switching strategy in HCV positive patients with recurrent graft hepatitis for reduction of the risk of hepatitis flare.

In terms of renel function, CNI withdrawal was associated with improvement of cCrCl in 57% of patients successfully converted to EVL monotherapy. However the magnitude of improvement was lower than expected (mean 4.03 ± 12.6 ml/min) and directly correlated with patients' baseline cCrCl rather than with age, gender, HCV-status, and interval from transplantation. These results are in keeping with a recent experience by Cejas and co. of switching to SRL monotherapy in 112 adult LT recipients with impaired renal function (cCrCl <90 ml/min) [27]. The authors reported no significant improvement in cCrCl (Cockroft) for patients with baseline values <40 ml/min as opposed to those with >40 ml/min. We could not find any threshold in cCrCl that correlated with the highest probability of improvement in renal function and our data show that the extent of change is correlated in a continuous rather than categorical way with baseline cCrCl. Some reasons to explain this are both the long interval from transplantation (mean 45.5 ± 31.2 months) and the age of the recipients (mean 54.9 ± 11 years) of the current experience. Therefore it seems reasonable that earlier policies (<12 months) of CNI minimization should be taken into account in the post-transplant course to improve renal function in LT patients, based on the assumption that ''the earlier, the better'' after thorough evaluation of the patient immunological risk. Based on the feasibility of EVL monotherapy in maintenance LT recipients, the hypothesis of EVL monotherapy vs. continuation of CNI-based immunosuppression is being tested in de novo LT in an ongoing, controlled multicenter trial.

In keeping with ongoing studies on the use of EVL in LT, conversion to EVL monotherapy is feasible and safe in adult, maintenance LT recipients with a minimum follow-up of one year. Issues needing further refinement include avoidence of inadvertent drug overexposure, especially in patients with CsA-based immunosuppression, and management of drug-related adverse effects. Growing experience with EVL, ongoing patient enrollment, and better patient profiling may help improve the feasibility and safety of conversion to EVL and help patients benefit from the advantages of antiproliferative immunosuppression.

Authorship

PDS and FF: designed the research study, analyzed the data. PDS, PC, SP, and FC: enrolled patients and performed the study. AP: performed the laboratory tests (with special regard to everolimus trough levels). DC: the pathologist for all biopsies performed throughout the study period. LB and JD: dispensed drugs and kept clinical records of follow-up visits, drug schedules and adverse events. EB and LC: collected the data. PDS: wrote the manuscript.

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