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Reduced calcineurin inhibitor exposure with antibody induction and recurrent hepatocellular carcinoma after liver transplantation

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a common indication for liver transplantation (LT), but post-LT tumor recurrence remains a concern. Early post-LT immunosuppression is suggested to affect recurrence risk. We evaluated the impact on HCC recurrence of an immunosuppression protocol introduced in 2010 with interleukin-2 receptor antibody (IL-2RA) induction and delayed-introduction of reduced-dose tacrolimus with mycophenolate.

Methods: We included consecutive HCC patients transplanted 2000-2017 in Gothenburg. The impact on HCC recurrence of IL-2RA induction and mean tacrolimus trough concentration during the first 20 post-LT days was analyzed by multivariable Cox regression and propensity score-adjusted analyses.

Results: The study comprised 235 patients (mean age 57 yrs, men 80%, mean MELD 13, within Milan criteria 57%). The cumulative 5-yr HCC recurrence rate among patients transplanted before and after 2010 were 28.6% and 19.7%, respectively. IL-2RA induction had no independent effect on HCC recurrence. High tacrolimus exposure (mean 20-day tacrolimus concentration ≥8ng/mL) was associated with increased HCC recurrence risk on univariable analysis (HR 2.22, 95% CI 1.23-4.01, p=0.008), but was non-significant on multivariable analysis (p=0.17). Outside Milan criteria, high tacrolimus exposure was significant for HCC recurrence (HR 3.68, 95% CI 1.34-10.11, p=0.012) independently of tumor characteristics and AFP level. This was confirmed on multivariable propensity score-adjusted analysis.

Conclusions: Reduced early tacrolimus exposure, facilitated by IL-2RA induction, was associated with reduced risk for HCC recurrence among patients outside Milan criteria. Prospective studies are needed to confirm if early tacrolimus-minimization strategies can help reduce HCC recurrence rates and help extend transplant criteria.

Abbreviations

AFP, alpha-fetoprotein

ALT, alanine transaminase

AST, aspartate transaminase

CI, confidence interval

CIT, cold ischemia time

CNI, calcineurin inhibitor

HCC, hepatocellular carcinoma

HR, hazard ratio

IL-2RA, interleukin-2 receptor antibody

IQR, interquartile range

LRT, locoregional treatment

LT, liver transplantation

MELD, model for end-stage liver disease

MMF, mycophenolate mofetil

NLTR, Nordic liver transplantation registry

TAC, tacrolimus

TACE, transarterial chemoembolization

TC, trough concentration

UCSF, University of California at San Francisco

Introduction

Hepatocellular carcinoma (HCC) is a common indication for liver transplantation (LT) [1, 2]. Selection criteria for LT are traditionally based on tumor size and number of nodules, with the Milan and UCSF criteria being among the most common ones [3]. Post-LT tumor recurrence has been reported to affect less than 10% of patients within Milan criteria and up to 30% of patients exceeding the Milan criteria [4-6]. Additional predictors of recurrence include AFP, vascular invasion, and degree of tumor differentiation. The type of post-LT immunosuppression regimen may also affect recurrence risk [7], but the most optimal regimen remains undefined.

Reportedly, recurrent HCC has a more rapid and aggressive progression in immunosuppressed patients following LT compared to patients without immunosuppression [3, 8]. Proposed mechanisms of recurrence following LT include pre-existing occult metastases or intraoperative release of tumor cells into the circulation [9]. An intact immunity may have the ability to eliminate these tumor cells. However, under the strong immunosuppression immediately after LT, tumor cells may escape immune clearance, which might enable tumor cells to grow and invade tissues (micrometastasis) [9]. In line with this, Rodríguez-Perálvarez et al. found that calcineurin inhibitor (CNI) exposure during the first post-transplant month, but not later, was associated with post-LT HCC recurrence [10].

The use of interleukin-2 receptor antibody (IL-2RA) induction (basiliximab or daclizumab) is one of the strategies that has been used to enable early CNI minimization, i.e. delaying the introduction of CNI and in reducing doses. Basiliximab is a monoclonal antibody that targets the interleukin-2 receptor on activated T-lymphocytes. It is itself a potent immunosuppressant, but its

effect on post-LT HCC recurrence is unclear as studies are scarce and the findings contradictory [11, 12].

We retrospectively analyzed the effect of IL-2RA induction and early tacrolimus (TAC) exposure on the rates of post-LT HCC recurrence among patients, liver transplanted at a single center, before and after a change in immunosuppression protocol.

Materials and Methods

This is a retrospective single-center study of consecutive patients with HCC who underwent LT at Sahlgrenska University Hospital in Sweden during the years 2000-2017. Patients were identified from the hospital's surgical registry and the Nordic Liver Transplant Registry (NLTR). Inclusion criteria were a diagnosis of HCC as confirmed by histopathology of the explanted liver, and an age ≥ 18 years old. Exclusion criteria were cases without evidence of HCC in the explanted liver despite that they had not received locoregional therapy before LT (i.e. HCC misdiagnosis), combined hepatocellular cholangiocarcinoma, death within the first 2 months post-LT, or a switch to non-tacrolimus-based immunosuppression within the first month following LT.

Immunosuppression protocols

The immunosuppression protocol used before 2010 comprised TAC introduction immediately after LT with target trough concentration (TC) 10-15 ng/mL during the first two months post-LT, and 5-10 ng/mL thereafter. Prednisolone was initiated at 200 mg/day, and then tapered stepwise to 20 mg/day on post-operative day 4, and to 5 mg/day at 3 months. Mycophenolate mofetil

(MMF) was only added in case of impaired renal function or in case of graft rejection (30% and 17% were on MMF at 3 and 12 months, respectively).

Since 2010 the protocol consists of IL-2RA induction (basiliximab 20 mg intravenously) at the LT day and at post-operative day 4. All patients receive 1000 mg methylprednisolone intraoperatively. TAC is started on post-LT day 3 with a target TC of 5-8 ng/mL during the first 3 months, and (3-)5 ng/mL thereafter. MMF (1g twice daily) is given to all patients starting immediately post-operatively, but doses can be reduced in case of side-effects (97% and 79% were on MMF at 3 and 12 months, respectively). For patients with primary sclerosing cholangitis (PSC) or autoimmune hepatitis (AIH), the maintenance protocol also includes, in addition to the standard protocol, oral prednisolone initiated at 20 mg on post-operative day 1 and tapered stepwise to 5 mg at 3 months. All LT patients with HCC in their native liver undergo follow-up CT/MRI every 6 months up to 5 years post-LT.

Data collection procedures

Data were collected from hospital records, NLTR, pre-transplant radiology reports and from histology reports of the explanted livers. We collected data on tumor sizes, number of nodules, degree of differentiation according to the Edmondson-Steiner classification, presence of vascular invasion, tumor viability, and fulfillment of Milan (a single lesion ≤5 cm, up to three lesions ≤3 cm each, no evidence of vascular invasion, nor any regional nodal or extrahepatic metastases) and UCSF criteria (a single lesion ≤6.5 cm, up to three lesions ≤4.5 cm each with a total tumor burden of no more than 8 cm, with no evidence of vascular invasion, nor any regional nodal or extrahepatic metastases) [3]. For a tumor with 100% necrosis, tumor size was regarded as zero, in

line with guidelines [13]. We also recorded Child-Pugh score, etiology of cirrhosis, viral hepatitis status, and preoperative locoregional treatments (LRT), as well as all TAC TC values during the first 20 days post-LT and TAC TC at 3 and 12 months, and other relevant pre- and post-LT laboratory data including AFP. Study follow-up was until September 2018.

Statistical methods

Statistical analyses were made using SPSS version 25.0 (Chicago, USA), and R software version 3.6.0. Continuous variables were expressed as mean ± standard deviation, or as median with interquartile range (IQR) if non-parametric distribution. Differences in subgroups were compared using Student's *t*-test or ANOVA for continuous variables, Chi-square test or Fisher's exact test for categorical variables and Mann-Whitney's U-test for non-parametric variables. Kaplan-Meier plots with log-rank test and univariable and multivariable Cox regression analyses were used to evaluate the influence of immunosuppression regimen on recurrence-free survival (death independent of cause or HCC recurrence), overall survival and HCC-recurrence rate.

Immunosuppression regimen was analyzed by comparing the protocols with or without IL2-RA (IL-2RA vs no IL-2RA), and by TAC TC during the first 20 post-LT days. We stratified early TAC exposure into 2 subgroups: those with mean TAC TC <8 ng/mL during the first 20 days (low TAC exposure) and those with mean TAC TC ≥8 ng/mL (high TAC exposure). This cut-off was based on the median TAC TC during the first 20 days for the entire cohort (8 ng/mL) and was also the level that best separated the groups with or without IL-2RA induction from each other.

Two sets of multivariable-adjusted analyses were performed. First, the effect of IL-2RA induction and early TAC exposure were adjusted for covariates reflecting tumor characteristics known to be associated with post-LT HCC recurrence according to the literature [3, 14], these consisted of AFP, size of largest tumor in cm, number of tumors, and tumor differentiation (grade 1-2, grade 3-4, unknown). Second, a propensity score was calculated for each patient, based on tumor characteristics and other clinical variables with a P-value <0.1 in univariable Cox regression analyses with HCC recurrence as the outcome variable. The effect of TAC exposure and IL-2RA was then adjusted for this propensity score in multivariable Cox regression analyses. Subgroup analyses were performed for patients within and outside Milan and UCSF criteria respectively. P-values <0.05 were considered statistically significant.

The study received approval from the regional ethical review board in Gothenburg (diary number 934-14).

Results

Out of 258 patients fulfilling study inclusion criteria, 23 were excluded for the following reasons: six with mixed cancers in the explanted liver, two with intra-operative death, nine with death within 2 months post-LT, and six who were switched to non-TAC-based immunosuppression within the first post-LT month. After exclusions, 235 patients were ultimately analyzed.

Mean age was 57 years with a male predominance (80%), and mean MELD score was 13 (Table 1). The main etiologies for underlying parenchymal disease were hepatitis C (58%), alcoholic liver disease (30%), and hepatitis B (11%).

The no-IL-2RA group consisted of 92 patients (39%) transplanted between 2000 and 2009, and the IL-2RA group of 143 patients (61%) transplanted 2010-2017. Compared to the no-IL-2RA group, patients in the IL-2RA group were slightly older, had lower Child Pugh class, had more often undergone pre-transplant locoregional HCC treatments (mainly TACE), had shorter CITs, and lower AFP levels (Table 1). In addition, donor age was higher and wait-list times longer in the IL-2RA group. With regard to tumor characteristics, degree of differentiation was higher, whereas differences in other tumor characteristics were non-significant (Table 2). However, there were 66% missing values on tumor differentiation in the no-IL-2RA group, compared to 8% in the IL-2RA group. Of HCV-antibody positive patients in the no-IL-2RA group, 3 (6%) were HCV-RNA negative at LT, 15 (29%) were HCV-RNA positive, while HCV-RNA status was missing for 33 (65%) patients. Respective figures in the IL-2RA group were 28 (33%), 12 (14%), and 45 (53%).

During a median follow-up of 55 months (range 2.6-216.9), there were 53 cases of HCC recurrence; 27 in the no-IL-2RA group and 26 in the IL-2RA group. Cumulative incidence of HCC recurrence was 8.1% at 1 year and 20.9% at 5 years post-LT. Corresponding figures in the no-IL-2RA group were 10.9% and 28%, and in the IL-2RA group, 7.1% and 19.6% (Figure 1). Among cases of tumor recurrence, 36% occurred within 1 year post-LT, and 83% within 3 years post-LT. Recurrence-free survival in the IL-2RA and no-IL-2RA groups were 91.6% and 87% at 1 year, 71.3% and 64.1% at 5 years, and 60% and 54.4% at 8 years, respectively (Figure 1).

The mean number of TAC TC measurements during the first 20 post-LT days were 9.8 ± 4.5 with the median trough level for the entire cohort being 8 ng/mL (IQR 6.5-10.4). In the no-IL-2RA

group, the median tacrolimus level was 10.6 ng/mL (IQR 8.9-11.9) compared to 7.0 ng/mL (IQR 5.7-8.2) in the IL-2RA group ($p < 0.001$). Supplementary figure 1 shows the median tacrolimus levels over different time periods of LT.

In the no-IL-2RA group, the median TAC TC at 3 months and 12 months post-LT were 10 and 7.3 ng/mL respectively. For the IL-2RA group, these numbers were 6.8 and 5.6 ng/mL. Of these follow-up TAC TCs, a large number of data were missing (33.7% at 3 months and 39.2% at 12 months). Neither the TAC levels at 3 months nor at 12 months post-LT had a significant impact on HCC recurrence (Supplementary table 1).

We divided the cohort into high and low TAC exposure groups based on the mean TAC TC during the first 20-days post-LT, 8 ng/mL. Of patients in the no-IL-2RA group, 86% also belonged to the high TAC exposure group, compared to 29% of patients in the IL-2RA group. Compared to patients with low TAC exposure, those with high TAC exposure had less frequently undergone pre-transplant LRT, had longer CITs, shorter wait-list times, higher AFP levels (22 vs 8 ng/mL), but were similar on other baseline characteristics (Supplementary table 2). There was no difference in ALT or AST levels in the first week post-LT between patients with high or low TAC exposure (Supplementary table 2). High TAC exposure was significantly associated with HCC recurrence and recurrence-free survival (Figure 2). Especially early HCC recurrence (<1 year) was related to TAC exposure, as only 4 patients (4 %) with low TAC exposure suffered recurrence within the first year, compared to 15 patients (13 %) with high TAC exposure ($p = 0.02$). In contrast, HCC recurrence diagnosed later than 3 years after LT was reported in 4 patients in each group (4 % vs 3 %, $p = 1.0$) (Supplementary figure 2).

On univariable Cox regression analysis, mean 20-day TAC TC (HR 1.09, 95% CI 1.00-1.19; p = 0.046) and high TAC exposure (HR 2.22, 95% CI: 1.23-4.01, p = 0.008) were associated with increased HCC recurrence risk, whereas IL-2RA use was not (HR 0.69, 95% CI 0.40-1.19, p = 0.186) (Table 2). The relationship between TAC TC during the first 20 days and HCC recurrence is visualized in Figure 3.

Other factors with a P-value <0.1 for HCC recurrence on univariable Cox regression analyses were diameter of the largest nodule, number of nodules, tumor differentiation grade, vascular invasion, AFP level, Child Pugh score, alcohol etiology, pre-transplant locoregional HCC treatments, and CIT (Supplementary table 2). These covariates were included in the propensity score. In addition, nonfulfillment of Milan and UCSF criteria was associated with HCC recurrence (Supplementary table 2).

In Cox regression analyses adjusted for diameter of largest nodule, nodule number, vascular invasion, AFP level, and differentiation, neither high TAC exposure nor IL-2RA induction had statistically significant impact on HCC recurrence (Table 2). The same was true in propensity score-adjusted analyses (Table 2).

Subgroup analyses by Milan and UCSF criteria

At the time of LT, 133 patients (57%) were within Milan criteria and 159 (68%) were within UCSF criteria. Five patients were excluded due to incomplete data on tumor burden. The 1- and 5-year cumulative HCC recurrence rates were 17% and 37% outside Milan criteria and 3% and

13% within Milan criteria. Patients within and outside Milan criteria had comparable TAC TC (median 7.8 vs 7.8 ng/mL). TAC exposure was significantly associated with increased rate of HCC recurrence and decreased recurrence-free survival among patients outside Milan criteria, but not among those within (Table 3 and Figure 4). This increased risk for HCC recurrence in patients outside Milan with high TAC exposure was confirmed in multivariable analyses and propensity-score-adjusted analyses (Table 3). The effect of IL-2RA was non-significant (Table 3).

A similar trend was seen when stratifying patients according to UCSF criteria, with a HR of 9.69 (95% CI 1.90-50.19, p = 0.007) for HCC recurrence among patients outside UCSF criteria with high TAC exposure (Supplementary table 3 and Supplementary figure 3).

Furthermore, among 41 patients HCV-antibody negative at LT and outside Milan criteria, high TAC exposure was associated with HCC recurrence (HR 8.32, 95% CI 1.52-45.67, P=0.01), while IL-RA was not (HR 1.11, 95% CI 0.32-3.88, P=0.87).

Discussion

In this retrospective single-center study, we found that high TAC exposure within the first 3 weeks post-LT was independently associated with increased HCC recurrence risk among patients outside Milan criteria. The immunosuppression protocol at our center was changed in 2010 from standard-dose TAC and steroids to a steroid-free protocol including IL-2RA induction and MMF with a minimization of early TAC exposure. This early TAC minimization seemed beneficial

with regard to the risk for HCC recurrence, whereas IL-2RA induction itself showed no detrimental effects for HCC recurrence.

Our findings are consistent with those of Rodríguez-Perálvarez et al. [10], who showed that high CNI exposure (>10 ng/mL for TAC or >300 ng/mL for cyclosporine) in the immediate post-LT period was related to a 3-fold risk of HCC recurrence. However, we did not see this effect in the overall population, only among patients outside Milan or UCSF criteria. As in the above-mentioned study [10], we found no association between TAC level at 3 months or 12 months post-LT and HCC recurrence, indicating that it is the very early CNI exposure that might be critical for HCC recurrence. We further show that IL-2RA induction with routine MMF is a feasible strategy to achieve a minimization of very early TAC exposure.

One third of all HCC recurrences occurred within the first post-LT year. Proposed mechanisms for this early recurrence include undetected pre-existing micrometastases or intraoperative release of tumor cells into the circulation [7, 9, 15]. An intact immunity may eliminate such tumor cells. However, the strong immunosuppression immediately after LT impairs this immune-mediated tumor cell clearance, thus enabling tumor cell growth and tissue invasion [9]. The importance of these effects would expectedly increase with increased hepatic tumor burden, but this remains to be shown. In line with this hypothesis, we found that early TAC exposure emerged as important especially for early (<1 year) HCC recurrence, and in HCC with high tumor burden outside Milan or UCSF criteria.

TAC has been reported to promote growth of HCC cells in vitro [16], possibly related to increased cyclin-dependent kinase 4 activity [17]. Several clinical studies have reported an association between CNI exposure and HCC recurrence risk after LT [10, 18, 19].

Although IL-2RA induction enables minimization of early CNI exposure, IL-2RA itself is a potent immunosuppressant. IL-2RA eliminates all CD25+ cells including tumor-specific effector cytotoxic T-cells [8, 20], and there is concern that this may counteract the benefits from CNI minimization with regard to HCC recurrence.

IL-2RA induction therapy with delayed and reduced-dose CNI after LT has previously been studied mainly as a renal-sparing measure, where trials have confirmed its antirejection efficacy and safety [20]. We have also previously reported that rejection rates were similar before and after introduction of the new immunosuppression protocol [21].

No trial has specifically addressed IL-2RA induction with CNI minimization for HCC recurrence. Observational studies have yielded conflicting results, but IL-2RA use was not systematic and the choice to use IL-2RA was often poorly defined in these studies. In a retrospective single-center study of 93 patients (43 receiving IL-2RA), Lee et al. [11] reported increased risk for HCC recurrence with IL-2RA induction. However, IL-2RA was used by clinician discretion, there was an imbalance in clinical characteristics between the groups, it was unclear whether IL-2RA use was accompanied by CNI minimization, and CNI exposure was not adjusted for in this specific analysis. In contrast, in a large multivariable analysis of the Scientific Registry of Transplant Recipients, IL-2RA induction was independently associated with improved survival after

transplantation for HCC, although there were no specific data on HCC recurrence or early CNI trough levels [22]. A strength of our study compared to the studies above is that IL-2RA use was systematic due to a change in center protocol, and we were able to analyze combined effects of IL-2RA and CNI exposure.

Our modern immunosuppression protocol included routine MMF to all patients. MMF has been shown to inhibit cancer cell proliferation in vitro [23-25], but clinical evidence is lacking. No independent effect of MMF on HCC recurrence was observed in the study by Rodríguez-Perálvarez et al. [10]. However, further research is needed to analyze whether MMF use benefits in terms of HCC recurrence independent of TAC minimization.

Limitations of our study include the retrospective design. Multivariable and propensity-score adjusted analyses were unable to fully eliminate possible confounding due to different time periods. Specifically, temporal improvements in radiologic and pathologic diagnostics might cause confounding. Also, the selection criteria for LT in HCC were more liberal in the older cohort, and the use of pre-transplant LRT has become more common in the recent era. Additionally, there were many missing data especially for tumor differentiation grade among the cases transplanted in the earlier study period. Finally, there has also been a general improvement in treatment results over time. Therefore, residual and unmeasured confounding still likely remains, and prospective studies are needed to confirm our findings. Although the study comprised 235 patients, statistical power was restricted in subgroup analyses by Milan and UCSF criteria. Nonetheless, the proportion of HCC outside Milan/UCSF criteria was higher than at many centers, and this is a considered a strength of our study.

In conclusion, reduced TAC exposure during the first 3 weeks after LT with the help of IL-2RA induction and routine MMF use was associated with reduced HCC recurrence among patients with HCC outside Milan criteria. If these findings are confirmed in prospective trials, tailoring immunosuppression may become one strategy to improve outcomes and extend current transplantation criteria in HCC.

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Table 1. Descriptive data of study patients with hepatocellular carcinoma who underwent liver transplantation 2000-2009 (no IL-2RA) or 2010-2017 (IL-2RA). Tumor characteristics as derived from histology reports of the explanted liver. Continuous variables are expressed as mean ± standard deviation, categorical variables as number (%), and asymmetric distribution as median [interquartile range].

Variables	Overall (n=235)	No IL-2RA (n=92)	IL-2RA (n=143)	*p*-value
Age (years)	57 ± 8.3	54.7 ± 8.7	58.4 ± 7.7	0.001
Sex				1.00
Female	46 (19.6%)	18 (19.6%)	28 (19.6%)	
Male	189 (80.4%)	74 (80.4%)	115 (80.4%)	
Recipient BMI	27.7 ± 4.7	27.7 ± 4.7	27.8 ± 4.7	0.861
Cirrhosis				0.016
No cirrhosis	11 (4.7%)	9 (9.8%)	2 (1.4%)	
Child-Pugh A	104 (44.8%)	36 (39.1%)	68 (48.2%)	
Child-Pugh B	75 (32.3%)	26 (28.3%)	49 (34.8%)	
Child-Pugh C	43 (18.5%)	21 (22.8%)	22 (15.6 %)	
MELD score	11.9 [8.7-15.5]	12.2 [9.4-16]	11.4 [8.5-15.2]	0.152
ALT_{max1w}	14 [7.4-25]	15 [10-21]	13 [6.1-26]	0.386
AST_{max1w}	19 [9-33]	20 [8.7-32]	16 [9.1-35.5]	0.971
Hepatitis C	136 (57.9%)	51 (55.4%)	85 (59.4%)	0.589
Hepatitis B	26 (11.1%)	13 (14.1%)	13 (9.1%)	0.287
Alcoholic liver disease	70 (29.8%)	24 (26.1%)	46 (32.2%)	0.381
Cold ischemia time (hours)	8.0 ± 2.6	8.1 ± 2.5	8.0 ± 2.7	0.678
Donor age (years)	56 ± 15.9	52.3 ± 14.3	58 ± 16.5	0.014
Donor BMI	24.9 [23.1-27.8]	24.7 [22.7-26.3]	25.2 [23.2-28.8]	0.060
Diameter of largest nodule (mm)	32 ± 20.3	35.4 ± 23.5	30 ± 17.9	0.054
Number of nodules	2.46 ± 2.34	2.33 ± 1.83	2.54 ± 2.6	0.537
Number of nodules (categorical)				0.670
Uninodular	95 (41.1%)	39 (43.8%)	56 (39.4%)	
2-3 nodules	78 (33.8%)	27 (30.3%)	51 (35.9%)	
>3 nodules	58 (25.1%)	23 (25.8%)	35 (24.6%)	
Milan criteria* (within)	133 (57.8%)	53 (60.9%)	80 (55.9%)	0.493
UCSF criteria (within)**	159 (69.1%)	60 (69.0%)	99 (69.2%)	1
Vascular invasion				1.000

Yes	80 (42.1%)	25 (42.4%)	55 (42%)	
No	110 (57.9%)	34 (57.6%)	76 (58%)	
Degree of differentiation:				<0.001
Grade 1-2	66 (28.1%)	21 (22.9%)	45 (31.5%)	
Grade 3-4	97 (41.3%)	10 (10.9%)	87 (60.8%)	
Unknown	72 (30.6%)	61 (66.3%)	11 (7.7%)	
Pre-transplant LRT	82 (35.2%)	7 (7.7%)	75 (52.8%)	<0.001
Recurrence of HCC	53 (22.6%)	27 (29.3%)	26 (18.2%)	0.073
Within 1 year	20 (8.5%)	11 (12%)	9 (6.3%)	
1-3 years	24 (10.2%)	12 (13%)	12 (8.4 %)	
3-5 years/>5 years	5 (2.1%)/4 (1.7%)	2 (2.2%)/2 (2.2%)	3 (2.1%)/2 (1.4%)	
High TAC exposure	118 (50.6%)	77 (85.6%)	41 (28.7%)	

BMI = body mass index, ALT_{max1w} = maximum value of alanine transaminase in the first week post-LT, AST_{max1w} = maximum value of aspartate transaminase in the first week post-LT, MELD = model for end-stage liver disease, LRT = locoregional treatment, HCC = hepatocellular carcinoma, AFP = alpha-fetoprotein, LT = liver transplantation, UCSF = University of California at San Francisco, IQR = interquartile range, IL-2RA, interleukin-2 receptor antibody, TAC = tacrolimus

Table 2. The association between early immunosuppression and recurrence of hepatocellular carcinoma after liver transplantation by Cox regression analysis.

Variables	HR	95% CI	p-value
UNIVARIABLE			
Mean 20-day tacrolimus TC (ng/mL)	1.09	1.00-1.19	0.046
IL-2RA induction	0.69	0.40-1.19	0.186
High tacrolimus exposure (TC ≥8 ng/mL)	2.22	1.23-4.01	0.008
MULTIVARIABLE – confounder adjusted*			
High tacrolimus exposure (TC ≥8 ng/mL)	1.75	0.79-3.86	0.167
IL-2RA induction	2.48	0.83-7.42	0.106
MULTIVARIABLE – propensity score-adjusted **			
High tacrolimus exposure (TC ≥8 ng/mL)	1.39	0.68-2.84	0.369
IL-2RA induction	1.22	0.55-2.69	0.628

HR = hazard ratio, CI = confidence interval, TC = trough concentration, AFP = alpha-feto protein, LT = liver transplantation, IL-2RA = interleukin-2 receptor antibody

* Adjusted for diameter of largest nodule, number of nodules, vascular invasion (yes/no/unknown), degree of differentiation (grade 1-2/3-4/unknown), AFP level pre-LT

** Adjusted for diameter of the largest nodule, number of nodules, tumor differentiation grade, vascular invasion, AFP, Child Pugh score, alcohol etiology, preoperative locoregional HCC treatments, and cold ischemia time

Table 3. Univariable and multivariable Cox regression analyses on the association between early immunosuppression and recurrence of hepatocellular carcinoma after liver transplantation, after stratifying cases according to Milan criteria. Tumor characteristics as derived from histology reports from the explanted liver.

	Within Milan			**Outside Milan**		
Variables	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
UNIVARIABLE						
Mean 20-day tacrolimus TC (ng/mL)	1.01	0.86-1.19	0.922	1.23	1.09-1.40	**0.001**
High tacrolimus exposure (TC ≥8 ng/mL)	1.25	0.46-3.37	0.658	3.35	1.56-7.16	**0.002**
IL-2RA induction	0.76	0.28-2.05	0.586	0.56	0.29-1.10	0.092
MULTIVARIABLE – confounder adjusted*						
Tacrolimus exposure without adjustment for IL-2RA						
High tacrolimus exposure (TC ≥8 ng/mL)	0.22	0.03-1.44	0.114	3.50	1.28-9.56	**0.014**
IL-2RA without adjustment for tacrolimus exposure						
IL-2RA induction	6.28	0.86-45.81	0.070	1.19	0.27-5.17	0.816
Tacrolimus and IL-2RA						
High tacrolimus exposure (TC ≥8 ng/mL)	0.43	0.06-2.94	0.390	3.68	1.34-10.11	**0.012**
IL-2RA induction	4.06	0.46-35.61	0.206	1.67	0.36-7.79	0.514
MULTIVARIABLE – propensity score-adjusted**						
Tacrolimus without adjustment for IL-2RA						
High tacrolimus exposure (TC ≥8 ng/mL)	0.51	0.14-1.83	0.304	2.67	1.11-6.43	**0.029**
IL-2RA without adjustment for tacrolimus exposure						
IL-2RA induction	1.30	0.34-0.98	0.699	0.82	0.28-2.37	0.716
Tacrolimus and IL-2RA						
High tacrolimus exposure (TC ≥8 ng/mL)	0.49	0.11-2.12	0.340	2.75	1.12-6.77	**0.028**
IL-2RA induction	0.92	0.22-3.91	0.906	1.16	0.40-3.39	0.790

HR = hazard ratio, CI = confidence interval, AFP = alpha-fetoprotein, LT = liver transplantation, TC = trough concentration, IL-2RA = interleukin-2 receptor antibody

* Adjusted for: size of largest nodule, number of nodules, vascular invasion (yes/no/unknown), degree of differentiation (grade 1-2/3-4/unknown), level of AFP pre-LT

** Adjusted for diameter of the largest nodule, number of nodules, tumor differentiation grade, vascular invasion, AFP, Child Pugh score, alcohol etiology, preoperative locoregional HCC treatments, and cold ischemia time

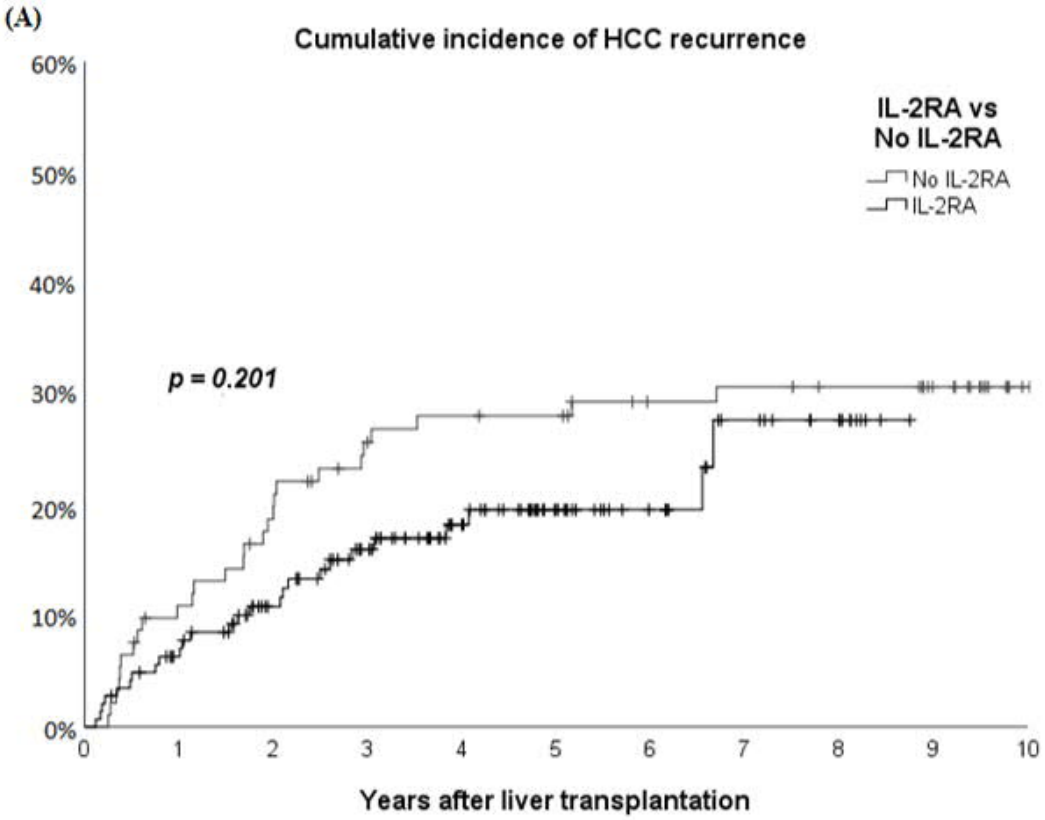
Figure Legends

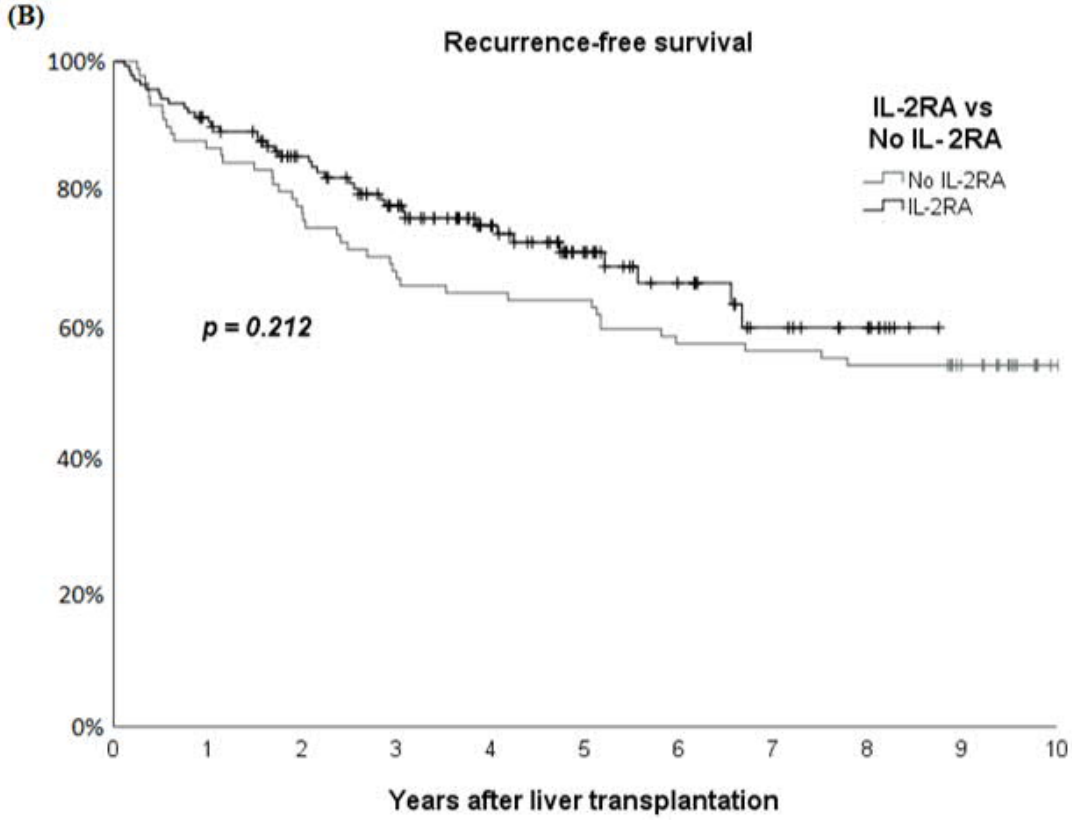
Figure 1: Cumulative incidence of hepatocellular carcinoma (HCC) recurrence (A) and recurrence-free survival (B) after liver transplantation according to interleukin-2 receptor antibody (IL-2RA) induction (yes or no).

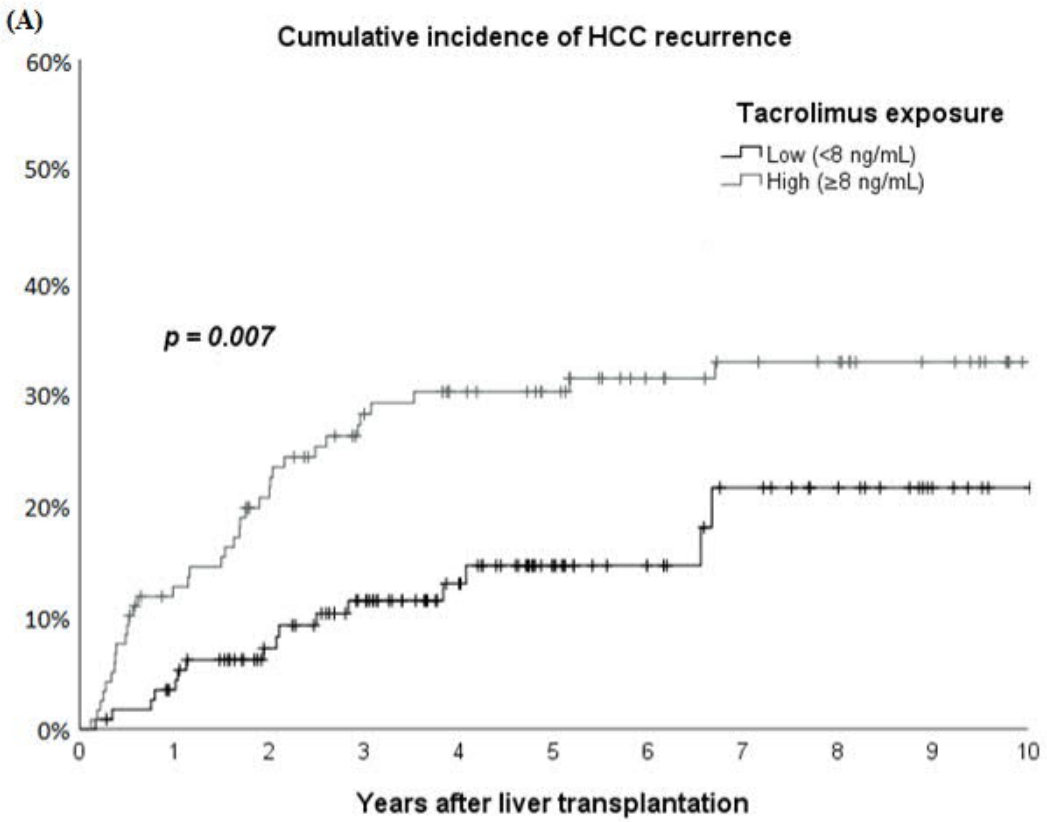
Figure 2: Cumulative incidence of hepatocellular carcinoma (HCC) recurrence (A) and recurrence-free survival (B) according to low or high tacrolimus exposures (mean tacrolimus trough concentration cutoff 8 ng/mL of all values available during the first 20 days after liver transplantation).

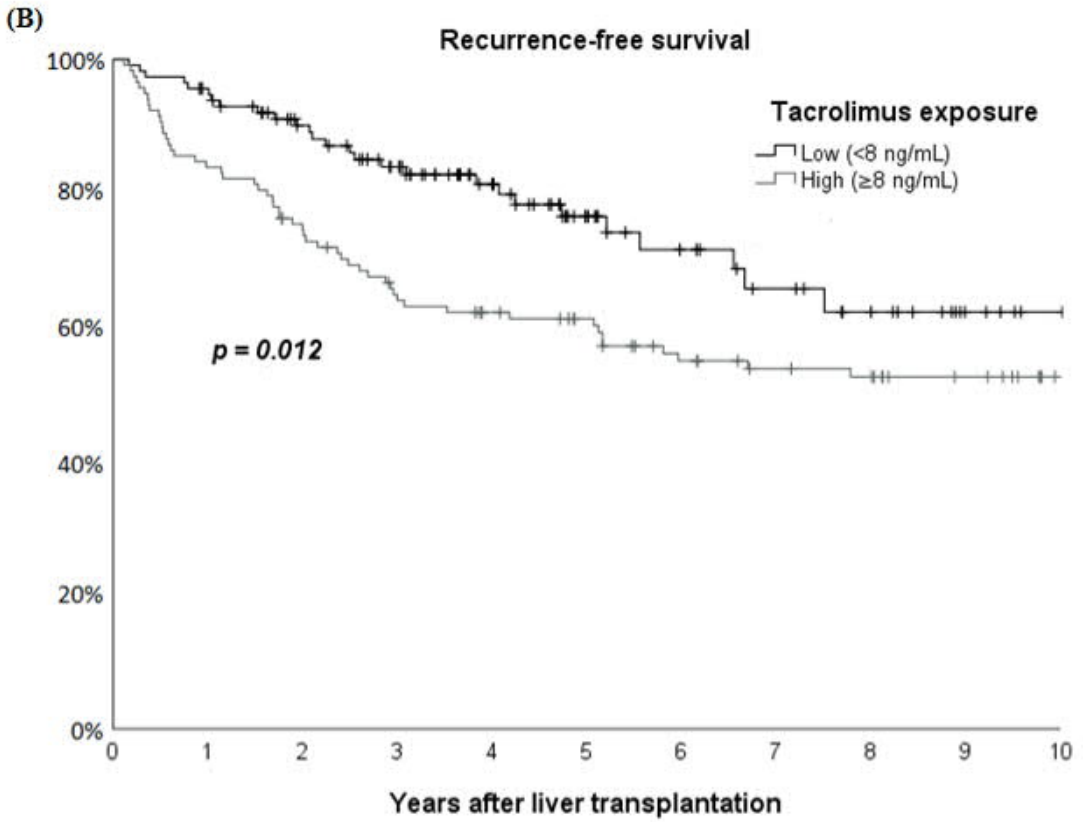
Figure 3: The relationship between mean tacrolimus trough concentration during the first 20 days after liver transplantation and recurrence of hepatocellular carcinoma (A) by Cox regression analysis, and as stratified by the use of IL-2RA (B).

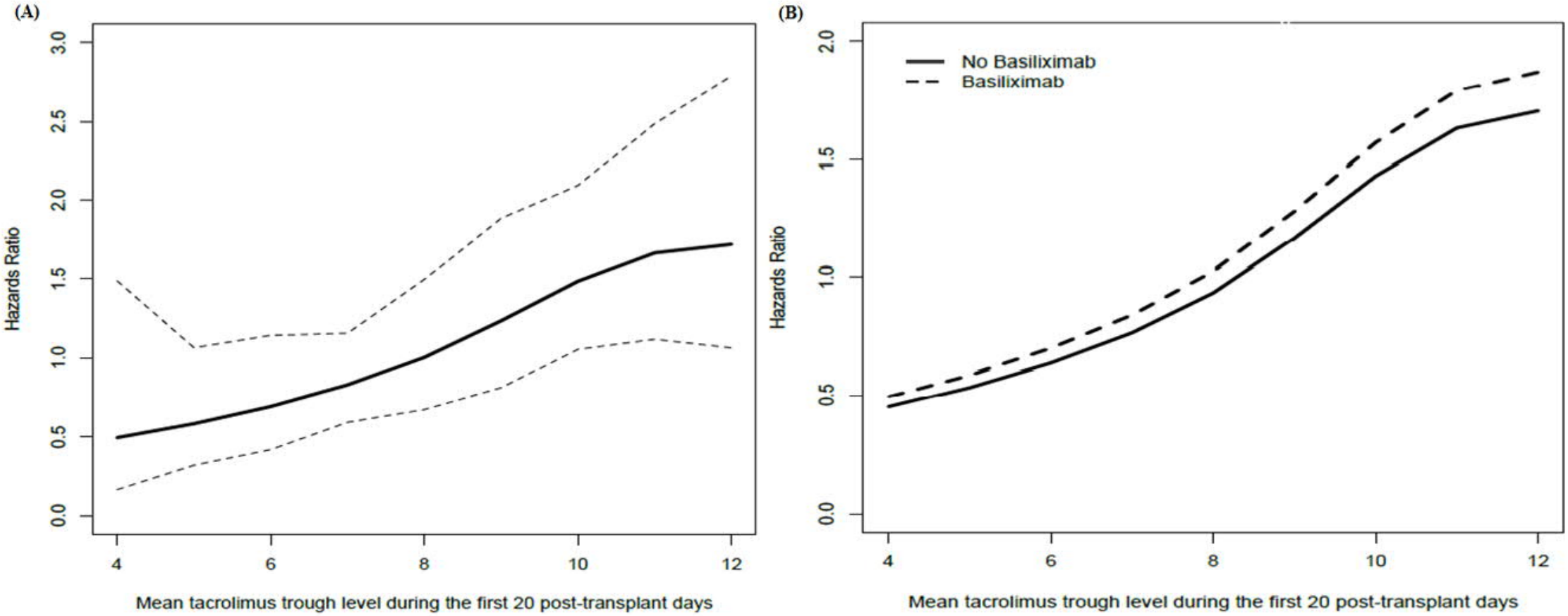
Figure 4: Cumulative incidence of hepatocellular carcinoma (HCC) recurrence (A and B) and recurrence-free survival (C and D) according to early tacrolimus exposure, stratified by fulfillment of Milan criteria at transplantation.

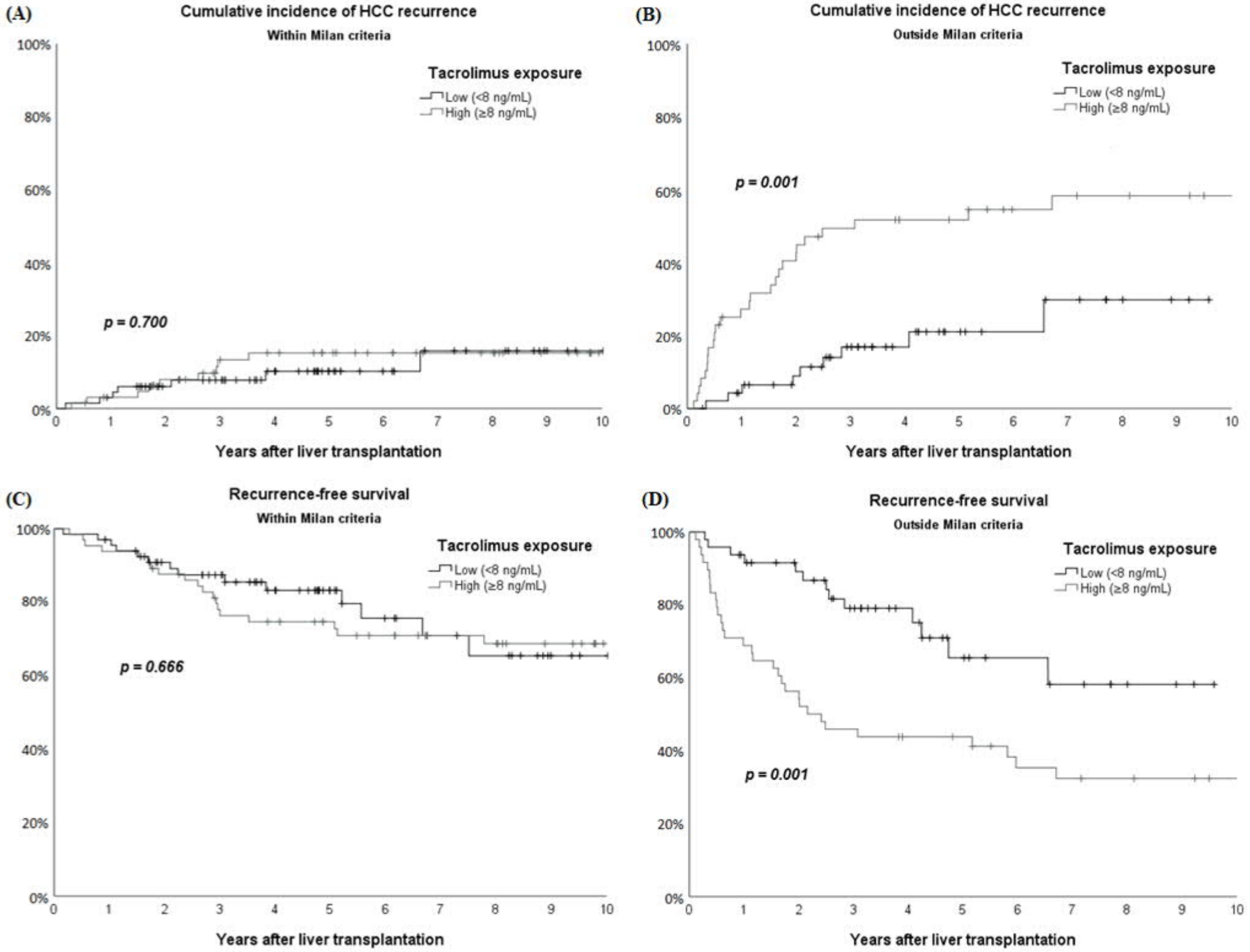

(A)
Cumulative incidence of HCC recurrence
60%
50%
40%
30%
20%
10%
0%
IL-2RA vs
No IL-2RA
No IL-2RA
IL-2RA
p = 0.201
0
1
2
3
4
5
6
7
8
9
10
Years after liver transplantation


(B)
Recurrence-free survival
100%
80%
60%
40%
20%
0%
IL-2RA vs
No IL- 2RA
No IL-2RA
IL-2RA
p = 0.212
0
1
2
3
4
5
6
7
8
9
10
Years after liver transplantation


(A)
Cumulative incidence of HCC recurrence
Tacrolimus exposure
Low (<8 ng/mL)
High (≥8 ng/mL)
p = 0.007
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
9
10
Years after liver transplantation


(B)
Recurrence-free survival
Tacrolimus exposure
Low (<8 ng/mL)
High (≥8 ng/mL)
p = 0.012
100%
80%
60%
40%
20%
0%
0
1
2
3
4
5
6
7
8
9
10
Years after liver transplantation


(A)
Hazards Ratio
0.0
0.5
1.0
1.5
2.0
2.5
3.0
4
6
8
10
12
Mean tacrolimus trough level during the first 20 post-transplant days
(B)
Hazards Ratio
0.0
0.5
1.0
1.5
2.0
No Basiliximab
Basiliximab
4
6
8
10
12
Mean tacrolimus trough level during the first 20 post-transplant days


(A)
Cumulative incidence of HCC recurrence
Within Milan criteria
Tacrolimus exposure
Low (<8 ng/mL)
High (≥8 ng/mL)
p = 0.700
100%
80%
60%
40%
20%
0%
0 1 2 3 4 5 6 7 8 9 10
Years after liver transplantation
(B)
Cumulative incidence of HCC recurrence
Outside Milan criteria
Tacrolimus exposure
Low (<8 ng/mL)
High (≥8 ng/mL)
p = 0.001
100%
80%
60%
40%
20%
0%
0 1 2 3 4 5 6 7 8 9 10
Years after liver transplantation
(C)
Recurrence-free survival
Within Milan criteria
Tacrolimus exposure
Low (<8 ng/mL)
High (≥8 ng/mL)
p = 0.666
100%
80%
60%
40%
20%
0%
0 1 2 3 4 5 6 7 8 9 10
Years after liver transplantation
(D)
Recurrence-free survival
Outside Milan criteria
Tacrolimus exposure
Low (<8 ng/mL)
High (≥8 ng/mL)
p = 0.001
100%
80%
60%
40%
20%
0%
0 1 2 3 4 5 6 7 8 9 10
Years after liver transplantation