Efficacy and Safety of Everolimus and Mycophenolic Acid With Early Tacrolimus Withdrawal After Liver Transplantation: A Multicenter Randomized Trial

Faouzi Saliba, Christophe Duvoux, Jean Gugenheim, Nassim Kamar, Sébastien Dharancy, Ephrem Salamé, Martine Neau-Cransac, François Durand, Pauline Houssel-Debry, Claire Vanlemmens, et al.

Received Date : 24-Oct-2016

Revised Date : 09-Jan-2017

Accepted Date : 09-Jan-2017

Article type : Original Article

Efficacy and safety of everolimus and mycophenolic acid with early tacrolimus withdrawal after liver transplantation: A multicenter, randomized trial

Faouzi Saliba[1], Christophe Duvoux[2], Jean Gugenheim[3], Nassim Kamar[4], Sébastien Dharancy[5], Ephrem Salamé[6], Martine Neau-Cransac[7], François Durand[8], Pauline Houssel-Debry[9], Claire Vanlemmens[10], Georges Pageaux[11], Jean Hardwigsen[12], Daniel Eyraud[13], Yvon Calmus[14], Fabienne Di Giambattista[15], Jérôme Dumortier[16], Filomena Conti[14]

1. Centre Hépato-Biliaire, AP-HP Hôpital Paul Brousse, INSERM, Unité 1193, Villejuif, France

2. Service d'Hépato-Gastro-Entérologie, AP-HP Hôpital Henri Mondor, Créteil, France

3. Département de Chirurgie Digestive et Transplantation Hépatique, Hôpital Archet, University of Nice Sophia Antipolis, Nice, France

4. Département de Néphrologie et Transplantation d'Organes, CHU Rangueil, Toulouse, France

5. Service d'Hépato-Gastroentérologie, CHRU de Lille, Lille, France

6. Service de Chirurgie Hépato-Biliaire et Digestive, Hôpital Trousseau, CHU Tours, France

7.Unité de Chirurgie Biliaire et de Transplantation Hépatique, Hôpital Magellan, CHU Bordeaux, Pessac, France

8. Service d'Hépatologie et Transplantation Hépatique, University Paris Diderot, INSERM U1149, Clichy, France

Th

9. Service de Chirurgie Hépatobiliaire et Digestive, CIC 1414, Hôpital Pontchaillou, Rennes, France
10. Service d'Hépatologie et Soins Intensifs Digestifs, Hôpital Jean Minjoz, Besançon, France
11. Service Hépato-Gastroentérologie, Hôpital Saint Eloi, Montpellier, France
12. Service de Chirurgie et Transplantation Hépatique, Hôpital la Timone, Marseille, France
13. Département d'Anesthésie-Réanimation, Service de Chirurgie Digestive et Hépato-Biliaire et de Transplantation Hépatique, Groupe Hospitalier Pitié Salpêtrière - Charles Foix, Assistance Publique-Hôpitaux de Paris, Paris, France
14. Service de Chirurgie Digestive et Hépato-Biliaire, Transplantation Hépatique, AP-HP Hôpital Pitié Salpêtrière, Paris, France
15. Novartis Pharma SAS, Rueil-Malmaison, France
16. Unité de Transplantation Hépatique, Hôpital Edouard Herriot, Lyon Cedex 3, France

Correspondence to:
Pr Faouzi Saliba
Hôpital Paul Brousse, Centre Hépato-Biliaire
12 avenue Paul Vaillant Couturier, 94800 Villejuif, France
Telephone: +33 1 4559 64 12 Fax: +33 1 4559 38 57 E-mail: faouzi.saliba@aphp.fr

Study registration: NCT NCT01625377 (ClinicalTrials.gov)

Running title: Everolimus with early tacrolimus withdrawal

Abbreviations

ALT	alanine transaminase
ANCOVA	analysis of covariance
AST	aspartate transaminase
BPAR	biopsy-proven acute rejection
CI	confidence interval
CMV	cytomegalovirus
CNI	calcineurin inhibitor
CsA	cyclosporine
EC-MPS	enteric-coated mycophenolate sodium
eGFR	estimated glomerular filtration rate
GGT	gamma-glutamyl transpeptidase
HAT	hepatic artery thrombosis
HCV	hepatitis C virus
ITT	intention-to-treat
KDOQI	Kidney Disease Outcomes Quality Initiative
LOCF	last observation carried forward
LS	least squares
MDRD	Modification of Diet in Renal Disease
MELD	model of end-stage liver disease
MPA	mycophenolic acid
mTOR	mammalian target of rapamycin
RAI	rejection activity index
ULN	upper limit of normal

Abstract

SIMCER was a six-month, multicenter, open-label trial. Selected *de novo* liver transplant recipients were randomized (week 4) to everolimus with low-exposure tacrolimus discontinued by month 4 (n=93), or tacrolimus-based therapy (n=95), both with basiliximab induction and enteric-coated mycophenolate sodium ± steroids. The primary endpoint, change in estimated GFR (eGFR, MDRD) from randomization to week 24 post-transplant, was superior with everolimus: mean eGFR change +1.1mL/min/1.73m^2 for everolimus versus -13.3mL/min/1.73m^2 for tacrolimus; difference 14.3 (95% CI 7.3, 21.3; p<0.001). Mean eGFR at week 24 was 95.8mL/min/1.73m^2 versus 76.0mL/min/1.73m^2 for everolimus versus tacrolimus (p<0.001). Treatment failure (treated biopsy-proven acute rejection [BPAR; rejection activity index score >3], graft loss or death) from randomization to week 24 was similar: everolimus 10.0%, tacrolimus 4.3% (p=0.134). BPAR was more frequent between randomization and month 6 with everolimus (10.0% versus 2.2%; p=0.026); the rate of treated BPAR was 8.9% versus 2.2% (p=0.055). Sixteen everolimus-treated patients (17.8%) and three tacrolimus-treated patients (3.2%) discontinued study drug due to adverse events. In conclusion, early introduction of everolimus at an adequate exposure level with gradual CNI withdrawal after liver transplantation, supported by induction therapy and mycophenolic acid, is associated with a significant renal benefit versus CNI-based immunosuppression but more frequent BPAR.

Introduction

Introduction of the calcineurin inhibitor (CNI) class of immunosuppressants made a major contribution to the development of liver transplantation, tripling survival rates compared to the pre-CNI era (1). CNI maintenance therapy, typically with tacrolimus, remains virtually universal following liver transplantation (2). Until recently, few alternative

immunosuppressive strategies have been available, despite awareness of the long-term complications associated with chronic exposure to CNI therapy. The most worrying of these is progressive renal deterioration due to CNI-related nephrotoxicity (3). An increase in cardiovascular risk factors such as new-onset diabetes mellitus (4), hypertension (5) and hyperlipidemia (6) is also of great concern. Withdrawal of CNI therapy after the initial high-risk period early after transplantation is an appealing option if rejection prophylaxis can be adequately sustained.

Mycophenolic acid (MPA) is widely used as an adjunct to CNI therapy, often to support steroid avoidance, but conversion to MPA within a CNI-free regimen is associated with an increased risk of acute rejection (7). The mammalian target of rapamycin (mTOR) inhibitors appear more promising for achieving long-term CNI-free maintenance therapy. Randomized trials have investigated various approaches to using the mTOR inhibitor everolimus to support CNI withdrawal after liver transplantation. The H2304 study randomized 719 *de novo* liver transplant patients to everolimus with tacrolimus minimization, everolimus with tacrolimus withdrawal, or a standard tacrolimus regimen (8, 9). Everolimus was introduced at day 30 post-transplant, and tacrolimus was withdrawn from month 4 over a three-week period. The rate of biopsy-proven acute rejection (BPAR) was significantly lower in the everolimus/reduced tacrolimus arm versus standard tacrolimus, but tacrolimus withdrawal resulted in a high incidence of acute rejection (26.4%) and recruitment to this group was discontinued. Renal function during the two-year study was significantly higher in the everolimus/reduced tacrolimus group than the control arm, and was even higher in the tacrolimus withdrawal group despite the fact that more than half of the patients had reverted to CNI therapy (8, 9). In the H2304 study, patients did not receive induction therapy and MPA was not permitted after randomization. In contrast, the PROTECT study showed that patients given basiliximab induction with gradual early withdrawal of CNI (tacrolimus or cyclosporine [CsA]) over an eight-week period did not experience increased BPAR (10, 11). Renal function was improved after CNI elimination in the PROTECT study based on

estimated GFR (eGFR) using the Modification of Diet in Renal Disease (MDRD) formula, but for creatinine clearance (Cockcroft-Gault formula) there was no significant difference from controls (10). A smaller third trial maintained patients on both everolimus and CsA to month 1, then abruptly discontinued CsA; basiliximab induction was given and oral steroids were mandatory to day 35 (12). Results showed significantly higher eGFR than in CsA-treated controls without increased BPAR. Thus, it appears that everolimus-based CNI-free immunosuppression can be achieved successfully after liver transplantation but the optimal adjunctive regimen and the timing for CNI withdrawal is not clarified. Notably, none of these studies administered concomitant MPA as part of the everolimus-based regimen.

SIMCER was a multicenter trial in which *de novo* liver transplant recipients were randomized at week 4 to everolimus with low-exposure tacrolimus, discontinued by month 4, or to a conventional tacrolimus regimen, all with MPA and the option of corticosteroid therapy. The aim of the study was to compare renal function between the two treatment groups, based on the change in eGFR between randomization and week 24 post-transplant.

Methods

Study design and conduct

SIMCER was a six-month prospective, multicenter, randomized, open-label, study in *de novo* liver transplant patients undertaken at 15 transplant centers in France during 2012 to 2015 (clinicaltrials.gov NCT01625377). The study protocol was approved by the French Health Products Safety Agency (Afssaps/ANSM) and the relevant institutional review board. Written informed consent was obtained from all participants.

Eligibility criteria

Patients aged 18 years or older who underwent a primary whole or split liver transplant from a deceased donor were eligible for enrollment at the time of transplant. Key exclusion criteria were multiple or previous organ transplantation, a non-heart beating donor, transplantation due to autoimmune hepatitis, primary sclerosing cholangitis or primary biliary cholangitis, eGFR ≤30 mL/min/1.73m^2, alpha-fetoprotein >1,000 ng/mL in patients transplanted for hepatocellular carcinoma (13), body mass index ≥35 kg/m^{2}and severe uncontrolled hypercholesterolemia (>9 mmol/l) or hypertriglyceridemia (>8.5 mmol/l) in the six months prior to transplantation. At the point of randomization, patients were randomized if they were receiving tacrolimus and enteric-coated mycophenolate sodium (EC-MPS), with or without steroids. Enrolled patients were not randomized if proteinuria was >0.8 g/24h or urinary protein/creatinine ratio was >50 mg/mmol, if they had experienced steroid-resistant rejection and/or severe BPAR (Banff 1997 score ≥7 [14]) between screening and randomization, if total bilirubin was ≥5 times higher than the upper limit of normal (ULN) and/or prothrombin time and/or factor V was <30%, if platelet count was <50,000/mm^3, neutrophil count was <1,000/mm^3 or leukocyte count <2,000/mm^3, or if Doppler ultrasound showed thrombosis of the hepatic arteries, hepatic veins, portal veins or inferior vena cava.

Immunosuppression and concomitant medication

Basiliximab induction (20 mg) was given on day 0 and day 4. Between transplant and randomization, all patients received tacrolimus (starting on day 3–5 post-transplant) targeting a trough concentration of 6–10 ng/mL, and EC-MPS at a dose of 720 mg b.i.d. (minimum dose 360 mg b.i.d.). Intravenous mycophenolate mofetil was permitted as necessary in the first 10 days post-transplant until *per os* feeding was resumed. Intravenous and oral steroids could be given according to local practice.

Randomization was performed at week 4 post-transplant using a validated, automated interactive web response system with investigators notified of the treatment group after stratification according to hepatitis C virus (HCV) status (positive or negative) and eGFR at time of transplant (<60 or ≥60 mL/min/1.73m^2 [MDRD]). Patients randomized to the everolimus regimen received an initial dose of 1 mg b.i.d., adjusted to target a trough concentration of 6–10 ng/mL. The tacrolimus dose was reduced by 50% on initiation of everolimus, with a further 50% reduction after four weeks (i.e. week 8 post-transplant). Tacrolimus was discontinued during week 12 post-transplant (and not later than week 16) if the everolimus trough concentration was in the range 6–10 ng/mL; otherwise study treatment was discontinued. Patients randomized to the control arm continued to receive tacrolimus to week 24 (trough concentration 6–10 ng/mL). All patients continued EC-MPS to week 24, with or without steroids (Figure S1).

Cytomegalovirus (CMV) infection prophylaxis, administered according to local practice, was mandatory for a minimum of three months if the recipient was CMV negative and the donor was CMV positive, and was highly recommended in all cases unless both recipient and donor were CMV-negative. All patients received prophylaxis for *Pneumocystis jirovecii* infections according to local practice until month 3.

Study endpoints

The primary endpoint was the change in eGFR (abbreviated MDRD formula [15]) between randomization and week 24 post-transplant. The principal secondary endpoint was a composite endpoint of treatment failure from randomization to week 24 post-transplant, defined as treated BPAR (rejection activity index [RAI] score >3), graft loss or death. Other secondary endpoints were any BPAR, BPAR RAI score >3, treated BPAR (any event or score >3), death, graft loss, and renal function assessed by eGFR (MDRD formula [15] and CKD-EPI formula [16]), creatinine clearance (Cockcroft-Gault formula [17]), serum creatinine, urinary protein/creatinine ratio, chronic kidney disease stage (Kidney Disease

Outcomes Quality Initiative [KDOQI] criteria [18]) and requirement for dialysis. A graft biopsy was to be carried out if acute rejection was suspected, and before or within 48 hours of starting anti-rejection treatment, with results graded locally according to Banff 1997 scoring (14). Safety was assessed by (serious) adverse events, particularly CMV infections, *de novo* cancer, recurrence of hepatocellular carcinoma or HCV, *de novo* diabetes (defined as fasting glycemia ≥1.26 g/L [7.0 mmol/L] and/or treatment with a hypoglycemic agent [19, 20]), and early discontinuation of the study treatment.

Statistical analysis

The primary variable, change in eGFR between randomization and week 24, was assessed by an analysis of covariance (ANCOVA) model with treatment arm and stratification group at randomization as factors and the eGFR value at baseline as the covariable, with results presented as least squares (LS) means with 95% confidence interval (CI) values for the difference between groups. The analysis was based on the intention-to-treat (ITT) population, using the last observation carried forward (LOCF) methods for missing eGFR values at week 24.

The sample size calculation estimated that 205 patients would provide 80% power to detect a mean (SD) between-group difference of 10 (24) mL/min/1.73m², based on published data (8), using a two-sided α level of 5% and allowing for a screening failure rate of 10% i.e. 184 patients would be randomized.

The principal secondary endpoint, treatment failure, was compared between treatment groups using the Chi-square test. The time from randomization to first treatment failure was described by a Kaplan-Meier analysis and compared between treatment arms using a log-rank test.

The ITT population included all randomized patients who received at least one dose of study treatment post-randomization and for whom eGFR was available at randomization with at least one subsequent eGFR value. The safety population included all randomized patients given at least one dose of study drug after randomization and who provided at least one post-treatment safety assessment.

Results

Patient population

In total, 241 patients were enrolled, of whom 188 (78%) were eligible for randomization at week 4 (93 everolimus, 95 tacrolimus) (Figure 1). The most frequent reason for randomization failure was not meeting randomization criteria (22 patients, including 15 in whom proteinuria was >0.8 g/24h or urinary protein/creatinine ratio was >50 mg/mmol). Of the 188 randomized patients, 183 were included in the ITT population and 184 were included in the safety population. Twenty-two patients (22/93, 23.7%) in the everolimus group and seven patients (7/95, 7.4%) in the tacrolimus group discontinued study drug prematurely (Figure 1).

Baseline characteristics were similar between groups (Table 1).

Immunosuppression and concomitant medication

Mean everolimus trough concentration was below the target range (6–10 ng/mL) until week 16 post-transplant when the mean (SD) level was 7.2 (3.5) ng/mL, remaining within range thereafter (7.7 [3.5] ng/mL at week 24) (Figure S2a). The proportion of patients with everolimus trough level <6 ng/mL was in the range 33.3% to 78.3% during the study. Mean (SD) tacrolimus trough concentration was similar at randomization in the everolimus group

(8.8 [3.1] ng/mL) and the tacrolimus group (8.7 [4.0] ng/mL), declining to 7.8 (2.7) ng/mL in the tacrolimus group at week 24 (Figure S2b). The initial mean (SD) dose of EC-MPS was 1312 (312) mg/day and 1349 (252) mg/day in the everolimus and tacrolimus group, respectively, declining slightly to 1131 (394) mg/day and 1125 (400) mg/day at week 24. Steroids were given to virtually all patients at time of transplant (everolimus 97.8%, tacrolimus 96.8%). At week 12, 78.0% of patients in the everolimus group and 84.9% of patients in the tacrolimus group were still receiving steroids (median dose 0.1 mg/kg/day in both groups [8.0 mg/day and 6.0 mg/day, respectively]). At week 24, 58.4% and 55.7% of patients in the everolimus and tacrolimus groups, respectively, continued to receive steroids (median dose 0.1 mg/kg/day [5.0 mg/day] in both groups.

The proportions of everolimus- and tacrolimus-treated patients receiving antihypertensive therapy were 52.7% and 45.3% at randomization, and 41.9% and 45.3% at month 6, respectively. For antidiabetic therapy, the proportions were 39.8% and 37.9% at randomization, and 29.0% and 30.5% at month 6. The use of lipid-lowering therapy was low in both groups (6.5% and 2.1% at randomization; 11.8% and 5.3% at month 6, respectively).

Renal function

Observed mean (SD) eGFR (MDRD) was similar in the two treatment groups at randomization (91.4 [36.6] mL/min/1.73m2 in the everolimus group versus 87.4 [39.7] mL/min/1.73m^2 in the tacrolimus arm; p=0.312), but was significantly higher in the everolimus cohort from week 6 onwards other than at week 8 (p=0.067) (Figure 2). Mean (SD) eGFR at week 24 was 95.8 (27.7) mL/min/1.73m^2 in the everolimus group versus 76.0 (24.5) mL/min/1.73m^2 in the tacrolimus arm (p<0.001). Values remained stable in the everolimus group from randomization to week 24 (mean [SD] change 0.1 [32.6])

mL/min/1.73m^2) but decreased by 11.8 (34.0) mL/min/1.73m^2 in tacrolimus-treated patients (LOCF method) (Table 2).

The everolimus group was significantly superior for the primary endpoint, change in eGFR from randomization to week 24: LS mean (SE) values were +1.1 (2.8) mL/min/1.73m^2 in the everolimus group compared to -13.3 (2.8) mL/min/1.73m^2 in the tacrolimus group, a difference of 14.3 mL/min/1.73m^2 (95% CI 7.3, 21.3; p<0.001). When the primary analysis was repeated in the subpopulation of patients with no major protocol deviations, results were similar: LS mean 1.0 (3.3) mL/min/1.73m^2 versus -12.6 (2.8) mL/min/1.73m^2, a difference of 13.6 (95% CI 5.9, 21.3; p<0.001).

In the everolimus group, no renal parameter changed significantly from randomization to week 24 (Table 2). In contrast, patients in the tacrolimus group showed significant decreases in eGFR, creatinine clearance and serum creatinine. Mean proteinuria remained stable in the everolimus group (Table 2), but was significantly higher than in tacrolimus-treated patients by week 24: 0.4 (0.8) g/L versus 0.2 (0.3) g/L (p=0.001). The urine protein/creatinine ratio, similarly, was unchanged from randomization to week 24 under everolimus (Table 2), but was significantly higher in the everolimus group versus the tacrolimus group at week 24 (36.1 [86.2] mg/mmol versus 17.2 [28.4] mg/mmol; p=0.003).

At week 24, 55.4%, 39.2% and 5.4% of patients in the everolimus group had CKD stage 1, 2 or 3, respectively, compared to 27.9%, 39.5% and 32.6% in the tacrolimus cohort (p<0.001) (Figure 3). No patient was classed as CKD stage 4 or 5 at week 24.

Efficacy

The incidence of the principal secondary endpoint, treatment failure (treated BPAR, graft loss or death) from randomization to week 24, was similar in both groups (everolimus 10.0%, tacrolimus 4.3%, p=0.134) (Table 3). Kaplan-Meier estimates for freedom from treatment

failure during this period were 89.0% in the everolimus group versus 95.5% in the tacrolimus group (log rank p=0.115).

The incidence of BPAR between randomization and week 24 was significantly higher in the everolimus cohort (10.0% [9/90] versus 2.2% [2/93] in the tacrolimus group; p=0.026); the incidence of treated BPAR was 8.9% [8/90] versus 2.2% [2/93] (p=0.055). Severity was recorded in eight of the nine everolimus-treated patients with BPAR, and was mild in 5/8 cases and moderate in 3/8 cases (Table 3). In the five cases of mild BPAR (Banff grade I), everolimus was continued in three cases and the patient was given oral or bolus steroid therapy, and was stopped in two cases (one patient re-started tacrolimus and BPAR was treated with oral steroids; subsequent immunosuppression was not reported in the second patient, who received bolus steroids). The three patients with moderate (Banff grade II) BPAR all discontinued everolimus (two re-started tacrolimus; information was unavailable in the third case) and received bolus steroid therapy. There were no severe episodes of BPAR in either group. The mean time from randomization to first BPAR was longer in the everolimus cohort (mean 80.4 [42.1]) days) than the tacrolimus arm (30.5 [27.6] days). Use of steroid therapy at month 3 post-transplant or at month 6 post-transplant showed no association with the incidence of BPAR at month 6 within either treatment group.

Among the eight patients in the everolimus group who had treated BPAR (RAI >3), the mean everolimus concentration during the study was <4.46 ng/mL (the lowest concentration quartile) in 5/7 cases (one moderate and four mild BPAR), between 4.46 and 5.78 ng/mL (i.e. the second lowest concentration quartile) in two cases (one mild, one moderate BPAR) and was only within target range in one case (mild BPAR). One patient with BPAR in the everolimus group was not treated, and was still alive with a functioning graft at the end of the study.

Between randomization and week 24, one tacrolimus-treated patient lost his graft due to hepatic vein thrombosis. There were two deaths: an everolimus-treated patient died from sliding syndrome and a tacrolimus-treated patient died with the cause reported as sudden death.

Adverse events

One or more serious adverse event occurred in 46.7% and 29.8% of patients in the everolimus and tacrolimus groups, respectively. Cholestasis, hepatocellular injury, anemia, hypokalemia, dyslipidemia and sepsis were more frequent in the everolimus arm, while diarrhea and renal failure were reported more often in the tacrolimus group (Table 4). Hepatic artery thrombosis (HAT) occurred in only one patient, starting 29 days before randomization (to the everolimus group), with no suspected relation to study drug.

The incidence of adverse events with a suspected relation to everolimus was 45.6% (most frequently aphthous stomatitis, anemia, impaired healing and peripheral edema), while 29.8% of patients in the tacrolimus group had one or more adverse events with a suspected relation to tacrolimus (most frequently renal failure, diarrhea and tremor) (Table S1). Impaired healing was reported in four everolimus-treated patients (delayed wound healing [2], asymptomatic impaired healing of the biliary fistula, and severe poor wound healing), with a suspected relation to study drug in each case.

The most frequent serious adverse events in the everolimus group were sepsis (n=5), biliary anastomosis complication (n=5), rejection (n=5) and renal failure (n=3). The cases of sepsis were related to biliary complications in three patients, and related to pulmonary infections in two patients; none suspected to be related to study drug. In the tacrolimus group, the most frequent serious adverse events were biliary stenosis (n=4), renal failure (n=4), diarrhea (n=4) and rejection (n=3).

CMV infection was reported in 17.8% and 11.7% of patients in the everolimus and tacrolimus groups, respectively. The incidence of *de novo* diabetes mellitus was similar between groups (everolimus 61.1%, tacrolimus 63.8%). One patient in the everolimus group was reported to have uterine leiomyoma during the study. There were no cases of hepatocellular carcinoma recurrence.

Sixteen patients in the everolimus group (17.8%) and three patients in the tacrolimus group (3.2%) discontinued study drug due to adverse events. The only adverse events which led to discontinuation in more than one patient were acute rejection (four everolimus-treated patients), pancytopenia (one patient in each group) and impaired healing (two everolimus-treated patients).

Discussion

In this multicenter, randomized trial, everolimus was introduced at one month after liver transplantation with tacrolimus reduction then discontinuation by month 3, in patients given basiliximab induction and MPA therapy. Under this regimen, renal function remained unchanged over the first six months post-transplant, in marked contrast to the decline in renal function observed in the tacrolimus-based control arm. The difference in eGFR by the end of the study was substantial (14.3 mL/min/1.73m^2), and although patients were only followed to week 24 post-transplant, there was no suggestion that values in the two groups were set to converge subsequently. BPAR, however, was significantly more frequent in the CNI-free cohort although episodes were mild in more than half the cases and the incidence (10.0%) was acceptable and substantially lower than reported for a CNI-free regimen of everolimus (9, 10). No graft losses occurred. These two findings indicate that a CNI-free regimen combining everolimus with MPA is a promising approach that offers renal protection without compromising mid-term liver graft function, with the caveat that the study was

performed in a selected group of patients. Discontinuation due to adverse events occurred in ~18% of everolimus-treated patients.

In contrast to the H2304 study (8), and the Spare-The-Nephron study of conversion to sirolimus (21), the immunosuppressive protocols in both treatment arms included basiliximab induction. The ReSpeECT study showed that inclusion of induction in a tacrolimus-based regimen improves renal function, with a trend to lower rates of BPAR (22). In the PROTECT study, inclusion of basiliximab induction, combined with slower CNI withdrawal, avoided an increase in rejection but a renal benefit was less clear-cut than in our CNI-free cohort (10). This may be due to the fact that half the everolimus-treated patients in PROTECT discontinued study medication, most often due to adverse events; those who continued the everolimus-based regimen showed better preservation of renal function. Here, inclusion of concomitant MPA permitted slightly lower levels of everolimus exposure than in the PROTECT trial (e.g. 7.7 ng/mL at month 6 compared to 9.3 ng/mL at month 8 in PROTECT [10]), possibly contributing to the lower rate of discontinuations due to adverse events in our cohort (23.7%). Unlike in PROTECT (10), where patients did not receive MPA, or in H2304 (8) where MPA was prohibited after randomization, MPA was also included in the tacrolimus control arm throughout the current study, an approach which is now common in clinical practice. When comparing outcomes between the two studies, however, it should be borne in mind that our population was followed only to month 6 post-transplant, whereas PROTECT analyzed patients at month 12, and it is feasible that more patients would have discontinued in the succeeding six months. In the Spare-the-Nephron trial, where 293 liver transplant patients were randomized to remain on CNI or switch to sirolimus (both with mycophenolate mofetil but without protocol-specified induction therapy) at weeks 4–12 post-transplant (21), results were generally similar to those seen here. Renal function at one year was again significantly higher in the CNI-free cohort, but with a higher rate of mild BPAR, and more frequent discontinuation due to adverse events (21).

The post-randomization incidence of BPAR in the everolimus/MPA arm of our study (10.0%) was lower than in the everolimus monotherapy arm in either the PROTECT trial (17.7%) (10) or the H2304 study (26.4%) (9). Again, the difference in follow-up times should be taken into account but since most acute rejection episodes occur early post-transplant it seems reasonable to conclude that combined administration of everolimus with MPA was more effective than everolimus alone. Even with basiliximab induction and concomitant MPA, however, the rate of BPAR was significantly higher in the everolimus group and three of the everolimus patients with treated BPAR experienced moderate rejection. Notably, however, six of these seven patients had a mean everolimus concentration below the minimum target of 6 ng/mL and five had concentrations below 4.4 ng/mL, underscoring the need to maintain adequate everolimus exposure in this context to ensure immunosuppressive efficacy.

A similar proportion of patients in both groups discontinued steroid therapy by week 24, an important consideration in view of the long-term morbidity associated with chronic steroid administration.

Adverse events with a suspected relation to study drug were more frequent under everolimus than tacrolimus, as was discontinuation due to adverse events. During the period of switch from CNI therapy to a CNI-free regimen, most patients in the everolimus group were receiving concurrent everolimus, MPA and tacrolimus (with steroids in some cases), which may have contributed to this observation. The most common adverse event leading to discontinuation of everolimus was acute rejection, followed by impaired healing, neither of which are unexpected although it is possible that with more experience the events could have been managed without switching therapy. Sepsis, while occurring only in the everolimus group, was not attributed to study drug. The incidences of neutropenia and thrombocytopenia were similar between groups, indicating that hematological tolerability of the everolimus/MPA regimen was not a concern. Proteinuria and the urine protein/creatinine ratio were both significantly higher under everolimus at the end of the study, consistent with

results in the H2304 study (8) but proteinuria reported as an adverse event was rare (2.2%). Use of lipid-lowering therapy was higher in the everolimus-treated cohort at month 6, as might be expected given the known lipogenic profile of mTOR inhibitors.

The study benefitted from randomization with appropriate stratification, and a large number of participating centers. We recognize that the six-month duration of the study is a limitation. Since rejection episodes occur primarily in the first six months post-transplant, and switch to another regimen is also more frequent during the first months, data from the first six months are highly relevant but longer follow-up is desirable to confirm if the renal advantage for the CNI regimen is maintained. The study population is now being followed to five years post-transplant in the observational CERTITUDE study, during which patients are assessed annually on the anniversary of transplantation and immunosuppression is at the investigators' discretion.

An important point to consider is that the study population excluded certain patient types at high risk for rejection (e.g. patients with autoimmune conditions, recipients of a previous transplant or those with previous severe BPAR) or mTOR-inhibitor-related adverse events (e.g. patients with body mass index >35 kg/m^2 or significant proteinuria) and the results do not necessarily apply in those groups. Additionally, patients with severe kidney disease (≤30 mL/min/1.73m^2) were excluded and the study population had a mean MELD score in the range 18–19 with good baseline renal function. A similar improvement in renal function may not be achieved in patients with a higher MELD score and substantially impaired renal function at the time of transplant.

In conclusion, results of the SIMCER study indicate that early introduction of everolimus with early stepwise withdrawal of CNI after liver transplantation, supported by induction therapy and concomitant MPA with or without steroids, may be a preferable strategy for achieving CNI-free therapy than everolimus with steroids alone. We observed a clinically relevant benefit for renal function versus a conventional CNI-based regimen in this selected

population using this approach, but this advantage was achieved at the cost of significantly more mild or moderate episodes of BPAR in the CNI-free group between randomization and month 6 points, largely driven by everolimus underexposure, and a higher rate of study medication discontinuation due to adverse events. Inclusion of MPA in the CNI-free regimen, with appropriate everolimus exposure targeting trough levels >6 ng/mL, may improve the tolerance and efficacy of everolimus-based CNI-free therapy. These results suggest that a regimen combining adequate exposure to everolimus with MPA can be an alternative to conventional CNI-based regimens in selected patients. Longer follow-up studies are necessary to determine how sustainable the beneficial impact on renal function will be, and whether such a combination favorably affects detrimental events after liver transplant such as the development of *de novo* tumors or hepatocellular recurrence.

Acknowledgments

With grateful thanks to the SIMCER study investigators (see Appendix).

Funding sources

The study was funded by Novartis Pharma SAS, Rueil-Malmaison, France.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. Faouzi Saliba has received speaker's honoraria and/or research grants from Roche, Novartis, Astellas, Gilead, Merck Sharp & Dohme, Gambro, Baxter and Vital Therapies. Christophe Duvoux has received speaker's honoraria and/or research grants from Novartis, Astellas, and Chiesi. Jean Gugenheim has received research grants from Astellas, Novartis, Roche, and Merck Sharp & Dohme. Nassim Kamar has received speaker's honoraria and/or research grants and has been member of advisory boards from Novartis, Astellas, Gilead, Merck Sharp & Dohme, Neovii, Sanofi, and Amgen. Yvon Calmus has been a member of advisory boards and/or received research grants from

Novartis, Astellas and Intercept. Sebastien Dharancy has received speaker's honoraria and/or research grants from Novartis, Astellas, Gilead, Merck Sharp & Dohme, BMS and Chiesi. Jérôme Dumortier has been a clinical investigator, speaker and/or consultant for Astellas, Gilead Sciences, Janssen Pharmaceuticals, Novartis and Roche. Ephrem Salamé has been a member of an advisory board for Novartis, and has received research grants from Novartis, Astellas, and Chjesi. François Durand received research grants, speaker's fees or consultant fees from Novartis, Astellas, Gilead and BMS. Pauline Houssel-Debry has been a speaker for Astellas, Gilead Sciences, and Novartis. Georges Pageaux has received speaker's honoraria and/or is a member of advisory boards for Novartis, Astellas, Gilead and BMS. Daniel Eyraud has received speaker's honoraria and/or research grants from Gilead and Merck Sharp & Dohme. Fabienne Di Giambattista is an employee of Novartis. Jérôme Dumortier has been a clinical investigator, speaker and/or consultant for Astellas, Gilead Sciences, Janssen Pharmaceuticals, Novartis and Roche. The other authors have no conflicts of interest to disclose.

Appendix: The SIMCER study investigators

Principal investigators are shown in italics.

Besancon: Jean Paul Cervoni, Vincent Di Martino, Stavros Dritsas, Carine Richou, *Claire Vanlemmens*; **Bordeaux:** *Martine Neau-Cransac*; **Chambray Les Tours:** Florian Charier,Anca Dobrin, Charlotte Nicolas, Jean-Marc Perarnau, Charlene Piat, Mauricio Sainz-Barriga, *Ephrem Salame*, Christine Silvain, Mathilde Soule; **Clichy:** Aurore Baron,*François Durand,* Claire Francoz,; **Creteil:** Roland Amathieu,Daniel Azoulay,Charlotte Costentin,*Christophe Duvoux*, Cyrille Feray, Monika Hurtova, Eric Levesque, Jean-Philippe Mavier, Jean-Claude Merle; **Grenoble:** Anka Antonino, Zineb Baidi,Aurélie Durand, Marie-Nöelle Hilleret, *Vincent Leroy*, Jean Pierre Zarski; **Lille:** Amélie Cannesson,*Sébastien Dharancy*, Guillaume Lassailly; **Marseille:** Emilie Arellano, Danielle Botta-Fridlund, Emilie Gregoire, *Jean Hardwigsen*; **Montpellier:** Michael Bismuth, Moussa Cisse, Stéphanie Faure, *Georges Pageaux*, Béatrice Vales; **Nice:** Imed Ben Amor,Saïd Ferkoune,Bernard Goubaux,*Jean Gugenheim*, Antonio Iannelli, Francesco Martini, Aline Myx-Staccini, Anne-Sophie Schneck; **Paris**: Marc Baussier, Denis Bernard, Simon Bizet, *Yvon Calmus*, Thibault

Camus, Filomena Conti, Hervé Desnault, *Daniel Eyraud*, Thomas Lescot, Jean-Pierre Masini, Fabiano Perdigao, Armelle Poujol-Robert, Olivier Scatton; **Rennes:***Karim Boudjema*, Christophe Camus, Pauline Houssel-Debry, Caroline Jezequel, Mohamed Lakehal, Marianne Latournerie, Michel Rayar; **Toulouse:** Isabelle Cardeau, Olivier Cointault, Arnaud Del Bello, Laure Esposito, Nassim Kamar, Laurence Lavaysserie, Marie-Béatrice Nogier, David Ribes, *Lionel Rostaing*; **Villejuif:**Maria-Teresa Antonini, Marc Boudon, Audrey Coilly, Jean-Charles Duclos-Vallee, Philippe Ichaï, Isabelle Ogier, Gilles Pelletier, Bruno Roche, *Faouzi Saliba,* Didier Samuel, Rodolphe Sobesky.

Figure legends

Figure 1. Patient disposition

Figure 2. Observed mean (SD) estimated GFR (MDRD) to week 24 (ITT population). D, day; W, week; RDN, randomization

Figure 3. Chronic kidney disease stage to week 24 according to Kidney Disease Outcomes Quality Initiative (KDOQI) criteria (18) (intention-to-treat [ITT] population). EVR, everolimus; TAC, tacrolimus

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Adverse events with a suspected relation to study drug occurring in ≥2.0% patients

Figure S1. Study design

Figure S2. Mean (SD) trough concentrations of (a) everolimus (b) tacrolimus to month 6. Shaded areas indicate target concentration range (safety population)

References

1. Iwatsuki S, Starzl TE, Todo S, Gordon RD, Esquivel CO, Tzakis AG, et al. Experience in 1,000 liver transplants under cyclosporine-steroid therapy: a survival report. Transplant Proc 1988;20:498–504.
2. Kim WR, Smith JM, Skeans MA, Schladt DP, Schnitzler MA, Edwards EB, et al. OPTN/SRTR 2012 Annual Data Report: liver. Am J Transplant 2014;14 Suppl 1:69–96.

3. Morard I, Mentha G, Spahr L, Majno P, Hadengue A, Huber O, et al. Long-term renal function after liver transplantation is related to calcineurin inhibitors blood levels. Clin Transplant 2006;20:96–101.
4. Kuo HT, Sampaio MS, Ye X, Reddy P, Martin P, Bunnapradist S. Risk factors for new-onset diabetes mellitus in adult liver transplant recipients, an analysis of the Organ Procurement and Transplant Network/United Network for Organ Sharing database. Transplantation 2010;89:1134–1140.
5. Ducloux D, Motte G, Kribs M, Abdelfatah AB, Bresson-Vautrin C, Rebibou JM,et al. Hypertension in renal transplantation: donor and recipient risk factors. Clin Nephrol 2002;57:409–413.
6. Moore R, Hernandez D, Valantine H. Calcineurin inhibitors and post-transplant hyperlipidaemias. Drug Saf 2001;24:755–766.
7. Goralczyk AD, Bari N, Abu-Ajaj W, Lorf T, Ramadori G, Friede T, et al. Calcineurin inhibitor sparing with mycophenolate mofetil in liver transplantation: a systematic review of randomized controlled trials. Am J Transplant 2012;12:2601–2607.
8. De Simone P, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, Saliba F, et al. Everolimus with reduced tacrolimus improves renal function in de novo liver transplant recipients: a randomized controlled trial. Am J Transplant 2012;12:3008–3020.
9. Saliba F, De Simone P, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, et al; H2304 Study Group. Renal function at two years in liver transplant patients receiving everolimus: results of a randomized, multicenter study.Am J Transplant 2013;13:1734–1745.
10. Fischer L, Klempnauer J, Beckebaum S, Metselaar HJ, Neuhaus P, Schemmer P, et al. A randomized, controlled study to assess the conversion from calcineurin-inhibitors to everolimus after liver transplantation – PROTECT. Am J Transplant 2012;12:1855–1865.
11. Fischer L, Saliba S, Kaiser GM, De Carlis L, Metselaar HJ, De Simone P, et al. Three-year outcomes in *de novo* liver transplant patients receiving everolimus with reduced tacrolimus: Follow-up results from a randomized, multicenter study. Transplantation 2015;99:1455–1462.
12. Masetti M, Montalti R, Rompianesi G, Codeluppi M, Gerring R, Romano A, et al. Early withdrawal of calcineurin inhibitors and everolimusmonotherapy in de novo liver transplant recipients preserves renal function. Am J Transplant 2010;10:2252–2262.
13. Duvoux C, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, et al; Liver Transplantation French Study Group. Liver transplantation for hepatocellular carcinoma: a model including α-fetoprotein improves the performance of Milan criteria. Gastroenterology 2012;143:986–994.e3; quiz e14–15.
14. Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, et al. The Banff 97 working classification of renal allograft pathology.Kidney Int1999;55:713–723.
15. Levey AS, Bosch JP, Lewis JB,Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461–470.
16. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150 604–612.
17. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41.
18. KDOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. http://www2.kidney.org/professionals/KDOQI/guidelines_ckd/p4_class_g1.htm
19. American Diabetes Association. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2007;26(suppl 1):1 s5–s20.

20. World Health Organization. Diabetes. Available at: www. who./int/ncd/dia/databases4.htm. Accessed 25 September 2015
21. Teperman L, Moonka D, Sebastian A, Sher L, Marotta P, Marsh C, et al. Calcineurin-free mycophenolate mofetil/sirolimus maintenance in liver transplantation: the randomized spare-the-nephron trial. Liver Transpl 2013;19:675–689.
22. Neuberger JM, Mamelok RD, Neuhaus P, Pirenne J, Samuel D, Isoniemi H, et al. Delayed introduction of reduced-dose tacrolimus, and renal function in liver transplantation: the 'ReSpECT' study. Am J Transplant 2009;9:327–336.

Table 1. Baseline characteristics (randomized patients)

	Everolimus (n=93)	**Tacrolimus (n=95)**
Male gender, n (%)	79 (84.9)	81 (85.3)
Age, years	56.5 (8.6)	55.5 (8.2)
Caucasian, n (%)	88 (94.6)	90 (94.7)
Body mass index, kg/m^2	25.6 (4.2)	26.5 (3.8)
End-stage disease, n (%)		
Hepatitis B	3 (3.2)	3 (3.2)
Hepatitis C	7 (7.5)	7 (7.4)
Alcoholic cirrhosis	49 (52.7)	50 (52.6)
Hepatocellular carcinoma	19 (20.4)	25 (26.3)
Other	15 (16.1)	10 (10.5)
MELD score	19.0 (9.8)	18.4 (8.7)
CHILD classification		
A	19 (21.1)	26 (28.0)
B	22 (24.4)	21 (22.6)
C	49 (54.4)	46 (49.5)
Missing	3	2
Diabetes at baseline, n (%)	26 (28.0)	28 (29.5)
Hepatitis C virus, n (%)	20 (21.5)	21 (22.1)
Split liver, n (%)	2 (2.2)	4 (4.2)
Cold ischemia time, hours	7.4 (2.3)	7.1 (2.3)
T-tube, n (%)	9 (10.1)	13 (14.6)

Continuous variables are shown as mean (SD)

Table 2. Renal endpoints (LOCF) (ITT population)

	Everolimus (n=90)			**Tacrolimus (n=93)**			**P value for everolimus vs tacrolimus at week 24[b]**
	Mean (SD) at week 24	Mean (SD) change from randomization	P value (change from randomization)[a]	Mean (SD) at week 24	Mean (SD) change from randomization	P value (change from randomization)[a]	
eGFR, MDRD, mL/min/1.73m^2	91.5 (30.4)	0.1 (32.6)	0.691	75.5 (25.6)	-11.8 (34.0)	0.002	<0.001
eGFR, CKD-EPI, mL/min/1.73m^2	86.0 (23.1)	2.4 (22.2)	0.430	74.2 (22.9)	-6.9 (20.1)	**0.002**	**<0.001**
Creatinine clearance, mL/min	85.8 (26.5)	0.7 (25.7)	0.553	75.4 (25.4)	-9.0 (30.6)	**0.007**	**0.003**
Serum creatinine, µmol/L	86.1 (35.1)	-1.3 (38.5)	0.458	100.7 (31.4)	7.2 (36.0)	**0.003**	**<0.001**
Proteinuria, g/L	0.4 (0.8)	0.2 (0.9)	0.221	0.2 (0.3)	0.0 (0.3)	0.278	**0.001**
Urine protein/creatinine ratio	36.1 (86.2)	21.9 (92.0)	0.349	17.2 (28.4)	-2.3 (27.9)	**0.002**	**0.003**

LOCF, last observation carried forward; ITT, intention-to-treat. Significant p values are shown in bold

[a] Wilcoxon signed rank test

[b] Student or Wilcoxon test

Table 3. Efficacy endpoints from randomization to week 24, n (%) (ITT population)

	Everolimus (n=90)	**Tacrolimus (n=93)**	**P value**
Treatment failure[a]	9 (10.0)	4 (4.3)	0.134[b]
BPAR	9 (10.0)	2 (2.2)	0.026[c]
Mild (RAI 4/5; Banff grade I)	5 (5.6)	1 (1.1	
Moderate (RAI 6/7, Banff grade II)	3 (3.3)	1 (1.1)	
Missing	1	0	
Treated BPAR	8 (8.9)	2 (2.2)	0.055[b]
Mild (RAI 4/5; Banff grade I)	5 (5.6)	1 (1.1)	
Moderate (RAI 6/7, Banff grade II)	3 (3.3)	1 (1.1)	
Graft loss	0	1 (1.1)	1.000[b]
Death	1 (1.1)	1 (1.1)	1.000[b]

BPAR, biopsy-proven acute rejection; RAI, rejection activity index; ITT, intention-to-treat

[a] Treated BPAR, RAI score >3, graft loss or death

[b] Fisher's test

[c] Chi squared rest

Table 4. Adverse events, n (%) (safety population)

	Everolimus (n=90)	**Tacrolimus (n=94)**
Any adverse event	81 (90.0)	85 (90.4)
Any serious adverse event	42 (46.7)	28 (29.8)
Premature discontinuation of study drug due to adverse events	16 (17.8)	3 (3.2)
Adverse events occurring in ≥5% of patients in either group		
Cholestasis	24 (26.7)	12 (12.8)
Hepatocellular injury	16 (17.8)	4 (4.3)
Anemia	13 (14.4)	7 (7.4)
Hypokalemia	11 (12.2)	2 (2.1)
Neutropenia	10 (11.1)	14 (14.9)
Leukopenia	10 (11.1)	(8.5)
Thrombocytopenia	8 (8.9)	10 (10.6)
Diarrhea	7 (7.8)	18 (19.1)
Hypertension	7 (7.8)	12 (12.8)
Peripheral edema	7 (7.8)	6 (6.4)
Liver transplant rejection	7 (7.8)	3 (3.2)
Dyslipidemia	7 (7.8)	1 (1.1)
Renal failure	6 (6.7)	15 (16.0)
Biliary anastomosis complication	5 (5.6)	5 (5.3)
Cytomegalovirus infection	5 (5.6)	5 (5.3)
Sepsis	5 (5.6)	0
Lymphopenia	4 (4.4)	6 (6.4)
Abdominal pain	6 (6.4)	3 (3.3)
Systemic inflammatory response syndrome	2 (2.2)	5 (5.3)