ORIGINAL ARTICLE

High trough levels of everolimus combined to sorafenib improve patients survival after hepatocellular carcinoma recurrence in liver transplant recipients

Hidetoshi Nitta[1,*], Aline Younès[1,*], Nada El-domiaty[1,2], Vincent Karam[1], Rodolphe Sobesky[1], Eric Vibert[1], Audrey Coilly[1], Teresa Maria Antonini[1], Eleonora De Martin[1], Daniel Cherqui[1], Hideo Baba[3], Olivier Rosmorduc[1], René Adam[1], Didier Samuel[1] & Faouzi Saliba[1]

1 AP-HP Hôpital Paul Brousse, Centre Hépato-Biliaire, Inserm UMR-S 1193, Université Paris- Saclay, Villejuif, France
2 Tropical Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
3 Department of Gastroenterological Surgery, Graduate School of Life Sciences, Kumamoto University, Kumamoto, Japan

Correspondence
Faouzi Saliba, AP-HP Hôpital Paul Brousse, Centre Hépato-Biliaire, 12 avenue Paul Vaillant Couturier 94800, Villejuif, France.
Tel.: +33 145596412;
fax: +33 145593857;
e-mail: faouzi.saliba@aphp.fr

*First two authors share first authorship.

ABSTRACT

Recurrence of hepatocellular carcinoma (HCC) following liver transplantation (LT) occurs in 10%–20% of patients transplanted for HCC. The treatment of HCC recurrence after LT remains a challenge. Consecutive patients who underwent LT for HCC between 2005 and 2015 at our center were recruited. Characteristics of patients with recurrence, modalities of treatment and outcome were collected retrospectively. Patient survival was analyzed according to HCC recurrence therapeutic strategy. Among 306 transplanted patients, 43 patients (14.1%) developed recurrence with a median survival time after recurrence of 10.9 months (95%CI: 6.6−18.6). Survival of patients treated with Sorafenib (SOR) and everolimus (EVL) (n = 19) was significantly better than that of the group treated with other strategies (n = 24) (P = 0.001). Multivariable analysis demonstrated that SOR plus EVL therapy and absence of dissemination at diagnosis of recurrence were independent predictive factors of prolonged survival after recurrence. Among the patients who treated with EVL, survival of patients with controlled EVL blood trough levels ≥5 ng/ml was significantly better compared to those with EVL trough levels <5 ng/ml (P = 0.021). Combination therapy of sorafenib and everolimus was an independent predictor for better survival after HCC recurrence. Patients with controlled everolimus trough level ≥5 ng/ml might get the best survival benefit.

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Key words
hepatocellular carcinoma, liver transplantation, mTORs inhibitors, Recurrence, survival, trough level

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Introduction

Liver transplantation (LT) has been considered as a curative therapy for patients with hepatocellular carcinoma (HCC) [1]. Since the introduction of the Milan criteria, the 5-year transplant survival resulted in near 70% [2]. Several attempts have been made to widen these criteria and enlarge patient's recruitment, this sometimes lead to increased HCC recurrence after LT [2–10]. HCC recurrence is still the main complications affecting the outcome after LT with recurrence rates of approximately 10–20% [10]. The prognosis of HCC recurrence is poor,

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with a median survival of less than one year after diagnosis and with the majority (67%) involving extrahepatic recurrence [11–13]. This could be referred also to the inevitable usage of immunosuppressants after LT [14,15]. It was shown that calcineurin inhibitors (CNI), have a cancer-promoting influence that might be related to their blood level concentration [16]. The treatment of recurrence after LT can result in prolonged survival but its strategies remain a challenge [17].

With the fact of high incidence of extrahepatic recurrence after LT, the effect of locoregional therapies, such as resection or ablation, don't achieve the needed targets. Until recently, sorafenib (SOR), a multi tyrosine kinase inhibitor, was considered as the only standard treatment strategy for advanced stage of HCC [18]. Some studies analyzed retrospectively the safety and efficacy of SOR for recurrent HCC after LT [19,20]. Some studies demonstrated beneficial effects of SOR on prolonged survival in post-LT patients with HCC recurrence, but the current evidence is still insufficient to draw definitive conclusions [21,22].

The mammalian target of rapamycin inhibitors (mTORi), such as everolimus (EVL) or sirolimus, might represent an alternative immunosuppressive agent; the antineoplastic effect of mTORi has also been confirmed by several studies [23,24]. Theoretically, such systemic therapies could be a favorable approach and their combination (SOR plus mTORi) might have some synergetic effect for this systematic disease. However, there were few reports about the efficacy and safety of the combination therapy for recurrent HCC after LT [25–27]. The aim of this study was to evaluate the predictive factors of survival after HCC recurrence and to evaluate the efficacy and safety of the combination therapy (SOR plus EVL) as a treatment for HCC recurrence after LT.

Patients and methods

Data source

A retrospective cohort study that included a total of 308 consecutive patients who underwent transplantation for HCC at the Hepato-Biliary Center of Paul Brousse Hospital, France during the period between February 2005 and December 2015.

HCC had to be confirmed on the pathology of the explanted native liver. Mixed hepato-cholangiocarcinoma on the pathological examination of the explant ($n = 2$) was excluded from the study. The data were collected from charts and the electronic database system. Data comprised recipient's demographics including age, sex, body mass index (BMI) and etiology of liver disease. Calculated model for end-stage liver disease (MELD) score, biochemical parameters like serum albumin (g/L), total bilirubin (μmol/l) and alpha-fetoprotein (AFP) (ng/L) levels at time of LT were all recorded. Type of the transplant either living, deceased donor or domino was documented. Tumor characteristics at time of transplant and at the pathological examination of the native liver were all considered. Tumor classification according to Milan criteria, University of California San Francisco (UCSF) criteria and AFP score at time of transplant were all recorded.

Patient and ethical approval

The study was approved by the local ethical committee in accordance with the ethical standards laid down in the Declaration of Istanbul 2008 as well as the 2000 Declaration of Helsinki. Written information consent was waived by the local ethical committee due to the retrospective design of the study.

Study design

The design of the study was discussed and approved by our local research committee. Patients were classified according to the recurrence status after LT into no recurrence group and recurrence group. The data of the patients who experienced HCC recurrence was closely analyzed regarding the patient's characteristics, site of recurrence and its treatment.

Diagnosis of HCC and pre-transplant treatment

Diagnosis of HCC was based on imaging data [ultrasound, contrast-enhanced computed tomography (CE-CT) and magnetic resonance imaging], serum AFP and clinical parameters; according to international guidelines [18]. The optimal treatment strategies for HCC patients were determined at multidisciplinary meetings involving surgeons, oncologists, hepatologists, radiologists, and a transplant coordinator [28]. Patients with single, peripheral tumor and compensated liver function were usually selected to undergo hepatic resection, and R0 resection, whereas patients ith multiple tumors with unpreserved liver function or severe portal hypertension were proposed for LT, as described previously [29,30].

Follow-up after liver transplantation

Patients were followed monthly for the first 6 months post-transplant after hospital discharge then every

3 months for the following two years and then every 6 months by surgeons and/ or hepatologists in the outpatient clinic. Alternatively, liver ultrasound or thoracic and abdominal CE- CT scans were examined and serum concentrations of AFP with liver function test were measured at follow-up visits. In patients with suspected recurrence by either elevation of AFP level or CE- CT scans, Fluorine-18 fluorodeoxyglucose positron emission tomography or bone scintigraphy were used for evaluation of extrahepatic metastasis. In the case of an uncertain diagnosis, HCC recurrence was confirmed by percutaneous tumor biopsy.

Post-transplant Immunosuppressive therapy and chemotherapy

Immunosuppressive regimens after LT consisted of a triple-drug regimen that included tacrolimus or cyclosporin, mycophenolate mofetil (MMF), and corticosteroids. Steroids were withdrawn 6 months after surgery. EVL was available for immunosuppressive therapy from 2006 in our institution initially in clinical trials and then mainly used for those patients with chronic kidney disease and particularly for treatment of patients with HCC recurrence or de novo cancer. The initial dose of EVL was 1 mg/day orally, and blood trough levels were measured by immunoassay methodologies to adjust the dose to maintain whole blood trough level at 3–8 ng/ml. EVL trough level were checked for all patients who were treated with EVL every time at clinical visits to the hospital. A mean EVL trough level for a patient was calculated as a sum of trough levels (ng/ml) at every trough level test / number of measurements.

SOR was administered by oncologists once HCC recurrence diagnosis was confirmed. The starting dose was 400 mg twice daily and then the dose rapidly adjusted according to the adverse events. The median dosage of SOR was 600 mg (400–800). In this study, the patients who underwent sorafenib more than 4 weeks were considered as SOR treatment.

Statistical analysis

Categorical and continuous variables were compared using the χ^2 test and Mann–Whitney U test, respectively. Cutoff values for continuous factors (age, body mass index, tumor size) were determined according to median values. AFP model was calculated according to a previous report.[7] Cumulative overall survival (OS) and recurrence-free survival (RFS) curves were constructed using the Kaplan–Meier method and compared by the log-rank test. Survival time was calculated from the date of LT to the date of the event of interest (death for OS, relapse for RFS), or the date of the last follow-up. Survival time after recurrence was calculated from the diagnostic date of recurrence to the date of death or the last follow-up. Univariable analysis of predictive factors for survival was performed using log-rank test. Variables that showed a P-value < 0.10 in univariable analysis were subjected to multivariable analysis using a Cox proportional hazard model. All variables associated with prognosis were candidates using a stepwise backward elimination procedure with a threshold of $P < 0.050$. The level of significance for all tests was set at $P < 0.050$. All statistical analyses were performed using JMP version 12 (SAS Institute, Cary, NC, USA).

Results

Patients and tumor characteristics

A total of 306 consecutive patients who underwent LT for HCC (83.7% were males) were recruited. The median follow-up time since LT was 55.2 months {95%CI: 49.2–62.4} months. Forty-three patients (14.1%) developed HCC recurrence. Patients' demographic data, clinical and tumoral characteristics according to the occurrence of recurrence are shown in Table 1.

Hepatitis C virus (HCV) infection as underlying etiology was more frequent in patients within the recurrence group compared to those in the no recurrence group (51.2% vs. 34.7%; $P = 0.042$). Other pretransplant factors that showed a statistically significant difference between the two groups were: serum albumin ($P = 0.035$), serum AFP ($P = 0.001$) and type of donor ($P = 0.014$) (Table 1).

Among the 306 patients, 230 patients (75.2%) had no HCC pretreatment history prior to LT (181/230 patients were either Child-Pugh score B or C at time of HCC diagnosis) and 76 patients (24.8%) had undergone bridging therapy before LT: trans-arterial chemoembolization (TACE), $n = 45$; TACE plus local ablation therapy (LAT) $n = 25$; LAT, $n = 5$; resection, $n = 1$.

As expected, HCC recurrence occurred more frequently in patients transplanted outside Milan or UCSF criteria. Higher percentage of patients had AFP score $\leq$ 2 among the no recurrence group (238 patients, 91.2%) compared to the recurrence group (27 patients, 62.8%) ($P < 0.0001$) at time of transplant. At the pathological examination of the explanted liver, microvascular invasion (MVI) was observed more in the recurrence group

Table 1. Baseline patients and tumor characteristics according to HCC recurrence.

	No recurrence group (n = 263)	Recurrence group (n = 43)	P-value
Age at transplant (years)	57.4 ± 8.3	57.9 ± 7.1	0.912
Sex (male / female)	219 (83.3%)/44 (16.7%)	37 (86.0%)/6 (14.0%)	0.642
BMI at transplant	26.5 ± 5.2	26.9 ± 4.4	0.701
HBV positive	43 (16.4%)	8 (18.6%)	0.716
HCV positive	91 (34.7%)	22 (51.2%)	0.042
MELD score	10.7 ± 5.1	12.4 ± 6.8	0.079
score ≥ 10	143 (54.4%)	21 (48.8%)	0.500
Total bilirubin (μmol/L)	49.5 ± 96.8	39.6 ± 75.0	0.389
Serum albumin (g/L)	32.6 ± 7.7	35.0 ± 7.7	0.035
Serum AFP (ng/ml)	110 ± 771	228 ± 481	0.001
Serum AFP ≥ 100 ng/ml	28 (10.8%)	13 (30.2%)	0.002
Type of donor	23 (8.7%)/44 (16.7%)/196 (74.5%)	1 (2.3%)/15 (35.0%)/27(62.7%)	0.014
Living / Domino / Deceased			
Pre-transplant tumoral characteristics			
Largest tumor size (mm)	24.9 ± 11.6	36.4 ± 27.1	0.001
Number of nodules	1.9 ± 1.3	2.7 ± 2.4	0.178
Number of nodules >3	22 (8.4%)	11 (25.6%)	0.003
Within Milan criteria	215 (81.8%)	26 (60.5%)	0.003
Within UCSF criteria	233 (88.6%)	30 (69.8%)	0.003
AFP score ≤ 2	238 (91.2%)	27 (62.8%)	<0.0001
Pathological features of the native liver			
MVI in the explant	94 (35.7%)	29 (67.4%)	<0.0001
Tumor differentiation			
Well	110/212 (51.8%)	16/41(39.0%)	0.470
Moderate	61/212 (28.8%)	12/41(29.3%)	
Poor	12/212 (5.7%)	7/41(17.1%)	
Necrotic	29/212 (13.7%)	6/41(14.6%)	

Categorical variables are expressed as number (%) and continuous variables as mean ± SD.

AFP, alpha-fetoprotein; BMI: body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; MELD, model for end-stage liver disease; MVI, microvascular invasion; UCSF, university of California San Francisco criteria.

compared to the no recurrence group (67.4% vs. 35.7%, $P < 0.0001$) (Table 1).

Patient survival and time to HCC recurrence

Kaplan-Meier analysis showed that the 5- and 10-year OS of the patients who underwent LT for HCC (n = 306) were 72.4% and 52.4%, respectively. The 5- and 10-year RFS were 69.8% and 46.6%, respectively (Fig. 1). The median time interval from LT to HCC recurrence was 13.2 months (2.4 −103.8). The median survival time (MST) after recurrence was 10.9 months (95% CI: 6.6–18.6) (Fig. 2). Eight patients developed HCC recurrence within 6 months. The MST after HCC recurrence for the remaining 35 patients was 13.3 months (95% CI: 6.6 – 19.1).

Among the 43 patients who developed HCC recurrence after LT, 36 patients died. Cause of death was 94.4% due to HCC recurrence (34 patients). Two male patients (60 and 53 years old) died due to myocardial infarction and malignant lymphoma, respectively.

Site and treatment of HCC recurrence

Most of the recurrences were extrahepatic (28/43 patients, 65.1%), both intra and extrahepatic (8 patients) and only 7 patients had intrahepatic recurrence. The most common sites of recurrence after LT were the lungs (n = 18) and liver (n = 14). At time of diagnosis of recurrence, 23 patients (53.5%) had a single organ metastasis and 20 patients (46.5%) had a disseminated form with two or more organs involvement (Table 2).

Thirty-three patients received a systemic chemotherapy as described in table 3. There were only 3 patients who underwent locoregional therapy; 1) A 53 years old man, who recurred in a lymph node adjacent to the pancreas tail at 8.7 years after LT, underwent distal pancreatectomy and survived 2 years without any sign of

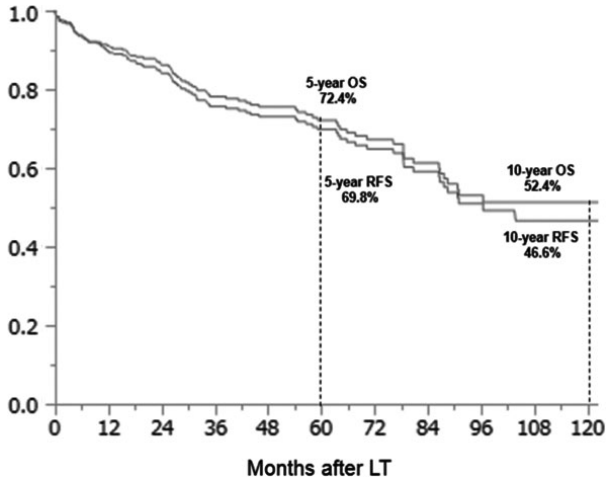

Figure 1 Kaplan–Meier analysis showing the 5- and 10-year overall survival and recurrence-free survival for the whole cohort (n = 306). LT, liver transplantation; OS, overall survival; RFS, recurrence-free survival.

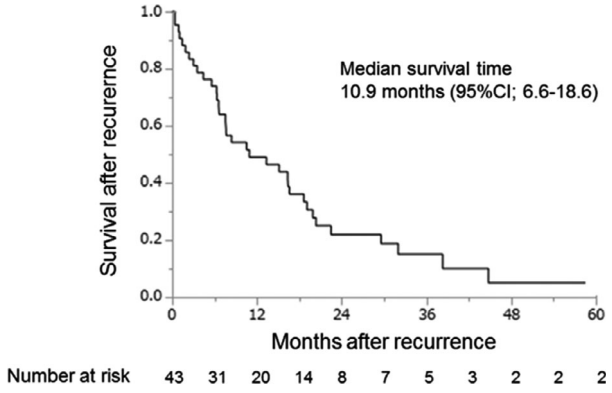

Figure 2 Kaplan–Meier analysis of patient survival after diagnosis of HCC recurrence (n = 43).

re-recurrence. 2) A 56 years old man, who recurred in a lymph node of the inferior mesenteric region 1.7 years after LT, underwent a lymph node resection. He developed 2 months later a re-recurrence and died after 6 months. 3) A 53 years old man, who had an intrahepatic recurrence (segment VIII) 19 months after LT, underwent percutaneous radiofrequency ablation (RFA). One year after, he developed a new intrahepatic recurrence and died 3.2 years later (Table 3). Among the seven remaining patients, one patient started panitumumab as 1st line treatment for peritoneal recurrence after 1.7 years from LT, then switch to SOR plus EVL as 2nd line treatment and died within 6 months of treatment. The other six patients had disseminated extrahepatic recurrence and received palliative supportive care (Table 3).

Table 2. First sites of HCC recurrence.

Site of HCC recurrence	Number of patients (n = 43)
Liver	7 (16.3%)
Lung	5 (11.6%)
Bone	4 (9.3%)
Lymph node	5 (11.6%)
Peritoneum	2 (4.7%)
Disseminated	20 (46.5%)
Lung + lymph node	5
Liver + lymph node	3
Bone + lung	2
Liver + lung	2
Bone + lymph node	1
Abdominal wall + peritoneum	1
Bone + lung + skin	1
Liver + lung + bone	1
Liver + lung + bone + lymph node	1
Peritoneum + lymph node	1
Peritoneum + lung + bone	1
Liver + Inferior Vena Cava	1

Immunosuppressive regimen after recurrence

After diagnosis of recurrence, MMF was stopped if present and CNIs were rapidly decreased then stopped within 1 to 3 weeks after the introduction of EVL. EVL was administered to 32 patients at the dose of 1 mg twice daily and adjusted to aim a trough level between 5 and 10 ng/ml. Eight patients were on EVL with CNI or MMF and 24 others were converted to EVL after diagnosis of recurrence. The median daily dose and trough level of EVL were 2 mg (1–7) and 6.4 ng/ml (2.5–9.7), respectively. The mean daily dose and trough level of EVL were 1.85 ± 1.37 mg and 6.66 ± 2.53 ng/ml, respectively. At the discretion of the oncologist and drug availability, 19/43 patients who received a combination therapy (SOR plus EVL). The OS after recurrence in the combination group (n = 19) was significantly higher than that of the other treatments group (n = 24) (P = 0.0006, the 2-year OS rate; 33.1% vs. 13.1%) (Fig. 3a). The OS after recurrence in the combination group (n = 19) was significantly higher than that of the patient who were on the EVL alone (n = 13) (P = 0.001, the 2-year OS rate; 33.1% vs. 7.7%) (Fig. 3b).

By analyzing the 35 patients who developed recurrence after 6 months; the survival rate of the combination therapy group (n = 16) was significantly better than that of the other treatments group (n = 19) (P = 0.010) (Fig. 3a'). The survival rate after HCC recurrence was

Table 3. Treatment of HCC recurrence after liver transplantation.

1st line	Number of patients	2nd line	Number of patients	3rd line	Number of patients
Sorafenib	17/43 (39.5%)	Sorafenib	7/15 (46.7%)	Capecitabine	2/4 (50.0%)
GEMOX	6/43 (13.9%)	GEMOX	4/15 (26.7%)	GEMOX	1/4 (25.0%)
GEM + bevacizumab	5/43 (11.6%)	GEMOX+ Bevacizumab	2/15 (13.3%)	Sorafenib + Doxorubicin	1/4 (25.0%)
GEMOX+ Bevacizumab	4/43 (9.3%)	XELOX+ Bevacizumab	1/15 (6.7%)		
GEMOX + Cetuximab	1/43 (2.3%)	GEMOX + Cetuximab	1/15 (6.7%)		
Resection (LN metastasis)	2/43 (4.7%)				
Panitumumab*	1/43 (2.3%)				
RFA (liver)	1/43 (2.3%)				
None	6/43 (13.9%)				
Total	43		15		4

GEM, gemcitabine; GEMOX, gemcitabine + oxaliplatin; LN, lymph node; RFA, radiofrequency ablation; XELOX, capecitabine + oxaliplatin
*Patient took part in a clinical trial.

significantly higher among the patients who were on the combination therapy ($n = 16$) compared to those who were on the EVL alone ($n = 11$) ($P = 0.008$) (Fig. 3b').

Everolimus trough level and patient survival

There was a marginally significant correlation between mean EVL trough blood level of each patient treated with EVL ($n = 25$) and survival after recurrence (correlation coefficient $R^2 = 0.167$, $P = 0.042$) (Fig. 4a). The MST of patients treated with EVL and had a mean trough level $\geq$ 5 ng/ml was significantly better compared to those patients who had a mean EVL trough level < 5 ng/ml (19.9 months vs. 10.7 months, $P = 0.021$) (Fig. 4b). Among patients who underwent a combination therapy (SOR plus EVL), the survival of those who had a mean EVL trough level $\geq$ 5 ng/ml was significantly better than those with a mean trough level < 5 ng/ml (MST: 22.5 months vs. 10.7 months, $P = 0.030$) (Fig. 5).

Adverse events

Adverse events that were attributed to SOR or SOR plus EVL are summarized in Table 4. There was no significant difference in the proportion of adverse events between SOR and combination therapy (SOR plus EVL) groups (42.1% vs. 40.0%, $P = 0.897$). Adverse events reported to be related to EVL were (stomatitis, $n = 2$), lower limb edema ($n = 2$), intestinal disorders ($n = 1$), hypercholesterolemia and/or hypertriglyceridemia ($n = 4$) and leucopenia ($n = 1$). None of the patients developed a rejection episode. All had a median EVL blood trough level >5 ng/ml (range = 5.30–10.60 ng/ml and mean = 7.45 ng/ml). None of the patients required discontinuation of EVL for adverse events in this cohort. SOR dosage was reduced in 9 (37.0%) patients, among them SOR was stopped in 5 in relation to serious adverse events (reported in Table 4), without difference among groups (Table S1).

Predictive factors of survival after recurrence

Univariable and multivariable analysis of predictive factors associated with survival after HCC recurrence ($n = 43$) are shown in Table 5. The combination therapy (SOR plus EVL) as a treatment of HCC recurrence [relative risk (RR), 0.32; 95% CI, 0.14–0.66, $P = 0.002$] and a single site recurrence at time of diagnosis (RR, 0.38; 95% CI, 0.18–0.80, $P = 0.011$) were independent predictive factors for better survival after HCC recurrence (Table 5).

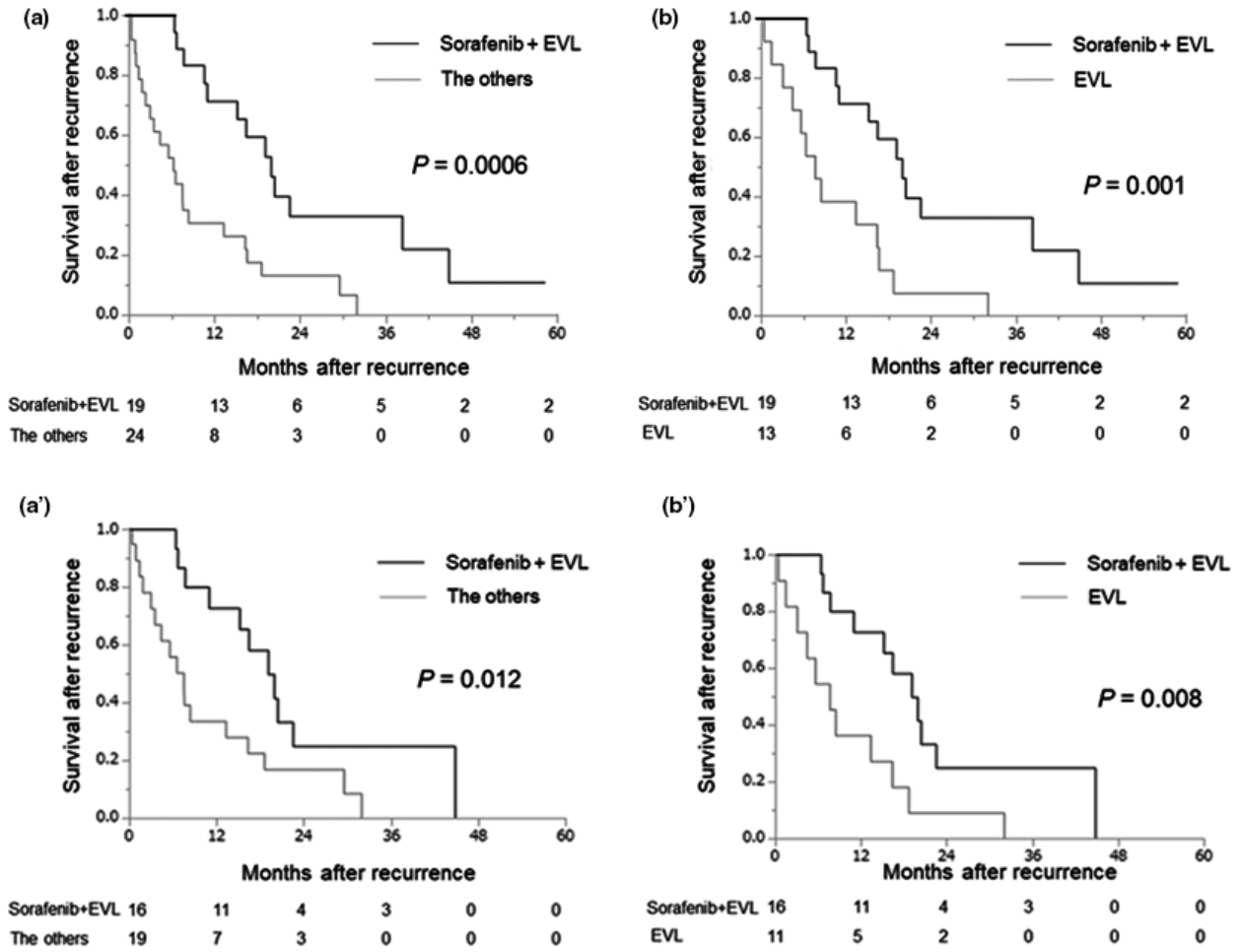

Figure 3 Patient survival after HCC recurrence according to the treatment. (a) Kaplan–Meier analysis comparing survival of the 43 patients treated with combination therapy (sorafenib plus Everolimus) vs. other treatments. (b) Kaplan–Meier analysis comparing survival of patients treated with combination therapy (sorafenib plus Everolimus) vs. Everolimus (EVL) alone. (a') Kaplan–Meier analysis comparing survival of the 35 patients who developed HCC recurrence after 6 months and treated with combination therapy (sorafenib plus Everolimus) vs. other treatments. (b') Kaplan–Meier analysis comparing survival of the patients who developed HCC recurrence after 6 months and treated with combination therapy (sorafenib plus Everolimus) vs. Everolimus (EVL) alone.

The predictive factors associated with survival after HCC recurrence among the 35 patients who developed HCC recurrence after 6 months was similar to that of 43 patients [the combination therapy (SOR plus EVL) as a treatment of HCC recurrence (RR, 0.35, 95% CI, 0.15–0.77, $P = 0.009$) and a single site recurrence at time of diagnosis (RR, 0.40, 95% CI, 0.17–0.91, $P = 0.027$) (Table S2).

Discussion

HCC recurrence is one of the critical complications after LT, because of its poor prognosis. The MST after HCC recurrence was previously reported as less than 1year [12, 31]. In this study, with long-term follow-up (median 55.2 months {95%CI: 49.2–62.4} months), the 5- and 10-year patient survival were respectively 72.4% and 52.4%. Forty-three patients (14.1%) developed HCC recurrence (83.7% was extrahepatic) with a MST after recurrence of 10.9 months. We found that MST of patients who received SOR plus EVL was significantly better than those patients treated by other treatments (19.9 vs. 6.3 months, $P = 0.001$). In the multivariable Cox proportional hazard analysis of factors associated with survival after recurrence, a single site recurrence at time of diagnosis and the use of a combination therapy (SOR plus EVL) were independent predictive factors for better survival.

Improvement of outcome of HCC recurrence after LT is still a main challenge. Several systemic therapies for advanced HCC in the nontransplant setting are currently under investigations [32]. SOR has been

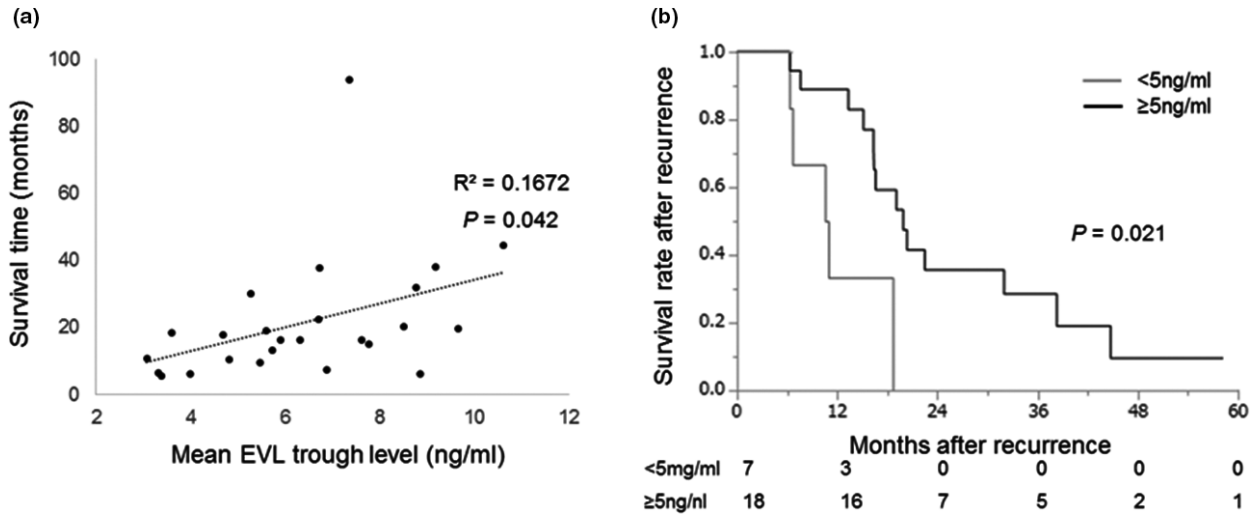

Figure 4 Relationship between survival and EVL blood trough level. (a) Scatterplot of mean EVL trough level and patient survival. (b) Kaplan–Meier analysis comparing patient survival according to mean EVL blood trough levels. EVL, Everolimus.

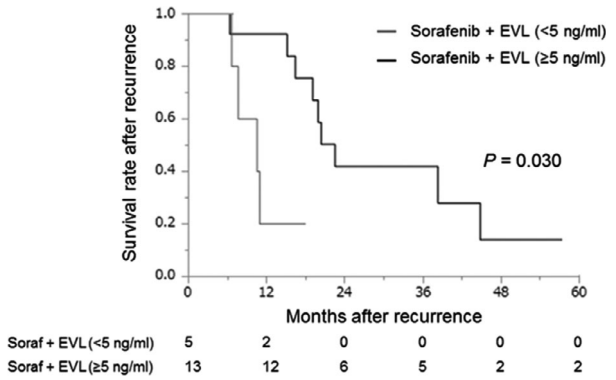

Figure 5 Patient survival after HCC recurrence according to EVL blood trough levels among the patients treated with the combination therapy (Sorafenib plus Everolimus) more than 3 months. EVL, Everolimus.

Table 4. Adverse events.

	SOR plus EVL (n = 19)	SOR (n = 5)	P-value*
Hand-Foot Syndrome (Grade ≥ 2)	3 (15.8%)	1 (20.0%)	
Fatigue (Grade 2)	2 (10.5%)	0	
Liver disorder (Grade 3)	1 (5.3%)	0	
Neutropenia (Grade 3)	1 (5.3%)	0	
Allergic reaction	1 (5.3%)	0	
Stroke	0	1 (20.0%)	
Total	8 (42.1%)	2 (40.0%)	0.897

Categorical variables are expressed as number (%).

*P-values were calculated using the chi-square test. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. EVL, Everolimus; SOR, Sorafenib.

approved as the only systemic therapy which has high efficacy and safety in treating advanced HCC and HCC recurrence after LT [33,34]. However, some previous studies reported heterogeneous outcome on the treatment of HCC recurrence after LT using SOR [20,35–37].

Berenguer et al. reported in a literature review that there is accumulating evidence linking increased exposure to immunosuppression after LT and carcinogenesis, particularly concerning CNIs, whereas exposure to mTORi may decrease this risk due to its antineoplastic effect [38]. mTORi was widely used as a therapeutic agent of several cancers as inhibiting a downstream effector of PI3K/Akt signaling. Combination with sorafenib, abrogates mTORi-induced activation of PI3K/Akt and Ras-MAPK signaling pathways [39]. Combination of rapamycin with sorafenib synergistically inhibits proliferation of cancer cells. Similar to PI3K/Akt signaling, RAF/MEK/ERK pathway is also a major cellular signaling pathway that regulates cell growth and angiogenesis in HCC. Some studies demonstrated that the RAF/MEK/ERK pathway could be activated as a consequence of mTOR inhibition, which might attenuate the antitumor effects of mTORi [39]. In the nontransplant setting, two randomized controlled trials compared for the first EVL versus placebo in sorafenib-exposed patients and for the second SOR with or without EVL and both showed no improvement in overall survival [40,41].

De'Angelis et al. summarized different small experiences with limited available data analyzing the efficacy

Table 5. Univariable and multivariable Cox proportional hazard analysis of factors associated with survival after HCC recurrence (n = 43).

		Univariable analysis			Multivariable analysis		
		Number of patients	MST after recurrence (month)	*P*	RR	95%CI	*P**
Variables at transplantation							
Age†	<60	23	16.6	0.094	NS		
	≥60	20	6.6				
Sex	Male	37	10.9	0.304			
	Female	6	NA				
BMI†	<27	22	7.6	0.503			
	≥27	21	15.1				
Diabetes	No	34	10.9	0.746			
	Yes	9	13.3				
MELD score†	<10	22	15.1	0.298			
	≥10	21	6.6				
HBs-Ag	−	35	15.1	0.330			
	+	8	7.6				
HCV-Ab	−	22	6.6	0.123	NS		
	+	21	15.1				
Type of transplant	Cadaveric	27	10.5	0.484			
	Domino	15	10.9				
	Living	1	NA				
Serum AFP (ng/ml)	<100	30	9.7	0.839			
	≥100	13	16.4				
Pretransplant tumoral characteristics							
Size of tumor (mm)†	<30	20	8.4	0.180	NS		
	≥30	23	16.3				
Number of nodules	1	19	16.6	0.661			
	≥2	24	10.9				
Within Milan criteria	Yes	26	8.4	0.190	NS		
	No	17	13.3				
Within UCSF criteria	Yes	30	8.4	0.096	NS		
	No	13	13.3				
AFP score	≤2	27	8.4	0.052	NS		
	>2	16	19.9				
Pathology of the native liver							
Tumor differentiation	Not poor	36	15.1	0.253			
	poor	7	7.6				
MVI	No	14	8.4	0.301			
	Yes	29	13.3				
Variables at time of HCC recurrence							
Age†	<60	19	16.6	0.090			
	≥60	24	6.6				
HCV RNA	−	26	10.9	0.135	NS		
	+	17	18.6				
AFP (ng/ml)	<100	27	10.5	0.585			
	≥100	16	15.1				
Recurrence	No	25	10.9	0.903			
<1 year	Yes	18	10.5				
Recurrence	No	10	17.7	0.446			
<2 year	Yes	33	10.5				
Extrahepatic recurrence	No	7	21.5	0.336			
	Yes	36	10.5				

Table 5. Continued.

		Univariable analysis			Multivariable analysis		
		Number of patients	MST after recurrence (month)	*P*	RR	95%CI	*P**
A single site recurrence at diagnosis	Yes	23	16.4	0.002	0.380	0.180–0.800	0.011
	No	20	5.6				
Treatment of HCC recurrence							
Locoregional therapy	No	40	10.9	0.263			
	Yes	3	38.3				
GEMOX	No	26	10.5	0.565			
	Yes	17	15.1				
SOR with EVL	No	24	6.3	0.0006	0.320	0.140–0.660	0.002
	Yes	19	19.9				
SOR without EVL	No	38	10.9	0.630			
	Yes	5	18.6				
EVL without SOR	No	30	15.1	0.035	NS		
	Yes	13	7.6				

AFP, alpha-fetoprotein; BMI, body mass index; CI, confidence interval; EVL, everolimus; GEMOX, gemcitabine + oxaliplatin; HBs-Ag, hepatitis B surface antigen; HCV-Ab, hepatitis C virus antibody; HCV RNA, Hepatitis C virus ribonucleic acid; MELD, model for end-stage liver disease; MST, median survival time; MVI, microvascular invasion; RR, relative risk; SOR, sorafenib; UCSF, university of California San Francisco criteria.

**P*-values were calculated by Cox regression model.

[†]According to the median value.

of SOR in combination with mTORi (sirolimus or everolimus) as a treatment for HCC recurrence [12]. The MST from HCC recurrence diagnosis was 18.2 ± 6.53 months in the combination group and the MST from the beginning of SOR therapy was 12 months (range 1.45–20.1 months) [20,21,27,35–37, 42–47]. The largest series of 31 patients treated with SOR in combination with EVL for HCC recurrence showed a MST since the start of SOR of 19.3 months and a median time to progression of 6.8 months [27]. In the present study, the MST of patients who received SOR and EVL was significantly better than those patients treated by other treatments (19.9 vs. 6.3 months, $P = 0.001$). In the multivariable Cox proportional hazard analysis, the combination therapy (SOR plus EVL) was an independent predictive factor for better survival (RR: 0.320, 95% CI 0.014–0.660). *Invernizzi et al.* reported similar results that the combination therapy (SOR plus EVL) is associated with better OS (MST: 18 months, 95% CI 8–27) after analyzing data of 37/50 patients treated with SOR in combination to EVL [25].

This study assessed for the first time the correlation between mean EVL trough concentrations and survival after HCC recurrence. A significant correlation between mean EVL trough blood level of each patient treated with EVL and survival after recurrence was shown (correlation coefficient $R^2 = 0.167$, $P = 0.042$). Interestingly, better survival was observed within the patients treated with EVL trough level more than 5 ng/ml compared to patients treated with EVL less than 5 ng/ml ($P = 0.021$). Moreover, under treatment with SOR, survival of the patients with EVL trough level ≥ 5ng/ml was also significantly better than EVL trough level <5 ng/ml (MST: 22.5 months vs. 10.7 months, respectively; $P = 0.030$). EVL concentration was adjusted to attain 5 to 15 ng/ml in order to achieve anti-tumoral effect [48]. *Cholongitas et al.* recently reported that among the patients who received EVL-based immunosuppression, the recipients with HCC recurrence, compared to those without HCC recurrence, had significantly lower mean trough levels of EVL at 7–12 months post-LT (3.9 vs 5.9 ng/ml, $P = 0.001$), while the patients with mean trough levels of EVL > 6 ng/ml had decreased HCC recurrence rates (log rank: 2.3, $P = 0.007$) [49]. Therefore, keeping EVL blood concentration above a certain level may be important to produce anti-tumoral effect, probably via inhibiting a downstream effector of PI3K/Akt signaling. Increase exposure to EVL would result into a more effective prevention of tumor recurrence but this need to be determined with a close surveillance to detect and

manage potential adverse events [26, 50]. *Deppenweiler et al.* provided that a trough level > 26.3 ng/ml was associated with 4-flod risk of toxicity events [51]. *De Simone* et al. observed in his small series that decreasing EVL exposure for patients experiencing SOR like adverse effects is necessary to avoid SOR toxicity [26]. Therefore, it is preferred to control appropriate trough level advisedly for the treatment of HCC recurrence.

In this present study, the most frequent adverse event observed was hand-foot syndrome (grade ≥2) followed by fatigue (grade 2) and a stroke. These adverse events lead to discontinuation or dose reduction of SOR. *Zavaglia et al.* reported that 91% of 11 patients required dose reduction of sorafenib due to adverse effects or intolerance for treatment of HCC recurrence after LT [37]. Some concerns emerged on an increase in adverse events due to association of SOR and mTORi. Interestingly, no significant statistical difference was observed between the combination group (SOR plus EVL) and SOR alone group regarding the adverse events (40% vs 42.1%, $P = 0.897$). Two studies showed similar results that coadministration of SOR with mTORi provided a favorable safety profile without an increase in drug-related adverse effects, neither in terms of frequency nor in terms of severity, compared with the group of patients treated with SOR alone [25,27]. In contrast, *De Simone et al.* reported a high frequency of side effects (71.4% of the patients had hand-foot syndrome) in their experience of 7 patients treated with the combination therapy (SOR plus EVL) for HCC recurrence after LT [26]. However, the number of patients in these few reports was too small to closely analyze the side effects of this combination therapy. Combination of SOR to EVL is challenging regarding side effects and requires close patient monitoring to adapt EVL dosage to SOR exposure and toxicity over longer follow-up periods.

Multimodal approach is required in treating HCC recurrence after LT. Recently, the role of combination systemic therapies in parallel with the curative therapies (ablation, TACE and stereotactic body radiation) has been explored in patients with recurrence [52].

This study has several strengths; this is a large series of patients treated by combination therapy (SOR plus EVL) and the first to assess the correlation of EVL trough concentrations and patient survival after LT. However, the main limitation of this study was its design as a retrospective single-center study. Therefore, multicentric series are needed to better assess the impact of EVL plus SOR on patient survival and help to fine tune treatment options for HCC recurrence after LT.

In conclusion, although the survival of patients who developed HCC recurrence after LT was poor, the combination therapy with SOR and EVL was an independent predictor for better survival. Patients with a controlled EVL blood trough level ≥5 ng/ml showed a significant better survival. This regimen warrants to be established while waiting for novel drugs to be validated for treatment of HCC recurrence after LT.

Authorship

HN: participated in research design; acquisition of data; analysis and interpretation of data; drafting of the manuscript and statistical analysis. AY: participated in research design; acquisition of data; analysis and interpretation of data and drafting of the manuscript. NE-D: participated in research design; analysis and interpretation of data and drafting of the manuscript. VK: participated in acquisition of data; analysis and interpretation of data. FS: participated in research design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript. RS, EV, AC, TA, EM, DC, HB, OR, RA and DS: participated in critical revision and approval of the manuscript.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Sorafenib dosage.

Table S2. Univariable and multivariable Cox proportional hazard analysis of factors associated with survival after HCC recurrence ($n = 35$).

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