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mTOR Inhibition Is Most Beneficial After Liver Transplantation for Hepatocellular Carcinoma in Patients With Active Tumors

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Objective: The aim of this study was to evaluate the survival benefit of sirolimus in patients undergoing liver transplantation (LT) for hepatocellular carcinoma (HCC) (exploratory analysis of the SiLVER-trial).

Summary and Background Data: Patients receiving LT) for HCC are at a high risk for tumor recurrence. Calcineurin inhibitors have shown evidence to promote cancer growth, whereas mammalian target of rapamycin (mTOR) inhibitors like sirolimus have anticancer effects. In the SiLVER-trial (Clinicaltrials.gov: NCT00355862), the effect of sirolimus on the recurrence of HCC after LT was investigated in a prospective randomized trial. Although the primary endpoint of improved disease-free survival (DFS) with sirolimus was

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not met, outcomes were improved for patients in the sirolimus-treatment arm in the first 3 to 5 years. To learn more about the key variables, a multivariate analysis was performed on the SiLVER-trial data.

Patients and Methods: Data from 508 patients of the intention-to-treat analysis were included in exploratory univariate and multivariate models for overall survival (OS), DFS and a competing risk analysis for HCC recurrence.

Results: Sirolimus use for ≥3 months after LT for HCC independently reduced the hazard for death in the multivariate analysis [hazard ratio (HR): 0.7 (95% confidence interval, CI: 0.52–0.96, $P = 0.02$). Most strikingly, patients with an alpha-fetoprotein (AFP) ≥10 ng/mL and having used sirolimus for ≥3 months, benefited most with regard to OS, DFS, and HCC-recurrence (HR: 0.49–0.59, $P = 0.0079$–0.0245).

Conclusions: mTOR-inhibitor treatment with sirolimus for ≥3 months improves outcomes in LT for HCC, especially in patients with AFP-evidence of higher tumor activity, advocating particularly for mTOR inhibitor use in this subgroup of patients.

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Keywords: AFP, Milan criteria, mTOR-inhibition, multivariate COX regression, Sirolimus

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Liver transplantation (LT) for hepatocellular carcinoma (HCC) is a well-established therapy with good long-term survival. In the algorithmic treatment approach, early-stage Barcelona Clinic Liver Cancer (BCLC) indicates transplantation in patients that have an HCC in cirrhosis within the Milan criteria.[1,2] In these patients, 5-year overall survival (OS) rates reach 60% to 75%.[3,4] There is evidence that immunosuppression type can influence outcomes.[5,6] Calcineurin inhibitors have been associated with an increased risk of HCC-recurrence,[7,8] whereas mammalian target of rapamycin (mTOR) inhibitors have antitumor effects.[9–11] Concerning mTOR-inhibitor use for LT in HCC, a low level of evidence had been available to support their application.[12] Therefore, a large international randomized controlled trial (RCT) (SiLVER-trial) was launched to investigate whether sirolimus-based immunosuppression improves outcomes.[13] Results showed that although long-term disease-free survival (DFS) was not statistically better, there was an improvement in OS and DFS during the first 3 to 5 years after LT. The OS showed a nearly 10% difference between the treatment groups, favoring patients in the sirolimus-arm (70.3% vs 79.4%, $P = 0.048$, HR: 0.7, 95% CI: 0.49–1.0). Importantly, however, the initial evaluation did not include multivariate analysis. Consequently, here we investigated predictive factors for OS in the intention-to-treat (intention-to-treat) cohort of the SiLVER-trial. The hypothesis was that the true survival benefit in the trial was dependent on sirolimus.

PATIENTS AND METHODS

The SiLVER-trial

The SiLVER-trial was the first multicenter RCT investigating sirolimus-based versus mTOR inhibitor-free immunosuppression in LT for HCC. The University of Regensburg sponsored the trial. Independent review board approval was obtained in 2005 (EudraCT-number: 2005-005362-36; Clinicaltrials.gov: NCT00355862). The study took place from January 2006 to March 2014. Overall, 525 patients were randomized into the trial, with 508 patients included in the ITT analysis.[14]

Hypothesis

It is common in transplantation trials that immunosuppressive regimens need to be switched due to side effects or other issues; this results in crossing over from one study arm to the other. In the SiLVER-trial, 78% of patients in the sirolimus treatment arm received sirolimus for at least 2 years. Conversely, 11% in the control arm (mTOR inhibitor-free) received sirolimus during the trial,[14] which accounted for approximately 30% crossover patients. The protocol was open for individual treatment after the patient experienced a recurrence of the tumor. Therefore, it was essential to perform a multivariate analysis for predictors of OS with the time-dependent variable of HCC recurrence. The hypothesis was that sirolimus use after LT for HCC is associated with better OS.

Follow-Up and Endpoints

The primary endpoint was OS in accordance with recommendations from the expert panel for the design of trials on HCC.[15] The median follow-up time was 72.4 months (95% CI 70.7–74.1). Secondary endpoints were DFS and HCC recurrence. The diagnostic criteria for HCC recurrence in the SiLVER-trial have been described elsewhere.[14]

Model-Building Process

Univariate Screening

Data of 508 patients from the ITT in the SiLVER-trial were included. In the first step, 91 items were screened to identify predictors of OS, DFS, and HCC recurrence. Items did not enter the analysis if data were missing for ≥10% of patients (n = 50 or more). Potential predictors of OS from the univariate analysis were analyzed in a bivariate model with HCC recurrence as a time-dependent variable of OS to obtain a more tumor-specific prediction of survival. In the final step, multivariate models of OS, DFS, and HCC recurrence, including subgroups, were performed.

Detailed Statistical Analysis

Univariate Screening

Cox proportional hazards analyses and log-rank tests were performed for OS and DFS. Sirolimus was considered as a time-dependent variable, where the risk of an event between patients treated with sirolimus for ≥3 months in total, to patients treated for <3 months before reaching the corresponding endpoint was compared. An optimal minimal treatment duration with sirolimus ≥3 months (starting from the date of randomization) was identified by testing different thresholds for treatment duration and its effect on endpoints. Since the occurrence of HCC would also significantly change the probability of death, bivariate analyses were performed for OS. With this, in addition to the risk factor of interest, HCC recurrence was included as a time-dependent variable. Predictors with a $P < 0.2$ were considered for the multivariate analyses. For the assumptions of proportional hazards, Kaplan-Meier curves for categorial variables and the corresponding Schoenfeld residuals were used to plot the deviance residuals, and to examine potential outliers.

Multivariate Analysis

Stepwise Cox proportional hazards multivariate analyses were performed to predict OS and DFS; stepwise competing risk analyses were performed for HCC recurrence with ''death'' as a competing event. For the stepwise modeling, a forward selection procedure was employed where, at each step, the model with the largest decrease in the Akaike's Information Criteria (AIC) was chosen. The process stops when none of the several predictors reduces the AIC. A modification of the AIC was used to compare alternative models. The original AIC statistic is defined by $AIC = 2k - 2\ln(\hat{L})$, where k is the number of parameters, and $\hat{L}$ is the maximized likelihood under the model. A smaller AIC value corresponds to an improved model based on the number of covariates and explained variation. In this

modified approach 3k instead of 2k was used to ensure that the rule of ten was satisfied.

Subgroup Analyses

Subgroup analyses for age groups (≤60, >60 years), sex and alpha-fetoprotein (AFP) levels before LT (<10, ≥10 ng/mL) were performed. The time-dependent ROC analysis was used to define the optimal cutoff point for AFP levels.[16] Age groups were defined according to the initial SiLVER publication.[14] Statistical analyses were performed using R software [Version 3.2.4, R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Packages: survival, MASS, kmi, timeROC].

RESULTS

Numbers At Risk and Patient Selection

All patients from the ITT analysis (n = 508) were included. The analysis of 91 items was planned when generating hypotheses for the trial, of which 100% entered the analysis due to the adequate data quality. The patient-specific data and the tumor-specific parameters in the explanted livers are displayed in Table 1; the data of 91 items and the 4 analyses are shown in Supplementary Table 1, http://links.lww.com/SLA/C394.

Factors Associated With OS

Sirolimus treatment ≥3 months (HR: 0.70; 95% CI 0.52–0.96; $P < 0.001$), and the Milan criteria (HR: 0.69; 95% CI: 0.51–0.94; $P = 0.02$) were associated with better OS (Table 2A, ''all patients''). HCC-recurrence (HR: 4.75; 95% CI: 3.40–6.64; $P < 0.001$), AFP ≥10 ng/mL (HR: 1.84; 95% CI: 1.36–2.48; $P < 0.001$), cardiovascular disease (CVD, HR: 1.84; 95% CI: 1.36–2.48; $P = 0.003$), chronic renal insufficiency (CRI, HR: 1.55; 95% CI: 1.02–2.36; $P = 0.04$) and donor age (HR: 1.02; 95% CI 1.01–1.02; $P < 0.001$) were associated with increased mortality (Table 2A). The Kaplan-Meier analysis showed a 5-year OS of 80% versus 67% favoring sirolimus treatment ≥3 months (Fig. 1). Vital tumor detection in the pathologic workup of the explanted diseased liver (HR: 1.65; 95% CI: 0.92–2.97; $P = 0.09$) and the patient sex (HR: 1.59; 95% CI: 0.94–2.69; $P = 0.09$) were not significantly associated with OS, although revealed a trend toward better outcome in full responders after bridging therapy and female patients.[17] Notably, sirolimus treatment ≥3 months and AFP ≥10 ng/mL were the thresholds with the statistically best HR.

Subgroup Analysis for OS—AFP

In patients with an AFP ≥10 ng/mL in Table 2B, male sex (HR: 3.86; 95% CI: 1.55–9.61; $P = 0.004$), HCC recurrence (HR: 4.32; 95% CI: 2.86–6.51; $P < 0.001$) and CVD (HR: 2.04; 95% CI: 1.20–3.45; $P = 0.008$) were associated with a significantly higher mortality risk. Sirolimus treatment ≥3 months (HR: 0.59; 95% CI 0.39–0.87; $P = 0.008$) and the Milan criteria (HR: 0.67; 95% CI 0.46–0.99; $P = 0.042$) were associated with better outcome. The Kaplan-Meier survival curves (AFP ≥10 ng/mL subgroup) in Figure 2A show 75% 5-year OS with sirolimus treatment ≥3 months, versus 58% with sirolimus treatment <3 months. In the subgroup of patients with an AFP<10ng/ml (Table 2C), HCC recurrence (HR: 5.90; 95% CI 3.32–10.50; $P < 0.001$), and donor age (HR: 1.03; 95% CI 1.01–1.04; $P = 0.001$) were associated with poorer outcome, whereas patients within the Milan criteria (HR: 0.57; 95% CI 0.36–0.92; $P = 0.02$) had better outcomes. Kaplan-Meier survival curves (AFP <10 ng/mL subgroup) in Figure 2B show 84% 5-year OS with sirolimus treatment ≥3 months versus 76% with sirolimus treatment <3 months.

Subgroup Analysis for OS—Patient Age

The multivariate analysis in the ''all patients'' analysis (Table 2A) revealed classic risk factors for death in the elderly like CVD and CRI. Accordingly, a dichotomized analysis of patients >60 years' old, versus ≤60 years, was carried out. In the subgroup >60 years in Table 2D, male sex (HR: 4.01; 95%-CI 1.25–12.82; $P = 0.019$), HCC recurrence (HR: 2.83; 95% CI 1.74–4.59; $P < 0.001$), an AFP ≥10 ng/mL (HR: 1.91; 95% CI 1.25–2.92; $P = 0.003$), CVD (HR: 2.10; 95% CI 1.30–3.41; $P = 0.003$), CRI (HR: 2.19; 95% CI 1.22–3.93; $P = 0.009$) and donor age (HR: 1.02; 95% CI 1.01–1.03; $P = 0.006$) were associated with poorer outcome. In the age >60 years' subgroup, Kaplan-Meier survival curves in Figure 3A show 63% 5-year OS with sirolimus treatment ≥3 months, versus 59% with sirolimus treatment <3months. The subgroup of patients ≤60 years (Table 2e) had better outcomes when on sirolimus ≥3 months (HR: 0.55; 95% CI 0.35–0.87; $P = 0.01$), but poorer outcomes after HCC recurrence (HR: 9.14; 95% CI 5.85–14.22; $P < 0.001$), in cases of AFP ≥10 ng/mL (HR: 2.16; 95% CI 1.42–3.28; $P < 0.001$) and with the usage of older donors (HR: 1.01; 95% CI 1.00–1.03; $P = 0.023$). In this subgroup of patients ≤60 years, the Kaplan-Meier survival curves in Figure 3B show 89% 5-year OS with sirolimus treatment ≥3 months, versus 73% with sirolimus <3 months. Factors associated with DFS and the competing risk analysis for HCC recurrence revealed very similar results versus the multivariate analysis of factors for OS (supplementary Tables 2 and 3, http://links.lww.com/SLA/C394). Notably, microvascular invasion was only a risk factor for DFS and the competing risk analysis for HCC recurrence.

DISCUSSION

In contrast to the confirmatory analysis of the SiLVER-trial in 2016,[14] an exploratory approach was used here. This exploratory analysis aimed to find new patterns in the data that were not necessarily hypothesized beforehand. Exploratory and confirmatory methods are complementary tools to discover novel and relevant findings.[18] The interpretation of models obtained via stepwise regression need to be interpreted carefully; P values may not have the same valence as in a confirmatory analysis, and there may be a variable interplay of data and models.[19,20] Nevertheless, this more detailed analysis allowed focusing more specifically on data found in the SiLVER-trial before. The key finding in this analysis was that sirolimus treatment ≥3 months is an independent factor for OS [HR of 0.70 (95% CI: 0.52–0.96)], when compared to sirolimus treatment <3 months, leading to a 30% reduced risk of death ($P = 0.024$). The effect was more pronounced when AFP was included in the model, whereby risk for death in patients with an AFP ≥10 ng/mL and sirolimus treatment ≥3 months, was reduced by 41% [HR 0.59 (95% CI: 0.39–0.87); $P = 0.008$]. These analyses support the assertion that sirolimus treatment improves outcomes in LT for HCC.

The analysis is based on the only available long-term (>5 years/patient) follow-up data from an RCT of >500 LT for HCC. The median follow-up was 72 months for each patient, and source endpoint data were monitored at the site for accuracy.[13,14] These high-quality criteria of RCTs plus the median follow-up per patient are considerable strengths of the trial. The SiLVER-trial data published in 2016[14] already revealed a survival advantage of sirolimus-based immunosuppression for up to 5 years after transplantation [OS 70.3% vs 79.4%, HR 0.70 (95% CI: 0.49–1.00), $P = 0.0479$]. However, the primary trial endpoint was powered for DFS after 5 years and did not reveal a statistically significant effect in the sirolimus arm over the longer term. Nonetheless, the anticancer effect of sirolimus becomes clearer in the current analysis, where the optimal minimum treatment was received by testing different thresholds for the treatment duration. Therefore, it is clear now that

TABLE 1. Patient and Tumor-Specific Parameters in Patients Undergoing LT for HCC From the ITT Cohort of the SiLVER-Trial, Grouped by Patients Receiving Sirolimus ≥3 Months and Patients Receiving Sirolimus <3 Months (Including No Sirolimus At All). Data Are Displayed as Percentages, in Mean ''cm'' as Indicated

		Sirolimus Treatment		
Patient-Specific Parameters		**≤3 mo (n = 284)**	**>3 mo (n = 224)**	***P***
Demography	Sex			0.015
	Female	49 (17.3%)	21 (9.38%)	
	Male	235 (82.7%)	203 (90.6%)	
	Age, y			0.887
	≤60	169 (59.5%)	131 (58.5%)	
	>60	115 (40.5%)	93 (41.5%)	
	AFP on the day of LT			0.807
	(0,10)	148 (53.6%)	118 (53.9%)	
	(10,100)	89 (32.2%)	68 (31.1%)	
	(100,1e+03)	27 (9.78%)	26 (11.9%)	
	(1e+03,1e+06)	12 (4.35%)	7 (3.20%)	
Pretransplant co-morbidity status	CVD	28 (9.89%)	18 (8.07%)	0.581
	Myocardial infarction	9 (7.14%)	6 (5.36%)	0.765
	Cardiac insufficiency	16 (10.9%)	6 (5.0%)	0.057
	Hypertension	88 (66.7%)	89 (76.7%)	0.108
	COPD	19 (6.69%)	18 (8.04%)	0.68
	Renal impairment	38 (13.4%)	22 (9.82%)	0.273
	HRS	17 (5.99%)	14 (6.25%)	1.000
	Chronic renal impairment	26 (9.15%)	16 (7.14%)	0.512
	Diabetes mellitus	90 (31.7%)	71 (31.7%)	1.000
	Dietary	17 (19.3%)	17 (24.6%)	
	Insulin	43 (48.9%)	34 (49.3%)	
	Medication	28 (31.8%)	18 (26.1%)	
	Hyperlipidemia	14 (4.93%)	20 (8.93%)	0.107
	Smoking	131 (46.8%)	111 (49.8%)	0.564
	Alcohol abuse	142 (50.5%)	110 (49.1%)	0.819
	Other drug abuse	28 (9.96%)	22 (9.87%)	1.000
	History of thrombosis	22 (7.86%)	15 (6.73%)	0.756
	Cirrhosis	271 (95.4%)	217 (96.9%)	0.544
Primary cause of cirrhosis	Alcoholic	87 (32.1%)	71 (32.7%)	0.665
	HBV	34 (12.5%)	26 (12.0%)	
	HCV	103 (38.0%)	83 (38.2%)	
Tumor-specific parameters in explanted livers				
Milan Criteria	Extended criteria	97 (34.2%)	85 (37.9%)	0.429
	Within Milan Criteria	187 (65.8%)	139 (62.1%)	
Risk group	High	116 (40.8%)	100 (44.6%)	0.442
	Low	168 (59.2%)	124 (55.4%)	
Vital tumor present		248 (87.3%)	200 (89.3%)	0.588
Grading	G1	58 (25.4%)	42 (23.7%)	0.793
	G2	133 (58.3%)	102 (57.6%)	
	G3	37 (16.2%)	33 (18.6%)	
Lesions	Mean tumor size, cm	0.93 (1.52)	1.09 (1.71)	0.257
	No. of tumors			0.771
	1	145 (52.0%)	104 (48.1%)	
	2	63 (22.6%)	50 (23.1%)	
	3	32 (11.5%)	33 (15.3%)	
	4	28 (10.0%)	20 (9.26%)	
	5	11 (3.94%)	9 (4.17%)	
	V2	29 (10.5%)	19 (8.72%)	0.607
	V1	70 (25.5%)	47 (21.6%)	0.355
Pretransplant treatment of lesions	RFA	70 (24.6%)	49 (21.9%)	0.531
	TACE	126 (44.4%)	107 (47.8%)	0.500
	PEI	16 (5.63%)	19 (8.48%)	0.279
	Chemotherapy	7 (2.46%)	1 (0.45%)	0.083
	Resection	28 (9.86%)	22 (9.82%)	1.000
	No bridging therapy	85 (29.9%)	58 (25.9%)	0.365
HCC recurrence and survival data	Time to recurrence, mo (IQR)	11.8 (5.7–18.4)	36.6 (19.1–43.7)	N.A.
	Survival time after recurrence, mo 95% CI]	14.4 (7.0–21.8)	24.9 (10.8–39.1)	
	Survival after recurrence, mo (95% CI)	14.4 (7.0–21.8)	24.9 (10.8–39.1)	0.056
	1-y OS	89.9%	100%	N.A.
	3-y OS	76.3%	87.7%	0.003
	5-y OS	67.0%	80.1%	0.002

There were no differences between the groups except an inhomogeneity between females in the 2 defined groups of analysis.

COPD inddicates chronic obstructive pulmonary disease; HBV, hepatitis B virus; HCV, hepatitis C virus; HRS, hepato-renal syndrome; G, grading; IQR, interquartile range; N.A., not applicable; PEI, percutaneous ethanol instillation; RFA, radio frequency ablation; TACE, transarterial chemoembolization; V1, microvascular invasion; V2, macrovascular invasion.

TABLE 2. Multivariate Cox Proportional Hazards Analysis of Prognostic Factors for Overall Survival in Patients Undergoing LT for HCC, Including Subgroups of AFP Levels at the Time Point of LT and Age Groups of Recipients

2a: All Patients, Concordance = 0.76

Variable	*P*	HR (95% CI)	Reference Group
Sex	0.09	1.59 (0.94–2.69)	Female
Recurrent HCC*	<0.001	4.75 (3.40–6.64)	No HCC
Sirolimus ≥3 mo*	0.024	0.70 (0.52–0.96)	Sirolimus <3 mo
AFP before LT (≥10 ng/mL)	<0.001	1.84 (1.36–2.48)	<10 ng/mL
Milan criteria	0.02	0.69 (0.51–0.94)	Extended criteria
CVD	0.003	1.84 (1.23–2.76)	No
CRI	0.04	1.55 (1.02–2.36)	No
Vital tumor detectable	0.09	1.65 (0.92–2.97)	No
Donor age	<0.001	1.02 (1.01–1.02)	

2B: AFP Before LT ≥10 ng/mL, Concordance = 0.73

Variable	*P*	HR (95% CI)	Reference Group
Sex	0.004	3.86 (1.55–9.61)	Female
Recurrent HCC*	<0.001	4.32 (2.86–6.52)	No HCC
Sirolimus ≥3 mo*	0.008	0.59 (0.39–0.87)	Sirolimus <3 mo
Milan criteria	0.042	0.67 (0.46–0.99)	Extended criteria
CVD	0.008	2.04 (1.20–3.45)	No

2C: AFP Before LT <10 ng/mL, Concordance = 0.72

Variable	*P*	HR (95% CI)	Reference Group
Recurrent HCC*	<0.001	5.90 (3.32–10.50)	No HCC
Milan criteria	0.02	0.57 (0.36–0.92)	High risk
Donor age	0.001	1.03 (1.01–1.04)	

2D: Patients Older Than 60 y, Concordance = 0.75

Variable	*P*	HR (95% CI)	Reference Group
Sex	0.019	4.01 (1.25–12.82)	Female
Recurrent HCC*	<0.001	2.83 (1.74–4.59)	No HCC
AFP before LT (≥10 ng/mL)	0.003	1.91 (1.25–2.92)	<10 ng/mL
Milan criteria	0.017	0.578 (0.37–0.91)	Extended criteria
CVD	0.003	2.10 (1.30–3.41)	No
CRI	0.009	2.19 (1.22–3.93)	No
Donor age	0.006	1.02 (1.01–1.03)	

2E: Patients Younger or Equal 60 y, Concordance = 0.75

Variable	*P*	HR (95% CI)	Reference Group
Recurrent HCC*	<0.001	9.14 (5.85–14.22)	No HCC
Sirolimus ≥3 mo*	0.01	0.55 (0.35–0.87)	Sirolimus <3 mo
AFP before LT (≥10 ng/mL)	0<0.001	2.16 (1.42–3.28)	<10 ng/mL
Donor age	0.023	1.01 (1.00–1.03)	

*Time-dependent variable.

the anticancer effect is likely dependent on the presence of sirolimus in patients that have active tumor at the time point of LT (elevated AFP), are at younger age, and are within the Milan criteria. Moreover, we found in cases of HCC recurrence that sirolimus treatment resulted in later tumor redevelopment and patients survived longer after the recurrence. The slowing of HCC redevelopment and longer life after reoccurrence not only supports sirolimus anticancer effects, it helps explain why patients with active tumors, and those at a young age, have the most benefit.

This current SiLVER Study analysis can be contrasted to retrospective findings from publications with lower patient numbers and lower evidence levels. In a registry analysis (109 patients receiving sirolimus vs 2382 patients without mTOR-inhibitors), Toso et al[5] showed that sirolimus was an independent predictor of OS.

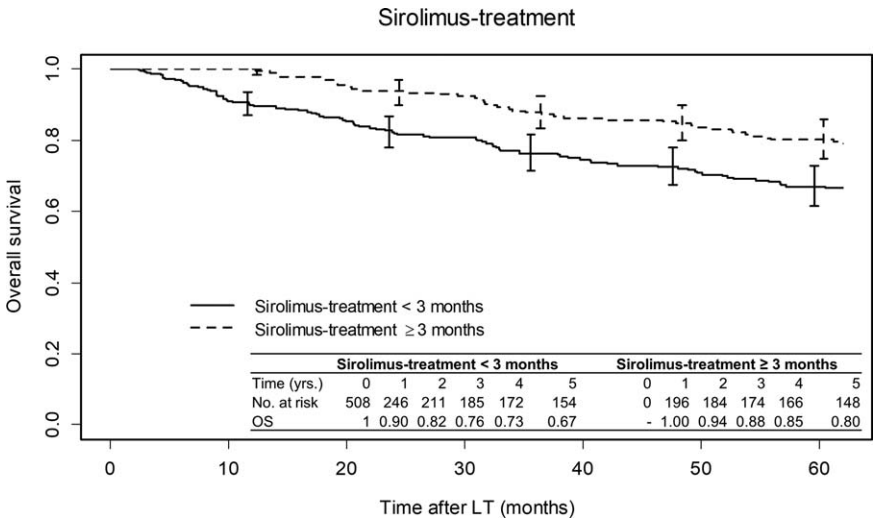

FIGURE 1. OS in patients with sirolimus use ≥3 months, compared to sirolimus use for <3 months or no treatment: HR: 0.7 (95% CI: 0.52–0.96, $P = 0.024$).

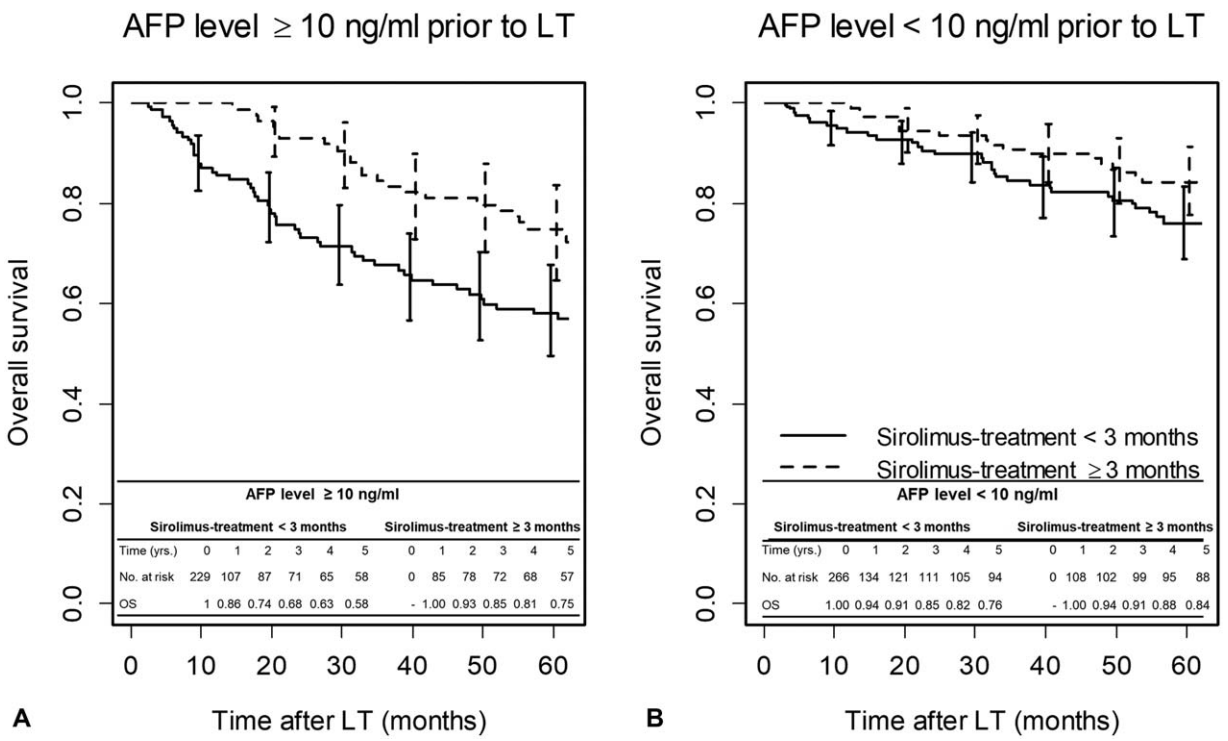

FIGURE 2. A and B: (A) OS in the subgroup of patients with AFP ≥10 ng/mL and sirolimus use for ≥3 months, compared to sirolimus use for <3 months or no treatment: HR: 0.59 (95% CI: 0.39–0.87, $P = 0.008$). (B) OS of patients with an AFP <10 ng/mL and sirolimus use for ≥3 months, compared to sirolimus use for <3 months or no treatment; there was no statistically significant benefit of sirolimus treatment.

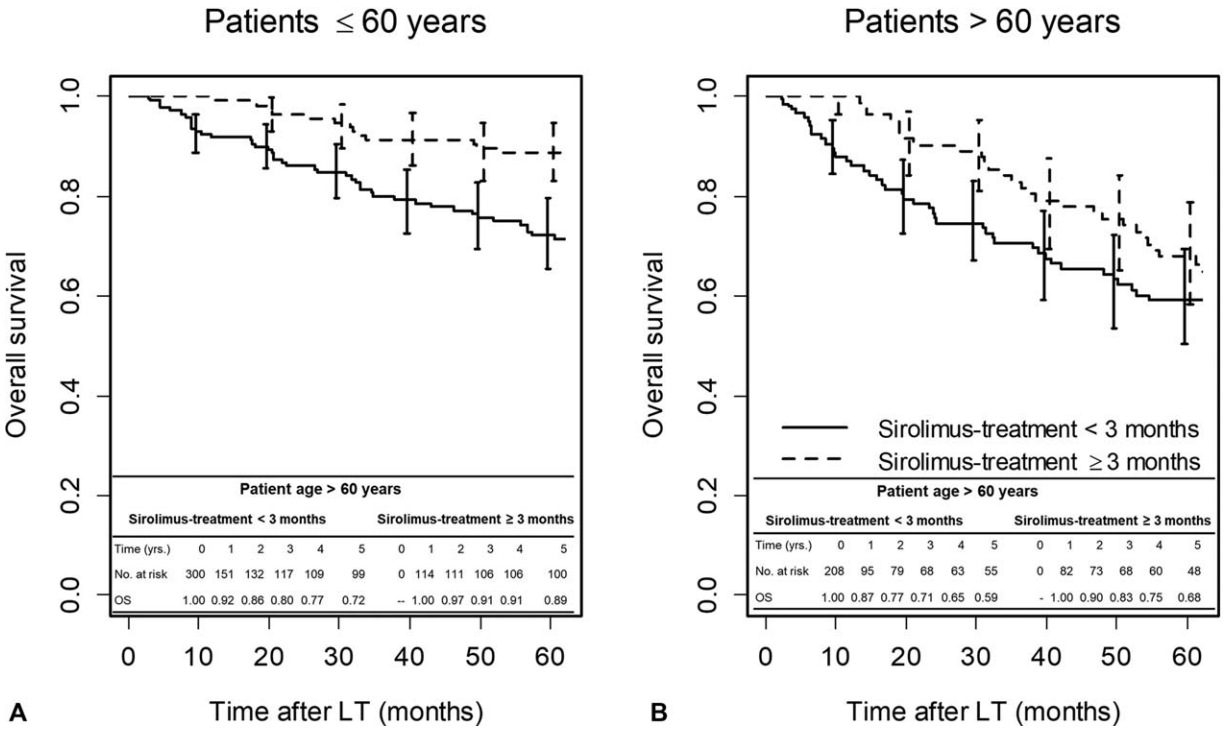

FIGURE 3. A and B: (A) OS of patients with an age >60 years and sirolimus use for ≥3 months, compared to sirolimus use for <3 months or no treatment; there was no significant benefit of sirolimus treatment. (B) OS in the subgroup of patients with an age ≤60 years and sirolimus use for ≥3 months, compared to sirolimus use for <3 months or no treatment: HR: 0.58 (95% CI: 0.37–0.93, $P = 0.025$).

Multivariate analysis detected a 36% reduced risk of death after LT for HCC with sirolimus treatment [HR: 0.63 (95% CI: 0.45–0.90); $P < 0.001$].[5] Chinnakotla et al and Zimmermann et al found similar results with better 5-year OS in patients on sirolimus treatment (80% vs 59% and 79% vs 62%, respectively).[21,22] Cholongitas et al and Heaton et al performed a meta-analysis and concluded that mTOR inhibitors might be beneficial in patients after LT for HCC.[17,18]

Another central finding in our multivariate analysis is that patients with an AFP ≥10 ng/mL show a substantial benefit from sirolimus-treatment ($P = 0.0079–0.0245$, HR: 0.49–0.59). AFP levels are known to be associated with tumor recurrence and OS in LT for HCC, which is consistent with our findings.[23,24] Merani et al showed in a cohort of 6817 patients that AFP >400 ng/mL are predictive for worse outcomes, especially when using the last AFP value before LT[25,26,27]; if the AFP was >400 ng/mL, the risk of death was increased by 50% [HR: 1.49 (95% CI: 1.29–1.72); $P < 0.001$]. Duvoux et al found a strong correlation of AFP values in combination with tumor-size and numbers, as well as macrovascular invasion.[21] Their cutoff AFP level for adverse outcome was defined as 1000 ng/mL which was translated into an outcome score of a more precise model to discriminate between high and low-risk for HCC recurrence and death after LT.[21,28] The group of Mazzaferro et al has worked extensively on risk prediction for HCC recurrence and OS after LT for many years and has established the Milan, and up-to-seven, criteria.[2,4] Their most recent work added AFP as a predictor to the METRO-ticket, giving additional precision to the estimation of DFS and OS after LT for HCC. They analyzed >1000 patients and found that AFP <200 ng/mL, in combination with a tumor burden not exceeding the up-to-seven criteria, to be associated with a 5-year OS of 78% versus 70% in patients exceeding those criteria.[29] Finally, Agopian et al showed that AFP-negative patients (<10 ng/mL) have the lowest risk of recurrence and death without recurrence (67% at 5 years after LT), as well as the lowest risk for recurrence (8.8%).[30,31] She et al also showed that the best outcome could be achieved in patients with AFP <10 ng/mL, which is consistent with our current findings.[32] AFP thresholds in our analysis were obtained with the highest HR for levels of 10 ng/mL from ROC analysis outperforming the mentioned thresholds from the literature. However, the predictive cutoff value for AFP requires more rigorous testing and refinement, since AFP levels do consistently predict HCC activity and recurrence likelihood. We therefore hypothesize that the apparent increased effectiveness of sirolimus in patients with evidence of ''active'' HCC (ie, AFP >10 ng/mL) is because ''left-over'' tumor is at least temporarily held in check by the known anti-cancer effects of mTOR inhibition[11,33]; less advantage of sirolimus may be evident when the tumor is ''inactive'' (ie, AFP <10 ng/mL), since it is less likely to recur with or without an mTOR-inhibitor present. At first this argument may appear to contradict the initial conclusion from the SiLVER-trial confirmatory analysis[14] that sirolimus is effective only in patients with less advanced HCC (within Milan criteria), but rather this multivariate analysis now specifies which subpopulation of patients within Milan criteria (those with an AFP >10 ng/mL) most likely benefit from sirolimus treatment. It should be added that data from RCTs to date, including the SiLVER-trial,[14] indicate that patients with more highly advanced HCC tumors (beyond Milan criteria) are not likely to benefit from mTOR inhibitor therapy.

In conclusion, this multivariate analysis of the SiLVER-trial data reveals that sirolimus treatment is beneficial when given ≥3 months after LT for HCC and is particularly advantageous for patients within Milan criteria with an elevated AFP >10 ng/mL. This conclusion applies to OS, DFS, and the risk for HCC recurrence, and should be considered when revising treatment guidelines.

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