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Mammalian target of rapamycin inhibitors are associated with lower rates of hepatocellular carcinoma recurrence after liver transplantation: a systematic review

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Summary

Calcineurin inhibitors (CNIs) have been associated in a dose-dependent fashion with an increased risk of post-transplant hepatocellular carcinoma (HCC) recurrence. The mammalian target of rapamycin inhibitors (mTORi) (sirolimus/everolimus) might represent an alternative immunosuppressive regimen with antineoplastic effect. In the present systematic review, the association between mTORi and HCC recurrence after liver transplantation (LT) was evaluated and compared against that of CNIs-treated patients. In total, 3666 HCC liver transplant recipients from 42 studies met the inclusion criteria. Patients under CNIs developed HCC recurrence significantly more frequently, compared with patients under mTORi (448/3227 or 13.8% vs. 35/439 or 8%, $P < 0.001$), although patients treated with CNIs had a higher proportion of HCC within Milan criteria (74% vs. 69%) and lower rates of microvascular invasion, compared with mTORi-treated patients (22% vs. 44%) ($P < 0.05$). Patients on everolimus had significantly lower recurrence rates of HCC, compared with those on sirolimus or CNIs (4.1% vs. 10.5% vs. 13.8%, respectively, $P < 0.05$), but everolimus-treated recipients had shorter follow-up period (13 vs. 30 vs. 43.2 months, respectively) and more frequently been transplanted for HCC within Milan criteria (84% vs. 60.5% vs. 74%, respectively, $P < 0.05$). Our findings favor the use of mTORi instead of CNIs to control HCC recurrence after LT, but comparative studies with longer follow-up are needed for final conclusions.

Introduction

Calcineurin inhibitors (CNIs) are considered the central immunosuppressive therapy for solid organ transplantation, including liver transplantation (LT) [1]. In clinical practice, CNIs are used in combination with other immunosuppressive drugs, achieving a significant reduction in the rates of rejection episodes and increasing the graft and patient survival after LT [2]. However, CNIs (i.e., cyclosporine and tacrolimus) are not without short- and long-term adverse effects including renal dysfunction, which is the most common long-term complication in patients after LT [3–5]. In addition, at the cellular level, CNIs enhance the proliferation of malignant cells through increased angiogenesis and cancer cell invasiveness, while clinical studies have demonstrated a CNIs dose-dependent increase in the post-transplant risk of hepatocellular carcinoma (HCC) recurrence [6].

Minimizing the effect of immunosuppressive regimens on tumor development may help to reduce the number of patients who develop HCC recurrence after LT. Mammalian target of rapamycin inhibitors (mTORi), such as sirolimus and everolimus, might represent an alternative immunosuppressive regimen, as *in vitro* studies have shown

that they inhibit angiogenesis and proliferation of neoplastic cells via reduction of several growth factors and enhance microvascular thrombosis, which is associated with lower metastatic potential [7,8]. In addition, several clinical studies have shown that mTORi may also exert an antineoplastic effect [9]. In a recent meta-analysis, sirolimus was associated with a lower incidence of HCC recurrence after LT, compared with CNIs [10]. A more recent meta-analysis [11] confirmed that sirolimus, compared with CNIs, was associated with lower HCC recurrence (OR = 0.30, 95% CI = 0.16–0.55, $P < 0.001$), lower HCC recurrence-related mortality (OR = 0.29, 95% CI = 0.12–0.70, $P = 0.005$), and lower overall mortality (OR = 0.35, 95% CI = 0.20–0.61, $P < 0.001$). However, in both meta-analyses [10,11], the association of everolimus with HCC recurrence after LT was not assessed, while possible contributing factors before or after LT [12–14] were not evaluated.

In this review, we systematically evaluated the available data to assess the association of the mTORi (i.e., sirolimus and everolimus) with the HCC recurrence after LT, and to compare mTORi against CNIs regimens in terms of HCC recurrence. In particular, we documented the rates of HCC recurrence with regard to different pre-LT HCC features and immunosuppression regimens after LT.

Methods

Data sources and searches

Medline/PubMed from January 2007 to October 2013 was searched to identify all medical literature included under the terms 'hepatocellular carcinoma recurrence' and 'tacrolimus' or 'cyclosporine' or 'sirolimus' or 'everolimus'. In addition, a manual search of all relevant review articles and of the retrieved original studies as well as of the abstracts from the major Hepatology and Liver Transplant congresses during the last 2 years was performed.

Study selection

All studies published in English were included if they fulfilled all of the following criteria: (i) they were randomized trials or observational cohort studies, (ii) they included adult patients who underwent LT for HCC, (iii) there was no use of other specific antineoplastic regimen post-LT against HCC recurrence, (iv) there were available data on the incidence of post-LT HCC recurrence in relation to the immunosuppressive regimen (CNIs or mTORi), and (v) they evaluated more than 10 patients with HCC who received CNIs or mTORi after LT. In each selected study, only the patients transplanted for HCC were evaluated, while patients transplanted for other indications were excluded. Literature search was performed by one reviewer (CM) who determined which studies could be potentially included after having screened titles and abstracts. Each study in the list of the preselected papers was evaluated by two independent reviewers (EC, PB) to determine whether it fulfilled all the inclusion criteria.

Data extraction and quality assessment

Data extraction from the finally selected papers was performed by one author (CM) according to a predefined form. Any queries in data extraction were arbitrated by discussion with another author (EC). Data extracted for selected studies included country and center(s), date of publication, type of study (randomized controlled trial, prospective cohort study, and retrospective cohort study), sample size, and the following information: (i) patient and tumor characteristics, that is, the number of patients with HCC who underwent locoregional therapies (LRT), number of cases that fulfilled Milan criteria or had demonstration of microvascular invasion in the explants, and (ii) patient and treatment protocols after LT (i.e., details on CNIs/mTORi treatment, induction therapy administration, duration of steroid treatment, the follow-up period, and the number of patients with HCC recurrence).

Data synthesis and analysis

We used a descriptive approach to summarize study characteristics and outcome (HCC recurrence in patients with regard to immunosuppression). Quantitative variables were expressed as mean values ± standard deviation (SD) and/or median values (range). Corrected chi-square test or Fisher's exact test was used to identify factors that were significantly associated with HCC recurrence. Significance testing was two-sided and set to less than 0.05.

Results

In total, 980 articles were initially identified from the literature search, but only 53 studies fulfilled the inclusion criteria (Fig. 1) [15–67]. There were four studies [19,37,58,65] from one single Italian center, which included patients from overlapping study periods, and it was decided to include only the first two studies [19,37], in which the impact of CNIs and/or mTORi on HCC recurrence was evaluated. However, from the second study [37], we evaluated only patients who received mTORi, excluding patients who received CNIs to avoid the overlap with the first study [19]. The other two studies [58,65] were also excluded because they were not focused on immunosuppression in relation to HCC recurrence. In one study [59], use of organs from executed prisoners could not be excluded, and it was therefore not included in the final review. Two studies were excluded because the rates of HCC recurrence

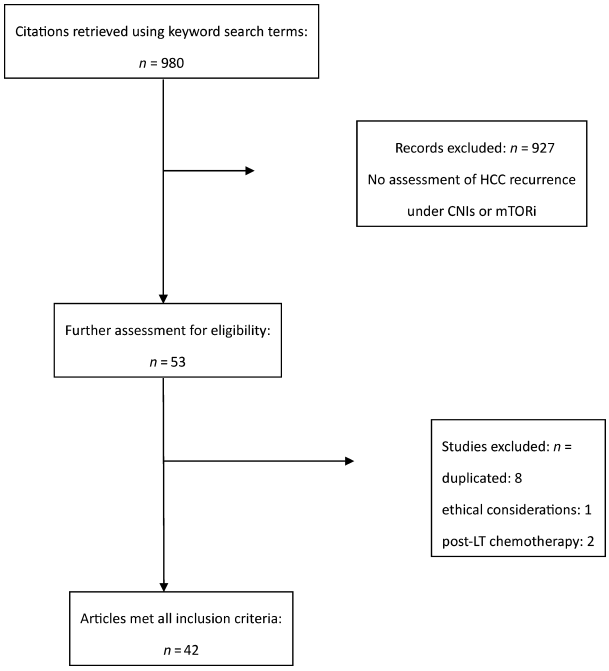

Figure 1 Study flow diagram. HCC, hepatocellular carcinoma; CNIs, calcineurin inhibitors; mTORi, mammalian target of rapamycin inhibitors.

were not available in patients who did not receive post-LT chemotherapy [60,61]. Six studies, three from a single center in China [20,57,64] and three from a single center in Korea [54,62,63], had overlapping study periods, and therefore, only the newest study from each center was included [20,54]. Two studies from a single U.S. center [30,66] had overlapping study periods, and in this case, the oldest study was included [30] because it focused on recurrence of HCC in association with CNIs/mTORi. Finally, two studies from the same center in Japan [48,67], both published in 2013, had overlapping study periods; in this case, we included the study [48] with the longer study period (1999–2012 vs. 1999–2011). Thus, 42 studies providing data regarding immunosuppression regimen (CNIs or mTORi) and HCC recurrence were included in our analysis [15–56].

Six studies were from the U.S.A [18,26,30,50,51,53]., five from Italy [19,35–37,47], five from Spain [25,29,33,44,55], three from France [23,32,42], UK/Ireland [15,22,31], and Turkey [21,46,56], respectively, two from China [20,38], Canada [34,40], Germany [43,45], and Japan [17,48], respectively, and one from Poland [39], Greece [41], Taiwan [49], Brazil [52], Korea [54], and Austria [27], respectively, while three were multicenter studies [16,24,28]. There were five randomized controlled trials (RCT), nine prospective cohort studies (PS), and 28 retrospective cohort studies (RS) (Table 1).

Characteristics of post-LT immunosuppression

In total, 3666 patients underwent LT for HCC and received CNIs or mTORi as immunosuppression (Table 1). Patients who received mTORi with or without CNIs were considered as patients treated with mTORi and were analyzed together, unless otherwise stated. CNIs were used in 3227 (88%) [15–35,44–56] and mTORi in 439 (12%) patients [16,26,28,30, 32,36–43] (Table 1). In particular, among the CNIs patients, 1489 received tacrolimus [15–25,50–54] and 157 cyclosporine [15,19,22,26,27], while in 1581 patients, details on the type of CNIs (tacrolimus/cyclosporine) could not be extracted [28–35,44–49,55,56]. Among the mTORi patients, 218 received sirolimus [30,36–40] and 196 everolimus [16,26,28,32,41,42], while details on the type of the mTORi (sirolimus/everolimus) could not be extracted in 25 patients [43] (Fig. 2).

Characteristics of patients on CNI therapy

Characteristics before LT

In 14 studies [17,21,33–35,44–48,50,51,53,54] including 1255 patients, LRT was used in 809 (64.5%) patients with available data on immunosuppression (tacrolimus: 276, tacrolimus/cyclosporine: 533 patients). Regarding the presence of microvascular invasion in the explant, data were available in nine studies [19,30,33,46–48,50,52,54] including 953 patients: invasion was detected in 202 (22%) patients (tacrolimus: 69, cyclosporine: 33, tacrolimus/cyclosporine: 100 patients). Finally, 1486 (74%) of 1999 patients from 23 studies [15–19,21,30,32–35,44–52,54–56] had HCC, which fulfilled Milan criteria (tacrolimus: 556, cyclosporine: 73, tacrolimus/cyclosporine: 857 patients).

Characteristics after LT

Induction therapy was given in 561 (17.3%) of 3227 patients [17,18,20,26–28,34,47,50]: 390 received antithymocyte globulin (ATG) (tacrolimus: 218, cyclosporine: 25, tacrolimus/cyclosporine: 147 patients), 132 received basiliximab (tacrolimus:78, cyclosporine:16, tacrolimus/cyclosporine: 38 patients), 28 were treated with daclizumab, and 11 received basiliximab or daclizumab (all on tacrolimus). Data regarding the duration of steroid administration were available for 2342 patients from 21 studies [15,16,18–20,22–24,26–28,34,35,46–49,52,54–56]: steroids were given for a median time of 3 (range: 0–12) months after LT. Maintenance postoperative blood levels of CNIs ranged from 5 to 15 ng/ml for tacrolimus and from 100 to 300 ng/ml for cyclosporine.

Characteristics of patients on mTORi therapy

Characteristics before LT

Based on the available data, 75 (51%) of 148 patients underwent LRT before LT [37,39–41] (sirolimus: 54, ever-

Table 1. Published studies using calcineurin inhibitors (CNIs) [cyclosporine (cyclo) and tacrolimus (tac)] or mammalian target of rapamycin inhibitors (mTORi) [sirolimus (sir) or everolimus (ever)] for prevention of hepatocellular carcinoma (HCC) recurrence after liver transplantation (LT).

Study 1st author, year [Ref no.]	Type of study	Patients, *N*	IR (mTORi or CNIs)	Within Milan criteria, *n*	LRT, *n*	Mean follow-up, mos	Duration of steroids, mos	Induction therapy, *n*	HCC recurrence, *n*
Zieniewicz, 2007 [39]	RS	26	sir	20	9	NA	NA	0	7
Toso, 2007 [40]	PS	70	sir	32	23	49	3	49 (daclizumab)	8
Escartin, 2007 [55]	RS	184	CNIs	184	NA	12	6	0	28
O'Grady, 2007 [22]	RCT	26	tac	NA	NA	NA	3	0	2
		27	cyclo						
Soliman, 2007 [27]	RS	25	cyclo	NA	NA	60	6	ATG (for 3 or 10 days)	5
Zimmerman, 2008 [30]	RS	52	CNIs	40	NA	24	NA	0	9
		45	sir	37		36			3
Vivarelli, 2008 [19]	RS	60	tac	43	NA	43.2	12	0	12
		79	cyclo	70					9
Silva, 2008 [33]	RS	257	CNIs	231	206	11	NA	0	33
Nocera, 2008 [36]	RS	18	sir	NA	NA	19	3	0	1
Chen, 2008 [38]	RS	28	sir	15	NA	NA	NA	0	0
Kiyici, 2008 [56]	RS	72	CNIs	35	NA	31.7	12	0	8
Varona, 2009 [44]	RS	65	CNIs	56	27	NA	NA	0	3
Vakili, 2009 [50]	RS	28	tac	21	5	40.8	NA	28 (daclizumab)	8
Trunecka, 2010 [24]	PS	81	tac	NA	NA	NA	12	0	1
Masetti, 2010 [26]	RCT	16	cyclo	NA	NA	12	1	16	3
		28	ever			12	1	28 (basiliximab)	2
Vivarelli, 2010 [37]	RS	31	sir	17	22	24	12	0	4
Cholongitas, 2011 [15]	PS	22	tac	12	NA	97	0	0	7
		10	cyclo	3					2
Schleider, 2010 [43]	PS	25	mTORi	NA	NA	84	NA	0	4
Aktas, 2011 [21]	RS	50	tac	22	9	NA	NA	0	12
Di Benedetto, 2011 [35]	PS	14	CNIs	10	9	NA	3	0	7
Boudjema, 2011 [23]	RCT	68	tac	NA	NA	NA	6	0	3
Ortiz de Urbina, 2011 [25]	RS	18	tac	NA	NA	NA	NA	0	0
Saliba, 2011 [42]	RS	44	ever	NA	NA	11.2	NA	0	0
Hoffmann, 2011 [45]	PS	78	CNIs	39	62	48.9	NA	0	14
Balci, 2011 [46]	RS	40	CNIs	18	7	46	6	0	9
Chan, 2011 [49]	RS	126	CNIs	93	NA	35.2	3	0	17
De Carlis, 2012 [47]	RS	118	CNIs	99	118	60	1	118 (ATG)	15
De Simone, 2012 [16]	RCT	35	tac	35	NA	14	6	0	0
		73	ever	73					2
Miyagi, 2012 [17]	RS	14	tac	9	4	60	NA	11 (basiliximab or daclizumab)	3
Mangus, 2012 [18]	RS	218	tac	145	NA	52	3 in 28 pts (the other no steroids)	218 (rATG)	31
Fischer, 2012 [28]	RCT	23	CNIs	NA	NA	12	0	23 (basiliximab)	1
		14	ever					12	0

Table 1. continued

Study 1st author, year [Ref no.]	Type of study	Patients, *N*	IR (mTORi or CNIs)	Within Milan criteria, *n*	LRT, *n*	Mean follow-up, mos	Duration of steroids, mos	Induction therapy, *n*	HCC recurrence, *n*
Castroagudin, 2012 [29]	RS	165	CNIs	NA	NA	52	NA	0	18
Ghanekar, 2012 [34]	RS	44	CNIs	0	0	NA	3	29 (rATG) 15 (basiliximab)	2
Sharma, 2012 [51]	RS	94	tac	88	25	26,4	NA	0	17
Felga, 2012 [52]	RS	130	tac	130	NA	NA	3	0	9
Doyle, 2012 [53]	RS	264	tac	NA	159	66	NA	0	19
An, 2012 [54]	RS	85	tac	51	74	28,3	6	0	15
Yoshizumi, 2013 [48]	PS	104	CNIs	52	104	58	6	0	19
Hu, 2013 [20]	PS	296	tac	NA	NA	36	Range from 0 to 6 months	78 (basiliximab)	64
Rodriguez-Peralvarez, 2013 [31]	RS	219	CNIs	NA	NA	NA	NA	0	38
Houssel, 2013 [32]	RS	20	CNIs	0	NA	NA	NA	0	5
		16	ever						4
Cholongitas, 2013 [41]	PS	21	ever	19	21	48	6	0	0

NA, not available; IR, immunosuppressive regimen; LRT, locoregional therapy; ATG, antithymocyte globulin; RCT, randomized controlled trial; PC, prospective cohort study; RC, retrospective cohort study.

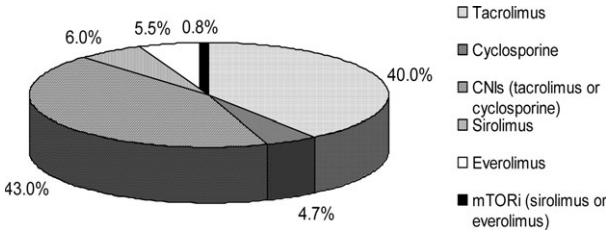

Figure 2 Pie chart showing the subgroups of patients under CNIs or mTORi after liver transplantation (LT) in patients whose primary indication for LT was hepatocellular carcinoma. CNIs, calcineurin inhibitors; mTORi, mammalian target of rapamycin inhibitors.

olimus: 21 patients). Regarding the presence of microvascular invasion in the explant, data were available in three studies [30,37,40]: invasion was detected in 64 (44%) of 146 patients (all under sirolimus). Finally, 213 (69%) of 310 patients had HCC, which met Milan criteria (sirolimus: 121, everolimus: 92 patients) [16,30,32,37–41].

Characteristics after LT

Induction therapy was given in 89 (20%) of 439 patients: 40 received basiliximab (all patients were on everolimus) and 49 received daclizumab (all patients were on sirolimus) [26,28,40]. Based on the available data from seven studies [16,26,28,30,37,40,42] including 256 patients, combination of mTORi with CNIs indefinitely was used in 118 (46%) (sirolimus: 59, everolimus: 59 patients), while 138 (54%) patients received mTORi without CNIs after a median of 1 month (range 0.3–58.8) post-LT. Data regarding the duration of steroid administration were available for 362 patients from six studies: steroids were used for a median time of 6 (range: 1–12) months after LT [16,26,36,37, 40,41]. Maintenance postoperative blood levels of mTORi ranged from 5 to 13.5 ng/ml [36,37,40] for sirolimus and from 3.9 to 8.1 ng/ml [16,26,28,41,42] for everolimus.

HCC recurrence on CNIs versus mTORi

All patients

Hepatocellular carcinoma recurrence was detected in 13.2% (483/3666) of patients transplanted for HCC who received CNIs or mTORi during a median follow-up of 36 (range: 11–97) months. HCC recurrence developed significantly more frequently in patients on CNIs than in patients on mTORi (448/3227, 13.8% vs. 35/439, 8%, $P < 0.001$) (Fig. 3). Before LT, patients on CNIs, compared with those on mTORi, had higher rates of HCC within Milan criteria before LT [1486/1999 (74%) vs. 213/310 (69%), $P = 0.04$], lower rates of microvascular invasion [22% (202/953) vs. 44% (64/146), $P < 0.001$] and had more frequently undergone LRT before LT [64.5% (809/1255) vs. 51% (75/148), $P = 0.0004$] (Table 2). After LT, the group of patients on

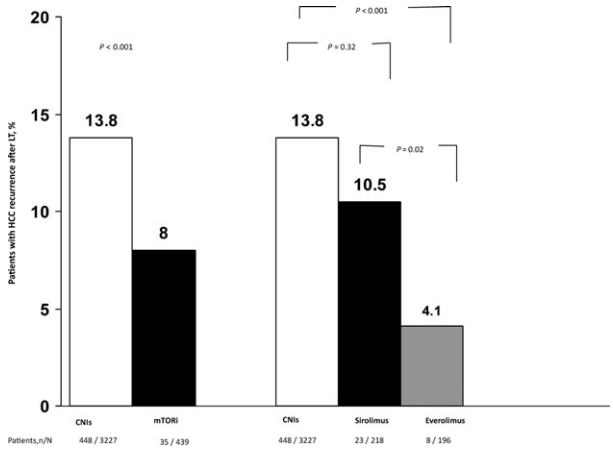

Figure 3 Recurrence of HCC after liver transplantation (LT) in relation to the type of post-transplant immunosuppressive regimen. HCC, hepatocellular carcinoma; CNIs, calcineurin inhibitors, mTORi, mammalian target of rapamycin inhibitors.

Table 2. Characteristics of patients who underwent liver transplantation (LT) for hepatocellular carcinoma and received mTOR inhibitors or calcineurin inhibitors (CNIs) as immunosuppression regimen.

	CNIs (tacrolimus or cyclosporine)	mTOR inhibitors	*P*
Before LT			
Within Milan criteria, *n/N* (%)	1486/1999 (74)	213/310 (69)	0.04
Vascular microinvasion, *n/N* (%)	202/953 (22)	64/146 (44)	<0.001
LRT, *n/N* (%)	809/1255 (64.5)	75/148 (51)	0.0004
After LT			
Induction therapy, *n/N* (%)	561/3227 (17.3)	89/439 (20)	0.52
Duration of steroids, months, median (range)	3 (0–12)	6 (1–12)	–
Duration of follow-up, months, median (range)	43.2 (11–97)	19 (11.2–84)	–

LRT, locoregional therapy; mTOR, mammalian target of rapamycin.

CNIs, compared with those on mTORi, had a similar proportion of patients who received induction therapy [17.3% (561/3227) vs. 20% (89/439), $P = 0.52$], with a shorter duration of steroids administration [median: 3 (range: 0–12) vs. 6 (range: 1–12) months], and a longer duration of follow-up [median: 43.2 (11–97) vs. 19 (11.2–84) months] (Table 2). Interestingly, mTORi, compared with CNIs, were associated with lower rates of HCC recurrence in both Asian [0% (0/28) vs. 18.8% (118/625), $P = 0.004$] and Caucasian patients [8.5% (35/411) vs. 12.7% (330/2602), $P = 0.02$]. However, although in RS/PS studies, the rates of HCC recurrence were lower in mTORs patients, compared with CNIs patients [9.5% (31/324) vs. 14.5% (439/3032), $P = 0.019$], this difference was not significant in RCT studies [3.47% (4/115) vs. 4.65% (9/195), $P = 0.77$]. Finally, based on the available data, among the patients with HCC recurrence, 81 patients died: 10 (37%) of the 27 mTORi-treated patients and 71 (41%) of the 172 CNIs-treated patients.

Based on the available data [15–17,21,30,32–34,39–41,46,49–52,54], among patients transplanted for HCC within Milan criteria, the rate of HCC recurrence was lower in patients on mTORi, compared with those on CNIs [3.8% (7/181) vs. 9.2% (71/788), $P = 0.03$). However, among patients transplanted for HCC outside Milan criteria, the rate of HCC recurrence was similar between the two groups [29.5% (15/51) vs. 29.2% (85/291), $P = 1.0$). Regarding administration of steroids, among patients who received steroids for ≤3 months after LT, the rate of HCC recurrence was similar between mTORi- and CNIs-treated patients [9.4% (11/116) vs. 13.5% (135/1000), $P = 0.28$). However, among recipients who received steroids for >3 months after LT, patients on mTORi had lower rates of HCC recurrence, compared with those on CNIs [4.8% (6/125) vs. 13.1% (144/1101), $P = 0.02$) [15,16,18–20,22–24,26–28,34–37,40,41,46–49,52,54–56]. Finally, no definite data could be extracted from the studies with mTORi regarding LRT, microvascular invasion, and induction therapy in association with recurrence of HCC.

Patients on mTORi immunosupression

Patients transplanted for HCC within Milan criteria had lower HCC recurrence, compared with those transplanted for HCC outside Milan criteria [total mTORi group: 7/181, 3.8% vs. 15/51, 29.5%, $P < 0.001$; sirolimus group: 5.6% (5/89) vs. 33% (11/33), $P = 0.0002$; everolimus group: 2.2% (2/92) vs. 22% (4/18), $P = 0.004$]. Based on the available data, the rate of HCC recurrence was similar between patients who received a combination of mTORi plus CNIs indefinitely with respect to those who received mTORi monotherapy (4/87 or 4.6% vs. 11/117 or 9.4%, $P = 0.29$). No data were available regarding the association of HCC recurrence and the mTORi dosage or blood levels. Finally, no difference in the rate of HCC recurrence was observed between patients who received steroids for ≤3 months, compared with those who received steroids for >3 months after LT [9.4% (11/116) vs. 4.8% (6/125), $P = 0.21$).

Sirolimus versus everolimus: Post-LT HCC recurrence was significantly more frequently observed in patients who received sirolimus than in those who received everolimus

Table 3. Characteristics of patients who underwent liver transplantation (LT) for hepatocellular carcinoma and received sirolimus or everolimus as immunosuppression regimen.

	Sirolimus	Everolimus	*P*
Before LT			
Within Milan criteria, *n/N* (%)	121/200 (60.5)	92/110 (84)	<0.001
Vascular microinvasion, *n/N* (%)	64/146 (44)	NA	–
LRT, *n/N* (%)	54/127 (42.5)	21/21 (100)	<0.001
After LT			
Induction therapy, *n/N* (%)	49/218 (22.4)	40/196 (20.4)	0.13
Duration of steroids, months, median (range)	3 (3–12)	6 (1–6)	–
Duration of follow-up, months, median (range)	30 (19–49)	13 (11.2–48)	–

NA, not available; LRT, locoregional therapy.

[10.5% (23/218) vs. 4.1% (8/196), $P = 0.02$] [16,26,28,30,32,36–42] (Fig. 3). However, the group of patients on sirolimus had lower rates of pretransplant HCC within Milan criteria compared with those on everolimus [60.5% (121/200) vs. 84% (92/110), $P < 0.001$] [16,30,32,37–41], and they had undergone LRT less frequently [42.5% (54/127) vs. 100% (21/21), $P < 0.001$] [37,39–41], but the latter was based on only one study with everolimus [41]. No definite conclusions could be drawn regarding microvascular invasion because there were no available data in the group of patients receiving everolimus. After LT, a similar proportion of patients received induction therapy whether sirolimus or everolimus were the mainstay of immunosuppressive therapy, [22.4% (49/218) vs. 20.4% (40/196), $P = 0.13$] [16,26,28,30,32,36–42], while sirolimus-treated patients had shorter duration of steroid administration [median: 3 (3–12) vs. 6 (1–6) months] [16,26,36,37,40,41] and longer post-LT duration of follow-up [median: 30 (19–49) vs. 13 (11.2–48) months] (Table 3).

Sirolimus versus CNIs: Patients on sirolimus had similar rates of HCC recurrence, compared with those on CNIs (10.5% vs. 13.8%, $P = 0.32$) (Fig. 3), but they had higher rates of pretransplant HCC outside Milan criteria (39.5% vs. 26%, $P = 0.001$) and microvascular invasion (44% vs. 22%, $P = 0.008$), while they had undergone LRT less frequently (42.5% vs. 64.5%, $P < 0.001$). Interestingly, among patients transplanted for HCC within Milan criteria, the rate of HCC recurrence was similar between sirolimus and CNIs groups of patients (5/89, 5.6% vs. 71/788, 9.2%, $P = 0.38$). After LT, the group of sirolimus-treated patients, compared with those on CNIs, had higher proportion of patients who received induction therapy [22.4% vs. 17.3%, $P = 0.02$] and with identical duration of steroid administration [median: 3 (3–12) vs. 3 (0–12) months].

Everolimus versus CNIs: Hepatocellular carcinoma recurrence was observed significantly less frequently in patients on everolimus than in patients on CNIs (4.1% vs. 13.8%, $P < 0.001$) (Fig. 3), but they had higher rates of HCC within Milan criteria (84% vs. 74%, $P = 0.01$), and they had undergone LRT more frequently (100% vs. 64.5%, $P < 0.001$), but the latter was based on only one study with everolimus [41]. Nevertheless, based on the available data, among patients whose tumor was within Milan criteria, the rates of HCC recurrence were lower in everolimus-treated patients, compared with CNIs-treated patients [2.2% (2/92) vs. 9.2% (71/788), $P = 0.02$), while the rates were similar in patients with HCC outside Milan criteria [22.2% (4/18) vs. 29.2% (85/291), $P = 0.63$]. Finally, after LT, both the group of patients on everolimus and those on CNIs had similar proportion of patients who received induction therapy (20.4% vs. 17.3%, $P = 0.5$), but the first group had, on average, a longer period of steroid administration [median: 6 (1–6) vs. 3 (0–12) months].

Discussion

The outcome of patients after LT has improved with the use of CNIs, but their administration is associated with several drawbacks including dose-dependent increased risk of renal insufficiency and HCC recurrence [2,4]. Particularly, high exposure to CNIs (mean trough concentrations of tacrolimus >10 ng/ml or cyclosporine >300 ng/ml) during the first postoperative period has been associated with increased risk of HCC recurrence [31]. The mTORi, such as sirolimus and everolimus, might represent an alternative immunosuppressive regimen, as they exhibit both immunosuppressive and renal-protective properties [68]. In addition, several *in vitro* and *in vivo* studies have shown that mTORi have multiple mechanisms of antitumor activity [68]. Practically, mTORi can be used alone or in combination with reduced CNIs dosage eliminating the preneoplastic effect of high exposure to CNIs.

Two recent meta-analyses [10,11] have shown that sirolimus is associated with significantly lower HCC recurrence rates, compared with CNIs. In the first meta-analysis [10] including three studies with 103 patients on sirolimus and 129 patients on CNIs-based immunosuppression, sirolimus was associated with a significantly lower risk of HCC recurrence (OR: 0.42, 95% CI: 0.21–0.83, $P = 0.01$). These results were confirmed in the more recent meta-analysis [11], in which 197 patients on sirolimus and 189 patients

on CNIs were analyzed. The authors found that patients on sirolimus had lower HCC recurrence rates, compared with patients on CNIs (OR: 0.30, 95% CI: 0.16–0.55, $P < 0.001$). However, in both meta-analyses [10,11], among the mTORi, only sirolimus was evaluated and no potential confounding factors (e.g., the proportion of patients with HCC meeting the Milan criteria or with microvascular invasion) were taken into account.

In this review, we systematically assessed the use of mTORi and CNIs regarding HCC recurrence after LT. Based on these data and such a univariate approach, mTORi (including both sirolimus and, for the first time, everolimus) were associated with lower rates of HCC recurrence after LT, compared with CNIs (35/439, 8% vs. 448/3227, 13.8%, $P < 0.001$). However, the comparison may not be straightforward because there were differences in the risk factors for HCC recurrence: patients treated with mTORi had been transplanted for HCC within Milan criteria in 69% of cases, significantly lower than the percentage observed in patients treated with CNIs, in whom 74% of cases had been transplanted for HCC within Milan criteria ($P = 0.04$). In addition, mTORi-treated patients presented higher rates of HCC with microvascular invasion [44% vs. 22%, $P < 0.001$] and had undergone LRT before LT (51% vs. 64.5%, $P = 0.0004$) less frequently. We understand that these data possibly reflect the tendency in daily clinical practice to use mTORi in patients at higher risk for developing HCC after LT. Thus, although patients on mTORi presented unfavorable HCC features more frequently compared with those under CNIs, they had lower rate of HCC recurrence after LT. However, we were not able to confirm this advantage of mTORi, compared with CNIs, in RCT studies [3.47% (4/115) vs. 4.65% (9/195), $P = 0.77$] (Table 1). Importantly, mTORi patients had numerically shorter follow-up, compared with CNIs patients (19 vs. 43.2 months). However, when only the studies with CNIs patients with less than 24 months of follow-up were included [16,26,28,30,33], the rates of HCC recurrence remained lower in mTORi-treated patients than CNIs-treated patients [35/439, 8% vs. 46/383, 12%, $P = 0.05$; median follow-up: 19 (11.2–84) vs. 14 (11–24) months].

It is well established that the Milan criteria are major determinants of the risk of recurrence of HCC in patients undergoing LT [12]. In this review, we confirmed the relevance of Milan criteria as prognostic factor of HCC recurrence after LT. In the present analysis, overall, HCC recurred more frequently in patients exceeding Milan criteria, compared with those within Milan criteria [29.2% (100/342) vs. 8.1% (78/969), $P < 0.001$], and this was seen also in the mTORi subgroup of patients (29.5% vs. 3.8%, $P < 0.001$). Interestingly, in patients with HCC within Milan criteria, the rates of HCC recurrence were lower in mTORi, compared with CNIs group of patients (3.8% vs. 9.2%, $P = 0.03$), but no difference was observed among patients who had HCC outside Milan criteria (29.5% vs. 29.2%, $P = 1.0$). Thus, it seems that patients transplanted for HCC within the Milan criteria who receive mTORi after LT experience the lowest rates of HCC recurrence. On the other hand, HCC outside the Milan criteria has high tendency of reappearance after LT and mTORi administration seems to have no 'beneficial' effect against HCC recurrence in these patients. According to these findings, mTORi should be the immunosuppression of choice particularly in patients with HCC within Milan criteria, while in patients with HCC exceeding the Milan criteria, at least from our analysis, using mTORi or CNIs as immunosuppression does not seem to have a different impact on HCC recurrence.

Overall, the rates of HCC recurrence were similar regardless of the duration of steroids administration (≤3 or >3 months) after LT (146/1116 or 13% vs. 150/1226 or 12.3%, respectively, $P = 0.32$). However, among patients who received steroids for >3 months after LT, the patients on mTORi had lower rates of HCC recurrence, compared with patients on CNIs (4.8% vs. 13.1%, $P = 0.02$). Thus, it could be proposed that steroids should not be given for more than 3 months in patients transplanted for HCC who receive a CNI-based immunosuppression regimen; otherwise, a mTOR-based regimen should be preferred.

The two previous meta-analyses had found that sirolimus was associated with lower rates of HCC recurrence, compared with CNIs [10,11]. However, it should be mentioned that in these meta-analyses, no other risk factors of HCC recurrence were evaluated [10,11]. In our systematic review, we found that patients on sirolimus had similar rates of HCC recurrence, compared with those on CNIs (10.5% vs. 13.8%, $P = 0.32$), but patients treated with sirolimus had, on average, poorer HCC-related prognostic factors, such as higher rates of HCC outside Milan criteria (39.5% vs. 26%, $P = 0.001$) and the presence of microvascular invasion (44% vs. 22%, $P = 0.008$). On the other hand, patients on everolimus had significantly lower recurrence rates of HCC, compared with those who received sirolimus or CNIs (4.1% vs. 10.5% vs. 13.8%, respectively, $P < 0.05$), but they had more frequently pretransplant HCC within Milan criteria (84% vs. 60.5% vs. 74%, respectively, $P < 0.05$) and shorter follow-up period (median: 13 vs. 30 vs. 43.2 months, respectively). However, when only the studies with CNIs-treated patients having less than 24 months of follow-up were included [16,26,28,30,33], the rates of HCC recurrence remained lower in everolimus-treated patients than CNIs-treated patients [(8/196), 4.1% vs. 46/383, 12%, $P = 0.0014$; median follow-up: 13 (11.2–48) vs. 14 (11–24) months]. Interestingly, based on the available data, among patients whose tumor bulk was within

Milan criteria, the rates of HCC recurrence were similar between sirolimus- and CNI- treated patients (5.6% vs. 9.2%, $P = 0.38$), but lower in everolimus-treated patients compared with CNI-treated patients (2.2% vs. 9.2%, $P = 0.02$). Thus, at least according to these findings, we are not able to conclude that sirolimus is superior to everolimus or vice versa to control HCC recurrence after LT. Again, it should be emphasized that everolimus-treated patients had shorter follow-up and more favorable prognostic factors, and thus, it cannot be suggested its superiority compared with the other immunosuppressive agents regarding HCC recurrence.

It has been debated whether the lower rates of HCC recurrence after LT under mTORi-based immunosuppression regimen is the direct result of their possible antiproliferative effect, or rather an indirect result of CNIs avoidance. Although a randomized study is needed to establish definite conclusions, we found that, based on the available data [16,28,32,36,38,40,41,43], among patients on mTORi, the rates of HCC recurrence were similar between patients who received a combination of mTORi plus CNIs indefinitely with respect to those who received mTORi monotherapy (4/87, 4.6% vs. 11/117, 9.4%, $P = 0.29$). Thus, based on the data available in literature, a direct antineoplastic activity of mTORi could be supported.

Our systematic review have some limitations including that few RCT studies were included, and factors, such as the dosage of immunosuppressive agents, could not be analyzed due to the lack of available data. However, it is the first systematic analysis of the literature data comparing mTORi (including both sirolimus and everolimus) and CNIs in a large number of patients with evaluation of particular characteristics associated with HCC recurrence. In conclusion, the present analysis favors the use of mTORi instead of CNIs to control HCC recurrence after LT in both Asians and Caucasians patients. However, this finding was not confirmed when only RCT studies were evaluated, and thus, the superiority of mTORi on HCC recurrence remains controversial. In addition, although everolimus was associated with negligible rates of HCC recurrence, everolimus-treated patients had significantly shorter follow-up and more favorable prognostic factors. Thus, longer follow-up is needed before any final conclusions can be reached, and comparative studies would help to identify which subgroups of patients may obtain the most benefit. Once more, despite the discussion on Milan-in–Milan-out criteria is still ongoing, the risk of HCC recurrence in patients transplanted for HCC within Milan criteria is surely low.

Authorship

EC and CM: performed the research. EC, KIRC and PB: wrote the paper. EC: analyzed the data.

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