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Sirolimus Use in Liver Transplant Recipients With Hepatocellular Carcinoma: A Randomized, Multicenter, Open-Label Phase 3 Trial

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Background. We investigated whether sirolimus-based immunosuppression improves outcomes in liver transplantation (LTx) candidates with hepatocellular carcinoma (HCC). **Methods.** In a prospective-randomized open-label international trial, 525 LTx recipients with HCC initially receiving mammalian target of rapamycin inhibitor–free immunosuppression were randomized 4 to 6 weeks after transplantation into a group on mammalian target of rapamycin inhibitor–free immunosuppression (group A: 264 patients) or a group incorporating sirolimus (group B: 261). The primary endpoint was recurrence-free survival (RFS); intention-to-treat (ITT) analysis was conducted after 8 years. Overall survival (OS) was a secondary endpoint. **Results.** Recurrence-free survival was 64.5% in group A and 70.2% in group B at study end, this difference was not significant ($P = 0.28$; hazard ratio [HR], 0.84; 95% confidence interval [95% CI], 0.62; 1.15). In a planned analysis of RFS rates at yearly intervals, group B showed better outcomes 3 years after transplantation (HR, 0.7; 95% CI, 0.48-1.00). Similarly, OS ($P = 0.21$; HR, 0.81; 95% CI, 0.58-1.13) was not statistically better in group B at study end, but yearly analyses showed improvement out to 5 years (HR, 0.7; 95% CI, 0.49-1.00). Interestingly, subgroup (Milan Criteria-based) analyses revealed that low-risk, rather than high-risk, patients benefited most from sirolimus; furthermore, younger recipients (age ≤60) also benefited, as well sirolimus monotherapy patients. Serious adverse event numbers were alike in groups A (860) and B (874). **Conclusions.** Sirolimus in LTx recipients with HCC does not improve long-term RFS beyond 5 years. However, a RFS and OS benefit is evident in the first 3 to 5 years, especially in low-risk patients. This trial provides the first high-level evidence base for selecting immunosuppression in LTx recipients with HCC.

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Hepatocellular carcinoma (HCC) is a common malignancy causing substantial morbidity and mortality worldwide that can be treated surgically in only about 30% of patients.[1] In many of those surgical cases, liver transplantation (LTx) is the only potentially curative treatment option, especially in patients where the tumor size, number, and spread are limited according to the Milan Criteria,[2] or other defined parameters.[3,4] Because the vast majority of these patients have liver cirrhosis, 2 otherwise terminal diseases are potentially cured by LTx. However, good outcomes in these patients are diminished by the problem of HCC recurrence or redevelopment in about 1 of 5 individuals. Indeed, immunosuppression needed to prevent organ rejection has long been associated with cancer,[5] and the most commonly used conventional immunosuppressive drugs are calcineurin inhibitors, which have specific tumor-promoting activities.[6,7] In contrast, although also immunosuppressive, mammalian target of rapamycin (mTOR) inhibitors are an exceptional

class of immunosuppressants with activities that can inhibit tumor growth, including antiangiogenic,[8] antiproliferative [9,10] and even, ironically, have proimmunogenic [11,12] effects. The mTOR inhibitors have proven effective in treating selective types of cancer, including renal cell adenocarcinoma.[13,14] Unfortunately, in HCC patients receiving LTx, the low level of evidence for a positive effect of mTOR inhibitors rests on retrospective data analyses [15-20] and small nonrandomized pilot studies,[21] leaving the question largely open as to whether mTOR inhibitors provide a benefit to LTx patients with cancer.

The aim of the present study, referred to as the Sirolimus in Liver Transplant Recipients with HCC study (SiLVER), was to perform a large prospective randomized trial comparing recurrence-free survival (RFS) in sirolimus (mTOR inhibitor)-containing versus mTOR inhibitor-free immunosuppression patients undergoing LTx for HCC. This trial was performed to

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completion according to our protocol at internationally located clinical sites over an 8-year period.

MATERIALS AND METHODS

Patient Selection

The LTx recipients were recruited from 45 transplant centers in Europe (42), Canada (2), and Australia (1) in a multicenter, randomized, open-labeled, parallel group trial (EudraCT: 2005-005362-36; Clinicaltrials.gov: NCT00355862). SiLVER was approximately an 8-year study, consisting of roughly a 3-year enrollment period (January 2006 to April 2009) with at least a 5-year follow-up; patients remained in the study for its entire duration, regardless of when they were randomized. The first patient was randomized in January 2006 and the last patient, last visit was conducted in March 2014. The study included all patients eligible for LTx, with the inclusion criteria being 18 years or older, histologically proven HCC before randomization and signed written informed consent. The main exclusion criteria were the presence of extrahepatic HCC and non-HCC malignancies within the past 5 years (excluding successfully treated nonmelanoma skin cancer). Multiple-organ recipients, patients with a known sirolimus hypersensitivity, hyperlipidemia refractory to management, evidence of infection, platelets less than 75 000/mm^3 and women of child-bearing potential not willing to take contraception were also exclusion criteria. Randomization was completed in April 2009.

Randomization

Patients were randomized into 2 groups. Group A was maintained for the study duration on a center-specific mTOR inhibitor-free, generally calcineurin inhibitor–based, immunosuppressive protocol. This control group of patients was compared to a second group (group B) that received mTOR inhibitor–free immunosuppression for the first 4 to 6 weeks, at which time, sirolimus was incorporated into the regime (target range, 4-10 ng/mL) either as a monotherapy or as a combination therapy with non-mTOR inhibitor–based drugs. As a safety precaution, the protocol included a Doppler ultrasound to show hepatic artery patency before initiating sirolimus. Guidelines were given to prevent overimmunosuppression in group B in case sirolimus was used in combination with other immunosuppressants; a regimen containing no more than 3 immunosuppressive agents (one being sirolimus) was allowed, and sirolimus monotherapy was encouraged. Investigators and patients were not masked to the study treatment. Details of the clinical protocol, including guidelines for immunosuppression use, have been published previously.[22]

The enrolled population included patients with HCC tumors that demonstrated liver cirrhosis and were within the guidelines of the Milan Criteria;[2] hereafter referred to as "low-risk" patients. Additionally, a fraction of recipients showed more extensive disease on posttransplant histopathological assessment. These recipients with tumors outside the limits of the Milan Criteria were also included into the study and are referred to as extended criteria or "high-risk" recipients; patients without liver cirrhosis (regardless of tumor size)[23,24] and patients undergoing salvage LTx were also considered high risk.[25] Minimization method using an interactive voice response system (IVRS) was applied for treatment group allocation to obtain minimal imbalance within each study site and regarding Milan Criteria. In all patients, including those receiving pre-LTx anti-tumor therapy (eg, chemoembolization, radiofrequency ablation) for histologically proven HCC, Milan Criteria stratification was based on post-LTx histopathological data. The IVRS randomization was performed between day 22 and day 42 after LTx, allowing for confirmation of HCC in the post-LTx explantation pathology assessment, when pre-LTx histological confirmation was not available.

Outcomes

The primary endpoint of RFS was defined as HCC recurrence or patient death. Patients underwent a standardized tumor-specific follow-up at every scheduled visit. These regular examinations included ultrasound, a chest X-ray, as well as α-fetoprotein measurements, along with a clinical examination to detect potentially related symptoms. In case of suspicious findings, a computed tomography scan/magnetic resonance imaging/positron emission tomography/bone scintigraphy was recommended in accordance with existing guidelines; a biopsy was also recommended to further confirm the HCC diagnosis. Confirmation of an HCC diagnosis was double-checked by on-site monitoring. For the purpose of the study, because of normal delays in establishing a definitive diagnosis of HCC, the first day of tumor suspicion constituted time of recurrence. All mentioned time measurements were calculated based on the day of LTx. In the first year after LTx, patients were followed up at months 1, 3, 6, 9, and 12; thereafter, patients had scheduled visits every 6 months until study end.

Main secondary endpoints of the study were: (1) overall survival (OS), and (2) RFS and OS in the high-risk and low-risk subgroups. In addition, the incidence of acute rejection episodes was recorded. All primary and secondary endpoint data were monitored on-site by the sponsor for

accuracy by verifying source data and electronic case report form (eCRF) entries.

Statistical Analysis

Sample size was calculated using the primary endpoint RFS, assuming proportional hazards and exponential distributions of RFS. The RFS time distributions of the 2 treatment groups were compared using a 2-sided (stratified) log-rank test at a 0.05 significance level. A 5-year RFS rate of 60% in patients treated with mTOR inhibitor–free immunosuppression was expected. An increase to a 5-year RFS rate of 72% due to sirolimus-containing immunosuppression was assumed. The improvement in the 5-year RFS rate from 60% to 72% corresponds to a hazard ratio (HR) of 0.643. For detecting an HR of 0.643 with a power of $1 - \beta = 0.80$ in a 3-stage group sequential design (2 interim analyses followed by 1 final analysis) with an α spending function of the O'Brien and Fleming type, it was necessary to observe 164 events (HCC recurrences or deaths). Adjusted significance levels were $\alpha = 0.0002$ (after 55 events), $\alpha = 0.0120$ (109 events), and $\alpha = 0.0462$ (164 events) for first, second, and final analyses, respectively. Assuming a planned accrual time of 2.5 years and a follow-up time of at least 5 years from the last patient recruited, a total of 405 patients were expected to yield the necessary number of events. With a lost to follow-up rate of about 20%, 255 patients per treatment group were required.

The primary and secondary endpoints were analyzed using the intention-to-treat (ITT) population. The ITT population included all patients randomized who provided informed consent. According to the statistical analysis plan, patients with major eligibility violations were to be excluded from the ITT analysis: (1) extrahepatic tumor (N1, N2, or M1) manifestation in histology, (2) no histologically proven HCC, and (3) primary malignancy other than HCC or skin cancer within 5 years prior to LTx.

The RFS was defined as the time interval between the date of LTx and the date of recurrence or death (as first event). Kaplan-Meier methods were applied to estimate RFS rates. Patients alive and recurrence-free at the time of the analysis were censored for RFS at the time of last patient contact. Patients who missed 2 consecutive visits (without prior HCC recurrence) were considered "lost to follow-up" and were censored at the last visit before this interruption. A 2-sided nonstratified log-rank test (primary confirmatory analysis) was applied to test the RFS time null hypothesis of no difference between the randomized treatment groups. Kaplan-Meier methods were used to analyze the secondary endpoint of OS, as well as for analyses of defined subgroups; similar methods were applied for the analysis of primary and secondary endpoints at yearly intervals (years 1, 2, 3, and so on). All endpoint and subgroup analyses were prespecified in the statistical analysis plan.

Study Oversight and Role of the Funding Source

This study was an investigator-initiated trial organized and sponsored by the University Hospital Regensburg. Parts of the study oversight were contracted through Chiltern International (Bad Homburg, Germany). An eCRF was developed together with Koehler eClinical (Freiburg, Germany), and they controlled the IVRS. Pfizer (formerly Wyeth) supplied sirolimus 1- and 2-mg tablets and provided a research grant, but was not involved in the trial design, analysis, interpretation, or writing of this report. Sirolimus storage, labeling, and distribution tasks were outsourced to B&C Clinipack (Wavre, Belgium). An independent data safety monitoring board (DSMB) was established to assess safety and planned interim efficacy data. Yearly DSMB meetings were held in strict confidence among the 4 DSMB members; only safety issues and a recommendation regarding continuation of the study were communicated in writing to the sponsor, with no information relating to efficacy results. To avoid study bias, access outside the DSMB to the study efficacy data set was not permitted, until after the final statistical analysis was initiated (May 2014).

RESULTS

A total of 528 patients were documented in the eCRF. Three patients without informed consent were excluded from analyses due to consent withdrawal, a request to delete his/her data, and an accidental randomization. Therefore, 525 patients were randomized into the study (Figure 1): 264 to group A and 261 to group B. At end of the trial, a total of 149 (56.4%) patients in group A and 138 (52.9%) patients in group B remained in the study. A total of 238 patients ended the study prematurely: 115 (43.6%) in group A and 123 (47.1%) in group B. The most common reason for premature withdrawal was patient death: 82 (31.1%) in group A and 64 (24.5%) in group B. A total of 93 patients (33 in group A and 60 in group B) withdrew from the study for reasons other than death.

From the 525 randomized patients a total of 17 patients were excluded from the ITT population according to preset criteria in the statistical analysis plan due to violations of major eligibility criteria: 10 patients (1.9%) without histologically proven HCC, 5 patients (1.0%) with extrahepatic tumor manifestation, and 2 patients (0.4%) with primary malignancy other than HCC or skin cancer; these cases were evenly distributed between groups A (3.0%) and B (3.4%).

A summary of the demographic data is given in Table 1 for all 525 randomized patients. Notably, most patients were men (86.1%) and white (95.8%). Approximately 60% of recipients were 60 years or younger, with a mean age of 57.7 years. Mean time on the waiting list for LTx was 0.53 years. Overall, the treatment groups were well balanced with regard to the baseline demographic data. The LTx surgical procedures used were also balanced between groups A and B, including the use of cell saver devices (**Table S1, SDC,** http://links.lww.com/TP/B206).

Comorbidity status is summarized in **Table S2 (SDC,** http://links.lww.com/TP/B206), showing an equal distribution between the groups. Most frequently reported comorbidities were cardiovascular diseases, hypertension, and diabetes mellitus. The treatment groups were also well balanced regarding the causes of the underlying liver disease, including cirrhosis because of HCV infection and alcohol use (**Table S3, SDC,** http://links.lww.com/TP/B206).

Pathological HCC specifics are summarized in **Table S4 (SDC,** http://links.lww.com/TP/B206). A total of 326 patients (64.2%) were within Milan Criteria, whereas 182 patients (35.8%) had tumors outside Milan Criteria. For most patients (64.3%), the number of lesions was 1 or 2, whereas 35.7% of patients had 3 lesions or more. The maximum

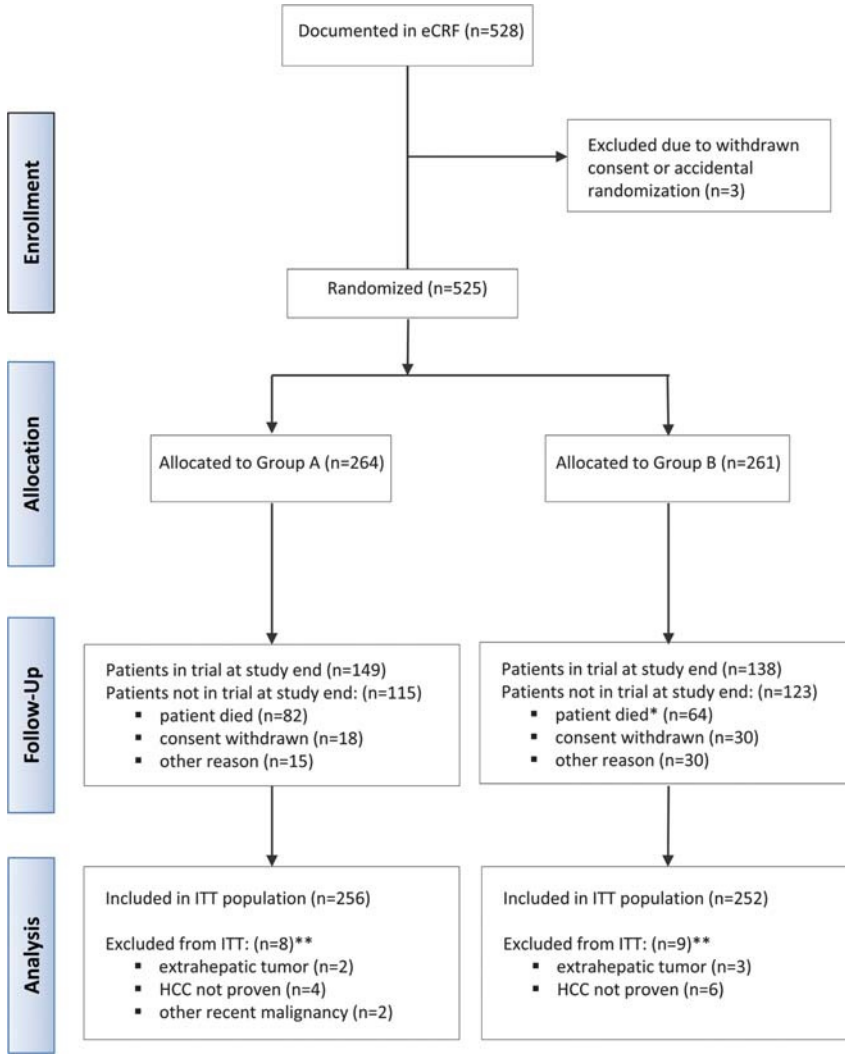


FIGURE 1. Patient disposition. * Reported for one patient who did not withdraw prematurely. ** Patients excluded from the ITT analysis due to violations of major eligibility criteria, as predefined in the statistical analysis plan.

tumor size was less than 3 cm for 45.7%, 3 to 5 cm for 43.9%, and greater than 5 cm for 10.4% of patients, and the most frequent tumor cell grading was G2 (46.3%). Overall, the treatment groups were well balanced for HCC specifics. Regarding HCC treatment before LTx, there was no appreciable difference in the proportion of patients that received treatment in group A (71.1%) versus group B (72.6%), or in the frequency distribution of treatment types (eg, transarterial chemoembolization, radiofrequency ablation) among the 2 groups (**Table S5, SDC,** http://links.lww.com/TP/B206).

Regarding compliance to the group assignments concerning mTOR inhibitor use, it is notable that 30 (11.4%) patients in group A received mTOR inhibitors (sirolimus or everolimus) at some time before HCC recurrence during the trial, and this was due in most cases to calcineurin inhibitor toxicity. As a measure of adherence in group B, during the first 2 years post-LTx, 206 (78.9%) patients were on sirolimus for 50% or greater of this period. At 3, 4, 5, 6, and 7 years after LTx, 70.1%, 66.3%, 66.9%, 70.0%, and 68.8% of remaining patients in group B were on sirolimus treatment, respectively, suggesting that at least 2 of 3 patients consistently remained on study medication during the course of the trial. The median sirolimus trough level ranged from 5.72 to 6.95 ng/ml for the study duration (**Figure S1, SDC,** http://links.lww.com/TP/B206), which is in line with previous reports.[26] Exposure to calcineurin inhibitors in groups A and B is shown in **Table S6 (SDC,** http://links.lww.com/TP/B206); as expected, calcineurin inhibitor doses were greater in group A than in group B during the study.

Before performance of the final analysis, 2 interim analyses of RFS were performed as planned on the randomized patients. The first interim analysis was planned after estimation of the 55th event from available data. Monitoring of the sites confirmed that the actual number of RFS events was 67: 42 patients (15.8%) and 25 patients (9.6%) in groups A and B, respectively. The nonstratified log-rank test did not reveal a statistically significant difference in RFS ($P = 0.0269$ at a significance level of $\alpha = 0.0002$ in the first interim analysis). The second interim analysis was performed after 119 confirmed events: 67 patients (25.4%) and 52 patients (19.9%) in groups A and B, respectively. The nonstratified log-rank test revealed no significant difference in RFS ($P = 0.1493$).

In the final analysis of the ITT population, the primary endpoint of RFS in group A was 64.5% (n = 165 patients) and 70.2% in group B (n = 177) at study end. No significant

TABLE 1.

Demographic data summary (randomized patients)

	Group A (N = 264)	Group B (N = 261)	Total (N = 525)
Age at time of consent, y			
Mean	57.3	58.1	57.7
SD	7.64	6.83	7.26
Median	58.0	58.8	58.5
Q1-Q3	53-63	54-63	53-63
Min to Max	22-72	37-75	22-75
N	264	261	525
Age class			
≤60 y	159 (60.2%)	152 (58.2%)	311 (59.2%)
>60 y	105 (39.8%)	109 (41.8%)	214 (40.8%)
N	264	261	525
Sex			
Female	45 (17.0%)	28 (10.7%)	73 (13.9%)
Male	219 (83.0%)	233 (89.3%)	452 (86.1%)
N	264	261	525
Race			
White	251 (95.1%)	252 (96.6%)	503 (95.8%)
African	3 (1.1%)	3 (1.1%)	6 (1.1%)
Asian	6 (2.3%)	4 (1.5%)	10 (1.9%)
Arabic	2 (0.8%)	2 (0.8%)	4 (0.8%)
Other	2 (0.8%)	—	2 (0.4%)
N	264	261	525
Time on waiting list, y			
Mean	0.58	0.49	0.53
SD	0.849	0.723	0.789
Median	0.28	0.30	0.29
Q1-Q3	0.1-0.7	0.1-0.6	0.1-0.6
Min to Max	0.0-6.9	0.0-7.3	0.0-7.3
N	260	256	516

N is the denominator for percentages (data not always available for all patients).

difference in RFS was observed among the treatment groups by the nonstratified log-rank test ($P = 0.2796$). The HR was 0.84 (95% confidence interval [95% CI], 0.62-1.15). A Kaplan-Meier plot for RFS is provided in Figure 2A (top). However, within the first years after transplantation, the RFS curve of group B is above the curve of group A, suggesting a better RFS in group B. A preplanned analysis of RFS rates over years confirmed what was observed in the Kaplan-Meier plot. Within the first 3 years after transplantation, a higher RFS rate was observed after 1 and 3 years ($P \leq 0.0499$) in group B compared to group A, and later on, this treatment difference was no longer apparent (Figure 2B, top). Hazard ratios ranged from 0.50 (95% CI, 0.29-0.87) after 1 year to 0.88 (95% CI, 0.65-1.21) after 6 years (Figure 2B, top). In general, the anatomical location of the HCC recurrence did not differ greatly between groups A and B, although there were proportionally slightly fewer recurrences found in the thoracic region of group B patients (**Table S7, SDC,** http://links.lww.com/TP/B206).

Overall survival was analyzed as a secondary endpoint using Kaplan-Meier methods. Patients without event were censored at the last date they were known to be alive. At study end, the OS rate in the ITT population was 68.4% in group A and 74.6% in group B (Figure 2A, bottom), which did not reach statistical significance ($P = 0.2096$, log-rank test; HR, 0.81; 95% CI, 0.58-1.13). However, additional planned analysis of OS rates over years showed better OS rates in group B compared with group A at 1, 4, and 5 years ($P \leq 0.0479$) after LTx, with values bordering on significance at 2 and 3 years; this difference was not significant after 5 years (Figure 2B, bottom). Hazard ratios ranged from 0.47 (95% CI, 0.22-0.99) after 1 year to 0.81 (95% CI, 0.58-1.13) after 7 or 8 years (Figure 2B, bottom). Interestingly, prespecified subgroup analyses revealed that patients without prior treatment of lesions demonstrated a significantly higher death rate in group A (28/74 [37.8%]) versus group B (14/69 [20.3%], $P = 0.0381$, log-rank test) over the study period. Additionally, further prespecified subgroup analyses showed that younger patients (≤60 years) had a significantly higher death rate in group A (44/155 [28.4%]) over the study duration compared to group B (25/145 [17.2%], $P = 0.0386$, log-rank test). Causes of death in groups A and B, including the younger and older subgroup, are summarized in **Table S8 (SDC,** http://links.lww.com/TP/B206). This analysis revealed that group B patients overall were not disproportionately susceptible to cardiovascular or infectious death causes, or other causes of death; however, consistent with the OS subgroup analysis, tumor-associated deaths were fewer in group B patients transplanted at 60 years or younger. A similar pre-LTx comorbidity distribution overall, and for the recipients 60 years or younger (**Table S9, SDC,** http://links.lww.com/TP/B206), suggests that the decrease in deaths observed in the younger age group is not due to randomization skewing.

A Kaplan-Meier plot was also generated as prespecified to look for differences in the ITT population for RFS in low- versus high-risk (based on Milan Criteria) subgroups. Interestingly, the low-risk group showed substantially better results in group B compared with group A, but results from the high-risk patients did not suggest a benefit from sirolimus treatment (Figure 3A, top). More specifically, RFS rates over the years showed a significant ($P \leq 0.0383$) treatment difference in low-risk group B patients compared with group A recipients during the first 4 years after LTx (Figure 3B, top); no statistically significant benefit was observed in group B in high-risk patients (Figure 3B, top); moreover, after 1 year, the groups A and B Kaplan-Meier curves for the high-risk patients cross and overlap (Figure 3A, top). Likewise, Kaplan-Meier curves were plotted for OS (Figure 3A, bottom), and analysis showed a survival advantage at 2 to 4 years in low-risk patients ($P \leq 0.0231$), but only a slight advantage at 1 year ($P \leq 0.0361$) in the high-risk group (Figure 3B, bottom).

Finally, when a subgroup analysis of those patients mainly on sirolimus monotherapy was performed, high RFS (82.9%) and OS (85.4%) rates were observed in the monotherapy population versus combination therapy patients (68.2% and 72.3%, respectively). Sirolimus monotherapy was defined by patients on sirolimus immunosuppression alone at the time of at least 50% of their protocol visits. However, this analysis is restricted by the fact that only 19.2% of group B patients received sirolimus monotherapy.

Overall, there were 8013 adverse events (AE), with 512 patients (97.5%) reporting at least 1 AE, 97.3% in group A and 97.7% in group B (Table 2, top). The frequencies of high-interest AEs are summarized in Table 2 (bottom). On an individual patient basis, the percentage of patients reporting related AEs was higher in group B (86.2%) compared with group A (61.0%) (**Table S10, SDC,** http://links.lww.com/TP/B206).

Adverse events leading to death were reported for 82 patients (31.1%) in group A and 64 patients (24.5%) in group B. Adverse events related specifically to immunosuppression in the treatment group that led to death were reported in 8 patients (3.0%) in group A and 7 patients (2.7%) in group B. All AEs were classified by primary system organ class and preferred term using MedDRA version 16.1. Notably, the DSMB did not identify specific safety concerns during the course of the trial.

Finally, 80 patients (30.3%) in group A and 84 patients (32.2%) in group B reported at least 1 episode of acute rejection. The mean number of rejection episodes was 1.3 (±0.9) in group A and 1.5 (±0.8) in group B. Considering only rejections after randomization, the proportion of patients with at least one episode was slightly higher in group B (23.4%) compared with group A (17.0%), but this difference did not reach statistical significance ($P = 0.0710$, χ^2 test).

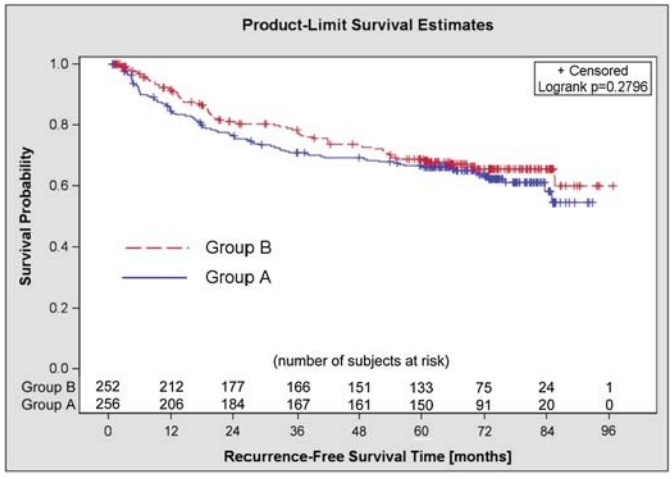
DISCUSSION

In this long-term prospective clinical trial, broad-based practical incorporation of sirolimus into an immunosuppressive regime for LTx recipients with HCC improved recurrence-free and OS in the first 3 to 5 years after transplantation, but thereafter did not indefinitely improve the usual morbidity and mortality associated with use of conventional, generally calcineurin inhibitor–based immunosuppression. Importantly, recipients at a low risk for tumor recurrence showed the clearest and most substantial benefit from sirolimus use during the early post-LTx period, versus high-risk recipients beyond Milan Criteria that received little or no benefit whatsoever. Additionally, our study shows that delayed sirolimus use according to our protocol is safe in this specific indication.

With evidence from basic science research and retrospective data indicating that the anticancer effects of mTOR inhibition could reduce HCC recurrence in LTx,[27] investigations to date have often examined the use of mTOR inhibitors in patients with advanced HCC. A number of these small trials or collections of patients have indicated a possible beneficial effect with relatively advanced tumors.[21,28-30] Indeed, it follows that mTOR inhibitors might provide a means to offer LTx to persons with extended criteria HCCs that otherwise would not be transplanted. However, a clear, and perhaps unexpected, result from our study is that high-risk recipients

A

HCC recurrence-free survival (primary endpoint)



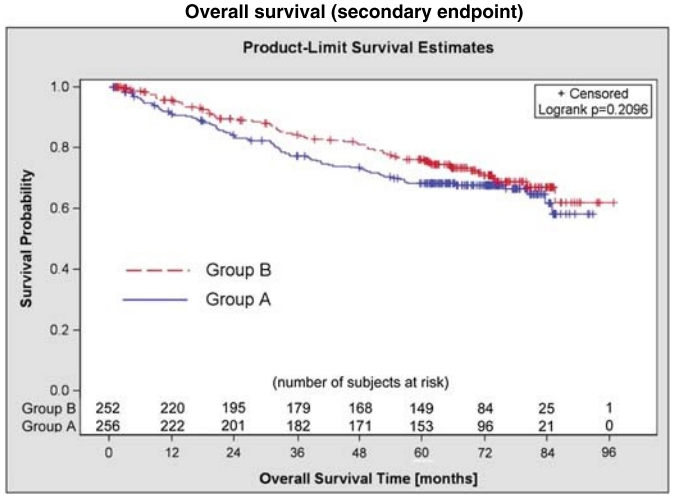
B

Recurrence-free survival over years (ITT population)

Time point after LTx	Group A (N=256)	Group B (N=252)	P-value (log-rank test)	HR [95% CI*]
1 year	218 (85.2%)	233 (92.5%)	0.0125	0.50 [0.29;0.87]
2 years	198 (77.3%)	209 (82.9%)	0.1434	0.75 [0.50;1.11]
3 years	185 (72.3%)	203 (80.6%)	0.0499	0.70 [0.48;1.00]
4 years	181 (70.7%)	192 (76.2%)	0.2193	0.81 [0.58;1.14]
5 years	175 (68.4%)	183 (72.6%)	0.3809	0.87 [0.63;1.19]
6 years	170 (66.4%)	178 (70.6%)	0.4325	0.88 [0.65;1.21]
7 years	166 (64.8%)	178 (70.6%)	0.2798	0.84 [0.67;1.15]
8 years	165 (64.5%)	177 (70.2%)	0.2796	0.84 [0.62;1.15]

*CI: Confidence Interval

Overall survival (secondary endpoint)



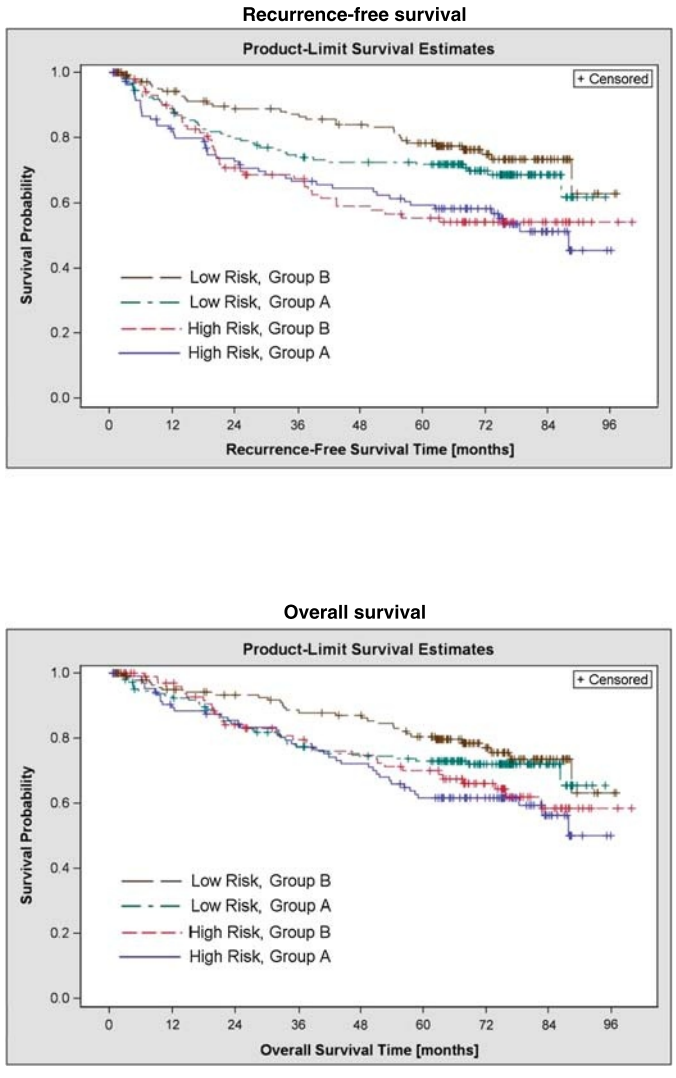
Overall survival over years (ITT population)

Time point after LTx	Group A (N=256)	Group B (N=252)	P-value (log-rank test)	HR [95% CI*]
1 year	234 (91.4%)	242 (96.0%)	0.0414	0.47 [0.22;0.99]
2 years	217 (84.8%)	228 (90.5%)	0.0775	0.64 [0.38;1.06]
3 years	201 (78.5%)	217 (86.1%)	0.0503	0.66 [0.43;1.00]
4 years	192 (75.0%)	210 (83.3%)	0.0468	0.68 [0.46;1.00]
5 years	180 (70.3%)	200 (79.4%)	0.0479	0.70 [0.49;1.00]
6 years	179 (69.9%)	192 (76.2%)	0.1982	0.80 [0.57;1.12]
7 years	176 (68.7%)	189 (75.0%)	0.2104	0.81 [0.58;1.13]
8 years	175 (68.4%)	188 (74.6%)	0.2096	0.81 [0.58;1.13]

*CI: Confidence Interval

FIGURE 2. Efficacy data on ITT population.

A



B

Recurrence-free survival over years (ITT population) - low risk

Time point after LTx	Group A (N=146)	Group B (N=146)	P-value (log-rank test)
1 year	128 (87.7%)	138 (94.5%)	0.0566
2 years	117 (80.1%)	131 (89.7%)	0.0383
3 years	109 (74.7%)	128 (87.7%)	0.0106
4 years	107 (73.3%)	124 (84.9%)	0.0280
5 years	106 (72.6%)	118 (80.8%)	0.1393
6 years	103 (70.5%)	114 (78.1%)	0.2103
7 years	102 (69.9%)	114 (78.1%)	0.1668
8 years	102 (69.9%)	113 (77.4%)	0.2047

Recurrence-free survival over years (ITT population) - high risk

Time point after LTx	Group A (N=110)	Group B (N=106)	P-value (log-rank test)
1 year	90 (81.8%)	95 (89.6%)	0.0970
2 years	81 (73.6%)	78 (73.6%)	0.9017
3 years	76 (69.1%)	75 (70.8%)	0.7606
4 years	74 (67.3%)	68 (64.2%)	0.6918
5 years	69 (62.7%)	65 (61.3%)	0.7939
6 years	67 (60.9%)	64 (60.4%)	0.8495
7 years	64 (58.2%)	64 (60.4%)	0.9257
8 years	63 (57.3%)	64 (60.4%)	0.8527

Overall survival over years (ITT population) - low risk

Time point after LTx	Group A (N=146)	Group B (N=146)	P-value (log-rank test)
1 year	135 (92.5%)	139 (95.2%)	0.3854
2 years	123 (84.2%)	137 (93.8%)	0.0169
3 years	114 (78.1%)	130 (89.0%)	0.0231
4 years	110 (75.3%)	127 (87.0%)	0.0223
5 years	108 (74.0%)	121 (82.9%)	0.1003
6 years	107 (73.3%)	117 (80.1%)	0.2342
7 years	106 (72.6%)	116 (79.5%)	0.2360
8 years	106 (72.6%)	115 (78.8%)	0.2859

Overall survival over years (ITT population) - high risk

Time point after LTx	Group A (N=110)	Group B (N=106)	P-value (log-rank test)
1 year	99 (90.0%)	103 (97.2%)	0.0361
2 years	94 (85.5%)	91 (85.8%)	0.9332
3 years	87 (79.1%)	87 (82.1%)	0.6697
4 years	82 (74.5%)	83 (78.3%)	0.6553
5 years	72 (65.5%)	79 (74.5%)	0.2811
6 years	72 (65.5%)	75 (70.8%)	0.5752
7 years	70 (63.6%)	73 (68.9%)	0.6077
8 years	69 (62.7%)	73 (68.9%)	0.5356

FIGURE 3. Efficacy data on low-versus high risk subgroups (ITT population).

do not show an appreciable RFS benefit from sirolimus-based immunosuppression. Our findings are consistent with recent trial results showing that everolimus had no effect on advanced HCC in non-LTx recipients,[31] inferring together that mTOR inhibitors alone are not effective deterrents of advanced HCC. On the contrary, patients with tumors staged within Milan Criteria in our study did receive a substantial benefit. Consistent with the observation of sirolimus being most beneficial for lower risk patients, a predetermined subgroup analysis showed that younger recipients (≤60 years) have a greater advantage. From these results, we surmise that the anticancer properties of sirolimus are most able to slow down development of relatively "naive" tumors in their early stages, which is also in line with our finding that patients without pre-LTx treatment of HCC had discernably better outcomes, even at study end.

Sirolimus treatment in our study protocol was designed to reflect the "practical" use of immunosuppression in LTx. Therefore, sirolimus was allowed to be used alone, or in combination with approved standard immunosuppressants (eg, calcineurin inhibitors, antimetabolites), because a wide center-to-center variation is expected in normal practice. Indeed, our study suggests that the presence of sirolimus, even when used in combinatory immunosuppressive regimens, positively influences outcomes for LTx patients with HCC. The degree of advantage for patients on sirolimus can be measured if an "area between the curves" analysis[32] is performed on our ITT data set. Indeed, a post hoc exploratory "area between the curves" statistical analysis revealed that patients in group B (including all patients: high and low risk) had an average gain of RFS of 6.4 months versus group A patients; the gain in OS in the sirolimus group was 7.0 months. Sirolimus monotherapy may also offer a further advantage. When a planned subgroup analysis of those patients on sirolimus monotherapy was performed, it did show roughly 13% to 15% higher RFS and OS rates, versus combination therapy patients, but the low proportion (one fifth) of patients on sirolimus monotherapy makes it difficult to know

TABLE 2.

Number and distribution of selected relevant AEs (randomized population)

	Group A (N = 264)	Group B (N = 261)	Total
Total patients with at least 1 AE	257 (97.3%)	255 (97.7%)	512 (97.5%)
Total number of AEs	3871	4142	8013
Selected AE or AE category	(event number in respective group)		
Hyperlipidemia, dyslipidemia, hypertriglyceridemia, or hypercholesteremia	73	137	210
Renal failure	39	35	74
Renal impairment	35	27	62
Acute renal failure	14	11	25
Chronic renal failure	13	4	17
Renal disorder	4	2	6
Total	105	79	184
Peripheral edema	56	96	152
Diarrhea	72	76	148
Incisional hernia	52	91	143
Hypertension	61	77	138
Hepatitis C	60	57	117
Headache	43	48	91
Leukopenia	44	40	84
Anemia	38	45	83
Mouth ulceration, stomatitis, aphtous stomatitis	22	50	72
Pneumonia	25	37	62
Thrombocytopenia	24	36	60
Depression or depressed mood	15	38	53
Wound healing complications, or dehiscence	16	30	46
Insomnia or other sleeping disorder	27	11	38
Hepatic artery stenosis	6	6	12
Hepatic artery occlusion	3	4	7
Hepatic artery thrombosis	4	2	6
Total	13	12	25
Proteinuria	2	22	24
Acne	4	16	20
Portal vein thrombosis	12	2	14
Pneumonitis	0	2	2

whether monotherapy offers reliable advantages. Nonetheless, our results are consistent with experimental data [7] and clinical data,[33] suggesting that mTOR inhibitors can provide an advantage even when used in combination with other immunosuppressants. This is a critical observation if mTOR inhibitors are to have widespread usefulness from an everyday practical perspective in LTx recipients with HCC.

Determining the safety of sirolimus use was a trial objective. To reduce possible reported issues of wound healing problems [34] and occurrence of hepatic vessel thrombotic events,[35] sirolimus introduction was delayed until 4 to 6 weeks after LTx. Although wound healing problems did occur more often in the sirolimus arm with delayed use (Table 2), there was no safety concern noted by the DSMB; regarding thrombotic events, there clearly was no increase observed in group B with this protocol. Besides the expected documented AEs associated with sirolimus treatment,[36] the study group did not identify any serious safety concerns that should warn against the use of sirolimus in LTx patients with HCC.

In conclusion, results from this unprecedented trial in HCC patients undergoing LTx show that although flexible incorporation of sirolimus into an immunosuppressive regimen does not improve long-term HCC recurrence free and OS outcomes after 5 years in patients undergoing LTx, outcomes were improved in the first few years after transplantation, especially in patients with tumor features within Milan Criteria. Although the outcome advantage is eventually lost with time, the window of benefit spans up to 5 years and besides the well-known side effects of this medication, no contraindicative overall disadvantage of sirolimus therapy became apparent over the long term. This trial provides a foundation for sirolimus-based immunosuppressive treatment of LTx patients with HCC.

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