UCLA
UCLA Previously Published Works

Title

Evaluation of Patients With Hepatocellular Carcinomas That Do Not Produce α-Fetoprotein

Permalink

https://escholarship.org/uc/item/2qk64422

Journal

JAMA Surgery, 152(1)

ISSN

2168-6254

Authors

Agopian, Vatche G
Harlander-Locke, Michael P
Markovic, Daniela
et al.

Publication Date

2017

DOI

10.1001/jamasurg.2016.3310

Peer reviewed

Research

JAMA Surgery | Original Investigation | PACIFIC COAST SURGICAL ASSOCIATION

Evaluation of Patients With Hepatocellular Carcinomas That Do Not Produce α-Fetoprotein

Vatche G. Agopian, MD; Michael P. Harlander-Locke, MPH; Daniela Markovic, MS; Ali Zarrinpar, MD, PhD; Fady M. Kaldas, MD; Elaine Y. Cheng, MD; Hasan Yersiz, MD; Douglas G. Farmer, MD; Jonathan R. Hiatt, MD; Ronald W. Busuttil, MD, PhD

← **Invited Commentary**

✛ **Supplemental content**

IMPORTANCE Serum α-fetoprotein (AFP) is a biomarker for hepatocellular carcinomas (HCCs) associated with a more aggressive tumor phenotype and inferior outcomes after a liver transplant (LT). Data on the outcomes for patients with HCCs that do not produce AFP are limited.

OBJECTIVE To compare characteristics and outcomes among LT recipients with radiographically apparent HCC lesions with AFP-producing tumors or with tumors that do not produce AFP (hereafter referred to as non-AFP–producing tumors), and to identify factors influencing recurrence in LT recipients with non-AFP–producing tumors.

DESIGN, SETTING, AND PARTICIPANTS Retrospective analysis at a university transplant center of 665 adults with HCC who underwent an LT during the period from 1989 to 2013. Of the 665 LT recipients, 457 (68.7%) had AFP-producing tumors, and 208 (31.3%) had non-AFP–producing tumors (the maximum AFP level before an LT was ≤10 ng/mL). Dates of study analysis were from August 2015 to June 2016.

INTERVENTION Liver transplant.

MAIN OUTCOMES AND MEASURES Recurrence-free survival and recurrence rates.

RESULTS Patients with non-AFP–producing tumors had radiographic tumor characteristics similar to those of patients with AFP-producing tumors, but, pathologically, they had fewer lesions (25% vs 35% with >2 lesions; P = .03), smaller cumulative tumor diameters (4.2 vs 5.0 cm; P = .02), fewer microvascular (17% vs 22%) and macrovascular (2% vs 9%) invasions (P < .001), and fewer poorly differentiated tumors (15% vs 28%; P < .001). Patients with non-AFP–producing tumors also had significantly superior recurrence-free survival at 1, 3, and 5 years (88%, 74%, and 67% vs 76%, 59%, and 51%, respectively; P = .002) and lower 5-year recurrence rates (8.8% vs 22%; P < .001) than patients with AFP-producing tumors. When stratified by radiologic Milan criteria, 5-year survival was better, and recurrence lowest, among patients with non-AFP–producing tumors within the Milan criteria (71% survival and 6% recurrence), and survival was worse, and recurrence highest, for patients with AFP-producing tumors outside the Milan criteria (40% survival and 42% recurrence; P < .001). Significant predictors of recurrence among patients with non-AFP–producing tumors include radiologic (>2 tumors [HR, 4.98; 95% CI, 1.72-14.4; P = .003]; cumulative diameter [1.70 per log SD; 1.12-2.59; P < .001]; outside the Milan criteria [10.0; 3.7-33.3; P < .001]) and pathologic factors (>2 tumors [4.39; 1.32-14.6; P = .02]; cumulative diameter [2.32 per log SD; 1.43-3.77; P = .001]; microvascular [3.07; 1.02-9.24; P = .05] and macrovascular invasion [8.75; 2.15-35.6; P = .002]).

CONCLUSIONS AND RELEVANCE Nearly one-third of patients with radiographically apparent HCC have non-AFP–producing tumors that have more favorable pathologic characteristics, lower posttransplant recurrence, and superior survival compared with patients with AFP-producing tumors. Posttransplant HCC recurrence for patients with non-AFP–producing tumors is predicted by important radiologic and pathologic factors, and is negligible for patients within the Milan criteria. Stratifying patients by AFP status in addition to radiological criteria may improve the selection process for and the prioritization of transplant candidates.

Author Affiliations: Dumont-UCLA (University of California, Los Angeles) Transplant Center and Liver Cancer Center, Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles (Agopian, Harlander-Locke, Zarrinpar, Kaldas, Cheng, Yersiz, Farmer, Hiatt, Busuttil); Ronald Reagan UCLA Medical Center, Division of Liver and Pancreas Transplantation, Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles (Agopian); Department of Biomathematics, University of California, Los Angeles (Markovic).

Corresponding Author: Vatche G. Agopian, MD, Ronald Reagan UCLA Medical Center, Division of Liver and Pancreas Transplantation, Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles, 757 Westwood Plaza, Ste 8501-B, Los Angeles, CA 90095 (vagopian@mednet.ucla.edu).

A liver transplant (LT) for hepatocellular carcinoma (HCC) has unequivocally been established as the gold standard therapy for select patients with underlying liver dysfunction, largely owing to the reproducibly excellent results when transplantation is limited to tumors meeting the widely accepted Milan criteria.[1-3] The prioritization of LT recipients with HCC by use of the Model for End-Stage Liver Disease (MELD) allocation system[4-6] has resulted in LTs for HCCs now accounting for nearly 25% of all transplants in the United States.[7]

Despite continuous refinements in the HCC MELD exception policy, LT recipients with HCC continue to be overprioritized compared with patients with liver failure who do not have cancer, with many studies revealing higher transplant rates despite lower risks of waitlist dropout and inferior survival.[8-10] The recently implemented regional Share MELD 35 policy,[11] intended to increase access to regional allografts for the sickest LT recipients, has had the occasional unintentional consequence of diverting lifesaving organs from patients with liver failure to LT recipients with HCC whose MELD scores have been allowed to mature to 35 or greater. This has led to the latest modification of the HCC allocation policy by the Organ Procurement and Transplantation Network, with a requirement of a mandatory 6-month waiting period prior to awarding of exception points and a capping of the MELD score at 34 for LT recipients with HCC with preserved underlying liver function.[12]

The contemporary practice of awarding equal prioritization for all LT recipients with HCC who qualify for MELD exception points, without taking into account their variable risks of waitlist dropout or posttransplant recurrence, aggravates the current problem. Multiple studies have demonstrated that radiologic size criteria alone do not reliably capture the biological behavior of tumors.[13,14] Important serum biomarkers, explant pathologic factors, and response to pretransplant locoregional therapy[15-20] have all been shown to significantly improve the prognostic ability of radiologic criteria alone, but none have formally been incorporated in the MELD prioritization scheme.

Serum α-fetoprotein (AFP) is a well-established prognostic marker of increased tumor virulence in HCC[15,21-27] and has been shown to be associated with worse tumor phenotype and aggressiveness. While multiple studies have shown inferior outcomes following an LT for HCC at various prognostic cutoffs, data regarding the characteristics and outcomes of patients with tumors that do not produce AFP (hereafter referred to as non-AFP–producing tumors) have been scarce. The specific aims of our study were to evaluate the proportion of LT recipients whose tumors do not produce AFP (≤10 ng/mL [to convert to micrograms per liter, multiply by 1.0]), compare their outcomes with those of patients with AFP-producing tumors, and identify factors that specifically affect posttransplant recurrence in these patients in order to allow for better stratification of patients for LT.

Methods

We retrospectively reviewed a prospectively maintained transplant database and identified all adult patients (18 years of age or older) who underwent an LT for HCC and who received a pretransplant diagnosis of HCC with available data on serum AFP level at UCLA from 1989 to 2013. The UCLA institutional review board approved the study. Informed consent was waived because the data were deidentified.

Recipients were classified as having an AFP-producing tumor (serum AFP level of >10 ng/mL at any time prior to LT irrespective of tumor treatment) or a non-AFP–producing tumor (serum AFP level of ≤10 ng/mL at all times prior to LT irrespective of tumor treatment) based on pretransplant AFP levels. The primary objective was to evaluate the proportion of radiographically apparent HCC lesions that were non-AFP–producing tumors and the effect of AFP status on posttransplant outcomes. Only recipients with explant pathology confirming viable HCC or patients with a pretransplant radiographic HCC diagnosis and directed locoregional tumor treatment with explant pathology confirming complete tumor necrosis were included. Variables for analysis included recipient demographics (age, sex, primary end-stage liver disease diagnosis, body mass index, diabetes, and hypertension), laboratory results (physiologic MELD score, total cholesterol level, AFP level, and neutrophil to lymphocyte ratio), radiologic characteristics (number of lesions, maximum tumor diameter, cumulative tumor diameter, and proportion within radiologic Milan criteria), treatment-specific factors (number and type of locoregional therapy), and pathologic characteristics (number of lesions, maximum tumor diameter, cumulative tumor diameter, extent of pathologic necrosis, tumor grade/differentiation,[28] presence of microvascular and macrovascular invasion, and American Joint Committee on Cancer T stage[29]). The selection of locoregional therapy modality is based on the size, number, and location of lesions, without consideration for serum AFP.

Posttransplant immunosuppression included our standard 3-drug regimen with methylprednisolone sodium succinate, mycophenolate mofetil, and a calcineurin inhibitor.[30] Occasionally, LT recipients were initiated on a mammalian target of rapamycin inhibitor with withdrawal of calcineurin inhibitors if the explant pathology had poor prognostic features. Tumor surveillance included intravenous contrast-enhanced axial abdominal imaging (computed tomography or magnetic resonance imaging), noncontrast computed tomography of the chest, and a serum AFP sample obtained every 3 months prior to the LT and every 6 months following the LT.

Key Points

Question What are the radiologic and pathologic features and posttransplant outcomes of hepatocellular carcinomas (HCCs) that do not produce α-fetoprotein (AFP)?

Findings In this single-center retrospective analysis of 665 liver transplant recipients, the patients with tumors that did not produce AFP had radiographic characteristics similar to those of patients with AFP-producing tumors but significantly more favorable pathologic features and posttransplant outcomes.

Meaning Stratifying patients with HCC by AFP status in addition to radiologic criteria may improve transplant candidate selection and prioritization.

Comparisons were made among LT recipients with AFP-producing tumors and LT recipients with non-AFP-producing tumors, with subanalyses stratified by both AFP status and radiologic size criteria. Continuous variables were compared using the Wilcoxon rank sum test and reported as median values with interquartile ranges (IQRs). Categorical and ordinal variables were compared using the χ^2 test/Fisher exact test or the Cochran-Armitage test for trend, respectively, and summarized as percentages. Patient survival curves were computed using Kaplan-Meier methods and were compared using log-rank tests. Cumulative incidence curves for recurrence were computed in each specified group while taking into account the competing risk of non-HCC-related mortality, and were compared using the Fine-Gray test. Cox regression analysis was used to identify factors associated with recurrence for both patients with AFP-producing tumors and patients with non-AFP-producing tumors, controlling for the competing risk of non-HCC-related mortality. An interaction P value was reported to indicate whether the hazard ratios (HRs) for any covariates differentially affected recurrence between patients with AFP-producing tumors and patients with non-AFP-producing tumors. $P < .05$ was considered statistically significant.

Results

Of 665 patients with radiographically apparent HCC undergoing an LT during the study period, 457 (68.7%) had AFP-producing tumors, and 208 (31.3%) had non-AFP-producing tumors. The overall median follow-up time was 27.3 months (IQR, 8.8-67.8 months), with a median follow-up of 43.4 months (IQR, 12.1-78.5 months) for recipients without recurrence or mortality at last follow-up.

Comparison of Patient and Tumor Characteristics by AFP Status

The characteristics of the LT recipients, the donors, and the procedure are shown in the eTable in the Supplement. Patients with non-AFP-producing tumors were more likely than patients with AFP-producing tumors to have diabetes (35.0% [72 of 206] vs 24.2% [109 of 451]; $P = .004$), alcoholic (14.5% [30 of 207] vs 5.6% [25 of 450]) or nonalcoholic (7.7% [16 of 207] vs 1.6% [7 of 450]) steatohepatitis as the underlying cause of their liver disease ($P < .001$), and a greater neutrophil to lymphocyte ratio (3.0 vs 2.7; $P = .04$) and were less likely than patients with AFP-producing tumors to have hepatitis C viral infection (47.8% [99 of 207] vs 64.7% [291 of 450]; $P < .001$). There were no significant differences in age, sex, MELD score, total cholesterol level, the presence of hypertension or obesity, or any donor and procedure characteristics between the 2 groups of patients.

Radiologic, treatment, and pathologic characteristics are shown in **Table 1**. Patients with non-AFP-producing tumors had radiologic characteristics that were similar to those of patients with AFP-producing tumor, but they had significantly more favorable pathologic features, including fewer numbers of tumors (24.6% [51 of 207] vs 34.8% [158 of 454] with >2 lesions; $P = .03$ for trend), smaller cumulative tumor diameters (4.2 vs 5.0 cm; $P = .02$), greater complete pathologic response to pretransplant locoregional therapy (24.8% [51 of 206] vs 16.0% [73 of 456]; $P = .01$), less microvascular (17.4% [36 of 207] vs 21.9% [100 of 457]) and macrovascular (2.4% [5 of 207] vs 9.4% [43 of 457]) invasions ($P < .001$ for trend), fewer poorly differentiated tumors (14.5% [25 of 172] vs 28.1% [107 of 381]; $P < .001$ for trend), and lower overall T stage (T1: 46.9% [97 of 207] vs 35.9% [164 of 457], T2: 39.1% [81 of 207] vs 43.1% [197 of 457], T3a: 11.1% [23 of 207] vs 10.9% [50 of 457], and T3b/T4: 2.9% [6 of 207] vs 10.1% [46 of 457]; $P < .001$ for trend). Patients with AFP-producing tumors were more likely than patients with non-AFP-producing tumors to receive transarterial therapy without ablation (38.1% [174 of 457] vs 27.5% [57 of 207]; $P = .01$).

HCC Recurrence and Posttransplant Survival

Of the 665 patients who underwent an LT, 103 (15.5%) experienced HCC recurrence, with a median time to recurrence of 15.9 months. Patients with non-AFP-producing tumors had significantly superior recurrence-free survival at 1, 3, and 5 years (88%, 74%, and 67% vs 76%, 59%, and 51%, respectively) ($P = .002$; **Figure 1**A), a lower cumulative incidence of recurrence at 5 years (8.8% vs 22%) ($P < .001$; Figure 1B), and a longer median time to recurrence for those who had developed recurrence (30.9 vs 13.4 months; $P = .01$) than did patients with AFP-producing tumors. When stratified by radiologic Milan criteria at presentation, in addition to AFP status, 1-, 3-, and 5-year recurrence-free survival was better for patients with non-AFP-producing tumors within the Milan criteria (89%, 77%, and 71%, respectively) and was worse for patients with AFP-producing tumors outside the Milan criteria (69%, 42%, and 40%, respectively) ($P < .001$; **Figure 2**A). Patients with AFP-producing tumors within the Milan criteria (78%, 64%, and 55%, respectively) and patients with non-AFP-producing tumors outside the Milan criteria (83%, 59%, and 47%, respectively) had intermediate but acceptable survival at 1, 3, and 5 years (Figure 2A). The 5-year cumulative incidence of recurrence, while controlling for the competing risk of non-HCC mortality, was highest for patients with AFP-producing tumors outside the Milan criteria (42%) and lowest for patients with non-AFP-producing tumors within the Milan criteria (6%) (overall $P < .001$), with AFP status being able to further discriminate posttransplant recurrence for both groups of patients within the Milan criteria (6% of patients with non-AFP-producing tumors vs 15% of patients with AFP-producing tumors; $P = .004$) and patients outside the Milan criteria (19% of patients with non-AFP-producing tumors vs 42% of patients with AFP-producing tumors; $P = .02$) (Figure 2B).

Analysis of LT recipients initially outside the Milan criteria revealed a trend toward improved 5-year survival (53% vs 39%; $P = .35$; **Figure 3**A) and significantly reduced recurrence (0% vs 43%; $P = .002$; Figure 3B) for patients with non-AFP-producing tumors that were downstaged compared with patients with non-AFP-producing tumors that were unable to be downstaged by pretransplant locoregional therapy. Similarly, for patients with AFP-producing tumors initially outside the Milan criteria, there was significantly superior 5-year recurrence-free survival (58% vs 23%; $P = .01$; Figure 3A) and lower

Table 1. Comparison of Radiologic, Treatment, and Pathologic Characteristics

Characteristic	Patients, No./Total No. (%)		P Value[a]
	AFP-Producing Tumors	Non-AFP-Producing Tumors	
Radiologic			
No. of lesions			
1	313/447 (70.0)	153/204 (75.0)	
2	79/447 (17.7)	35/204 (17.2)	.10
≥3	55/447 (12.3)	16/204 (7.8)	
Tumor diameter, median (IQR), cm			
Maximum	3.0 (2.4-4.0)	3.0 (2.3-4.2)	.58
Cumulative	3.7 (2.6-5.0)	3.5 (2.4-4.9)	.09
Milan criteria			
Within criteria	356/451 (78.9)	167/205 (81.5)	
Outside criteria			.36
Downstaged	46/451 (10.2)	23/205 (11.2)	
Not downstaged	49/451 (10.9)	15/205 (7.3)	
No. of pretransplant locoregional therapies			
0	117/456 (25.7)	47/208 (22.6)	
1	186/456 (40.8)	88/208 (42.3)	.37
2	101/456 (22.1)	44/208 (21.2)	
≥3	52/456 (11.4)	29/208 (13.9)	
Modality			
Transarterial therapy	175/456 (38.4)	57/207 (27.5)	
Thermal ablation	114/456 (25.0)	62/207 (30.0)	.01
Both	42/456 (9.2)	33/207 (15.9)	
Pathologic			
No. of tumors			
1	206/454 (45.4)	107/207 (51.7)	
2	90/454 (19.8)	49/207 (23.7)	.03
≥3	158/454 (34.8)	51/207 (24.6)	
Tumor diameter, median (IQR), cm			
Maximum	3.5 (2.5-5.0)	3.5 (2.5-4.5)	.63
Cumulative	5.0 (3.0-8.1)	4.2 (3.0-6.1)	.02
Tumor necrosis			
None/partial	383/456 (84.0)	154/206 (74.8)	.01
Complete (no viable tumor)	73/456 (16.0)	52/206 (25.2)	
Vascular invasion			
None	314/457 (68.7)	166/207 (80.2)	
Microvascular	100/457 (21.9)	36/207 (17.4)	<.001
Macrovascular	43/457 (9.4)	5/207 (2.4)	
Differentiation			
Well	67/381 (17.6)	42/172 (24.4)	
Moderate	207/381 (54.3)	105/172 (61.0)	<.001
Poor	107/381 (28.1)	25/172 (14.5)	
AJCC T stage			
T1	164/457 (35.9)	97/207 (46.9)	
T2	197/457 (43.1)	81/207 (39.1)	
T3a	50/457 (10.9)	23/207 (11.1)	<.001
T3b/T4	46/457 (10.1)	6/207 (2.9)	

Abbreviations: AFP, α-fetoprotein; AJCC, American Joint Committee on Cancer; IQR, interquartile range.

[a] Determined by the Wilcoxon rank sum test for comparisons of continuous variables, by the Cochran-Armitage test for trend for comparisons of ordinal variables, and by the χ^2 test for comparisons of categorical variables.

recurrence (13% vs 64%; P < .001; Figure 3B) for patients with AFP-producing tumors that were downstaged compared with patients with AFP-producing tumors that were unable to be downstaged.

Bivariate predictors of HCC recurrence for both patients with AFP-producing tumors and patients with non-AFP-producing tumors are shown in **Table 2.** For patients with non-AFP-producing tumors, the important radiologic factors in-
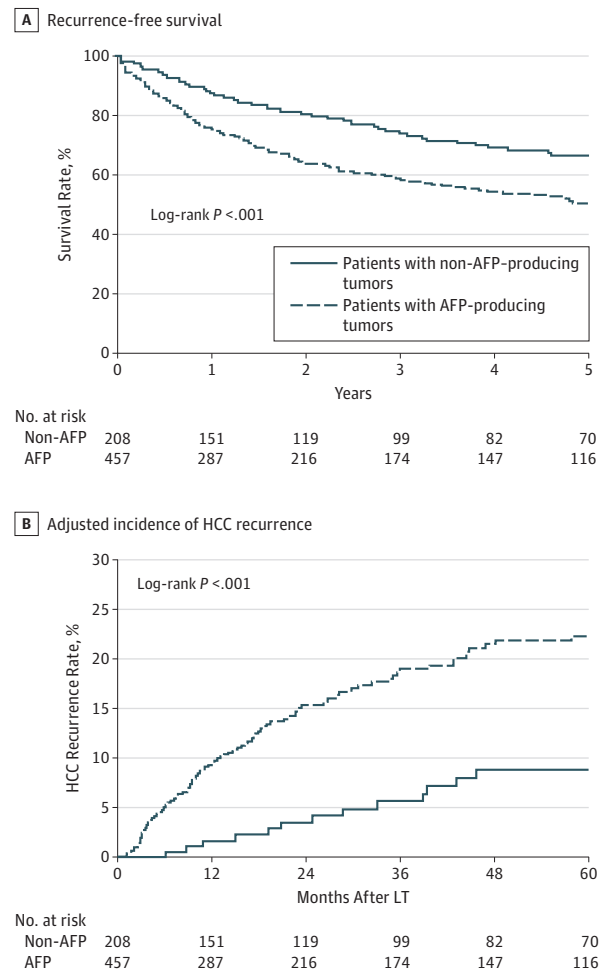
cluded more than 2 lesions (HR, 4.98 [95% CI, 1.72-14.4]; *P* = .003), increasing maximum tumor diameter (HR, 1.70 per log SD [95% CI, 1.16-2.50 per log SD]; *P* = .01) and cumulative tumor diameter (HR, 1.70 per log SD [95% CI, 1.12-2.59 per log SD]; *P* < .001), and tumors that were outside the Milan criteria at the time of LT (HR, 10.0 [95% CI, 3.7-33.3]; *P* < .001). Pathologic factors included more than 2 lesions (HR, 4.39 [95% CI, 1.32-14.6]; *P* = .02), increasing maximum tumor diameter (HR, 1.97 per log SD [95% CI, 1.23-3.15 per log SD]; *P* = .01) and cumulative tumor diameter (HR, 2.32 per log SD [95% CI, 1.43-3.77 per log SD]; *P* = .001), microvascular invasion (HR, 3.07 [95% CI, 1.02-9.24]; *P* = .05), and macrovascular invasion (HR, 8.75 [95% CI, 2.15-35.6]; *P* = .002). Patients with AFP-producing tumors had predictors of recurrence that were very similar to those of patients with non-AFP-producing tumors. While the HRs for any given predictor are slightly different between the patients with AFP-producing tumors and the patients with non-AFP-producing tumors, none of these covariates had a statistically significant differential effect on posttransplant recurrence between the 2 groups of patients, as evidenced by all the reported interaction *P* values greater than .05.

Of the 208 patients with non-AFP-producing tumors, 16 (7.7%) experienced a posttransplant recurrence; of these 16 patients, 9 (56.3%) had AFP levels of greater than 10 ng/mL, with a median postrecurrence AFP level of 15.8 ng/mL (IQR, 4.0-123.0 ng/mL). Conversely, although the majority (n = 55) of patients with AFP-producing tumors who experienced a posttransplant recurrence demonstrated elevated serum AFP levels, 14 patients (25.5%) experienced a proven recurrence with an AFP level of less than 10 ng/mL. Median survival following recurrence was significantly superior for patients with non-AFP-producing recurrent tumors compared with patients with AFP-producing recurrent tumors (70.6 vs 12.4 months; *P* = .002).

Discussion

Although an LT has been established as the ideal treatment for select patients with HCC, the scarcity of donor organs and the continued overprioritization of LT recipients with HCC by use of the MELD allocation system[9,10] obligate a change in the current stratification scheme to include markers of tumor biology beyond radiologic size criteria that better discriminate a patient's need for and predicted outcome following an LT. Serum AFP, an important HCC tumor biomarker, has indisputably been shown to be associated with increased risks of waitlist dropout[23,31,32] and posttransplant recurrence.[15,16,21,22,24-27,33] However, data on patients with HCCs that do not produce AFP are scarce. We report the largest single-center study evaluating LT recipients with non-AFP-producing tumors, who comprised nearly one-third of the 665 LT recipients with HCC. We evaluated the effect of AFP status on important pathological characteristics that drive tumor biology and on posttransplant cancer outcomes, and we identified important factors predicting HCC recurrence in LT recipients with non-AFP-producing tumors.
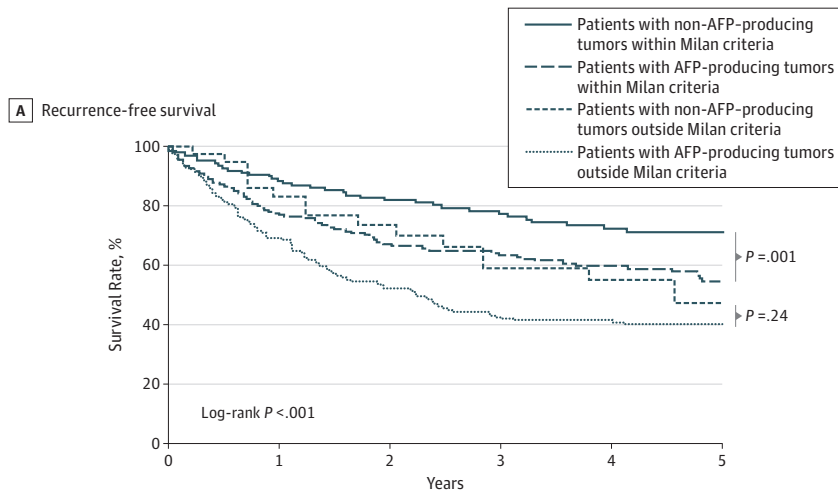
Figure 1. Kaplan-Meier Recurrence-Free Survival and Adjusted Incidence of Hepatocellular Carcinoma (HCC) Recurrence Stratified by α-Fetoprotein (AFP) Status

A Recurrence-free survival

No. at risk
Non-AFP	208	151	119	99	82	70
AFP	457	287	216	174	147	116

B Adjusted incidence of HCC recurrence

No. at risk
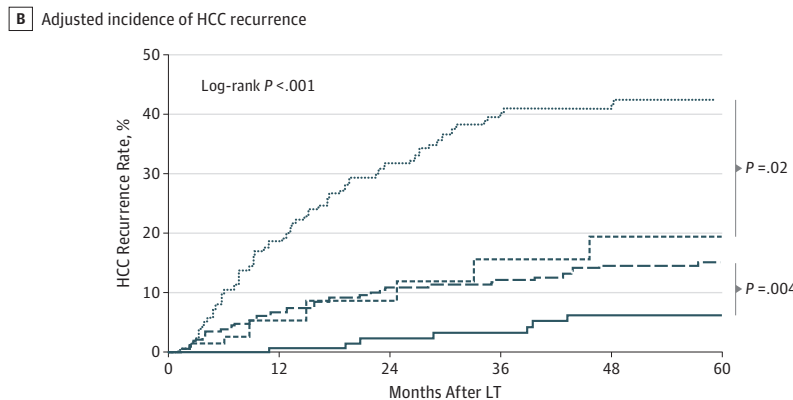|					
Non-AFP	208	151	119	99	82	70
AFP	457	287	216	174	147	116

The curves compare patients with AFP-producing tumors with patients with tumors that do not produce AFP (non-AFP–producing tumors). LT indicates liver transplant.

Numerous prior studies[1,15,26] have clearly established pathological tumor differentiation and microvascular invasion as 2 of the most important variables influencing posttransplant cancer outcomes; however, these variables cannot reliably be ascertained prior to an LT.[34,35] In the present study, despite no significant differences in any radiologic characteristics between non-AFP-producing tumors and AFP-producing tumors, we found that non-AFP-producing tumors were significantly less likely to be poorly differentiated or to show the presence of microvascular or macrovascular invasion. Our findings are consistent with the few prior studies demonstrating this association of AFP status with pathologic differentiation and vascular invasion. In a multicenter French study reported by Duvoux et al,[22] increasing AFP levels were significantly associated with the presence of poorly differentiated tumors, as well as the presence of microvascular and macrovascular invasion. Fujiki et al[36] demonstrated a

Figure 2. Kaplan-Meier Recurrence-Free Survival and Adjusted Incidence of Hepatocellular Carcinoma (HCC) Recurrence Stratified by α-Fetoprotein (AFP) Status and Radiologic Milan Criteria

A Recurrence-free survival

Legend:
- Patients with non-AFP-producing tumors within Milan criteria
- Patients with AFP-producing tumors within Milan criteria
- Patients with non-AFP-producing tumors outside Milan criteria
- Patients with AFP-producing tumors outside Milan criteria

Log-rank P <.001

P =.001
P =.24

No. at risk

Non-AFP within Milan criteria	167	121	96	81	66	56
AFP within Milan criteria	356	224	170	143	118	93
Non-AFP outside Milan criteria	39	29	22	17	15	13
AFP outside Milan criteria	96	60	44	32	29	24

B Adjusted incidence of HCC recurrence

Log-rank P <.001

P =.02
P =.004

No. at risk

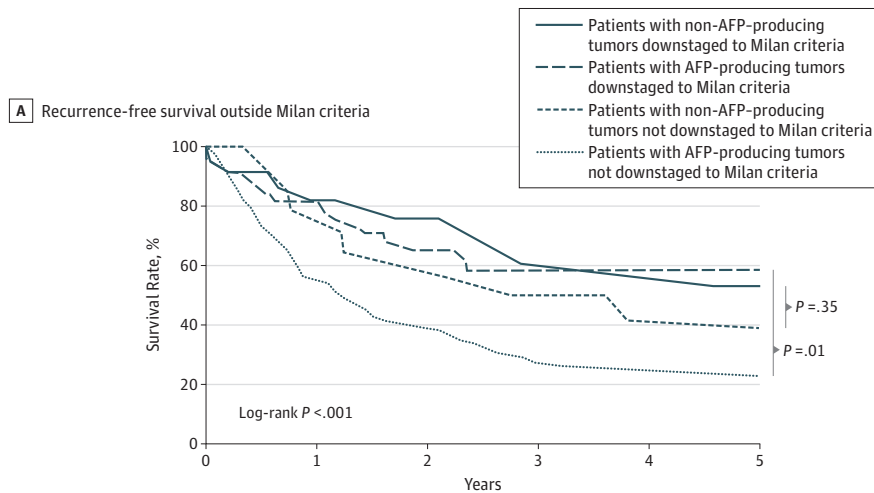|					
Non-AFP within Milan criteria	167	121	96	81	66	56
AFP within Milan criteria	356	224	170	143	118	93
Non-AFP outside Milan criteria	39	29	22	17	15	13
AFP outside Milan criteria	96	60	44	32	29	24

The curves compare patients with AFP-producing tumors with patients with tumors that do not produce AFP (non-AFP–producing tumors); AFP status was used to further discriminate survival among liver transplant (LT) recipients within or outside the Milan criteria (A), and HCC recurrence in patients within or outside the Milan criteria (B).

significantly increased risk of both microvascular invasion and poorly differentiated tumors in patients with an AFP level of higher than 800 ng/mL compared with patients with an AFP level of lower than 200 ng/mL. In a study of 211 patients with HCC within the Milan criteria undergoing an LT, Hameed et al[23] showed an increasing probability of vascular invasion with increasing serum AFP levels. Taken together with prior studies, our findings provide strong evidence that AFP status predicts important pathologic features independent of radiographic size and should be incorporated into the selection criteria for LT recipients with HCC.

In the present study, the patients with non-AFP–producing tumors had significantly improved disease-free survival and lower recurrence rates compared with the patients with AFP-producing tumors. Even for patients who did experience posttransplant recurrence, the median time to recurrence was significantly longer for the patients with non-AFP–producing tumors than for patients with AFP-producing tumors

Figure 3. Kaplan-Meier Recurrence-Free Survival and Adjusted Incidence of Hepatocellular Carcinoma (HCC) Recurrence Stratified by α-Fetoprotein (AFP) Status and Downstaging to Milan Status
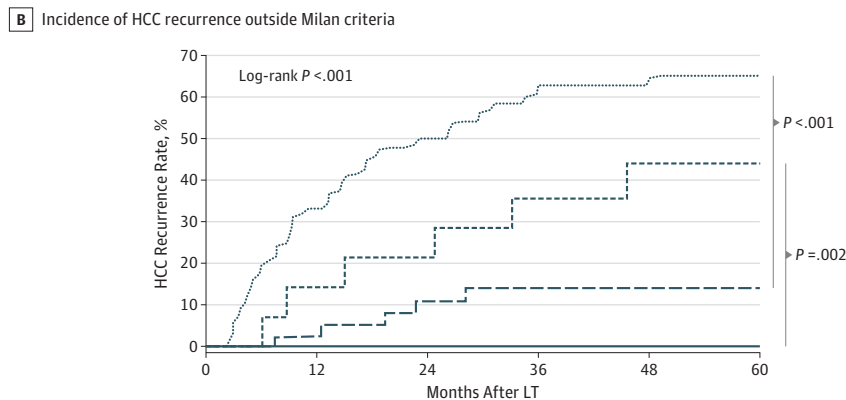
The curves compare patients with AFP-producing tumors with patients with tumors that do not produce AFP (non-AFP-producing tumors), for a subset of liver transplant (LT) recipients initially outside the Milan criteria. The ability to successfully downstage recipients to the Milan criteria was important for recurrence-free survival outside the Milan criteria (A) and the incidence of HCC recurrence (B).

(30.9 vs 13.4 months; *P* = .01), which supports the idea that non-AFP-producing tumors are less virulent. Interestingly, the AFP status of the recurrence itself also affected postrecurrence survival, with superior survival for patients with non-AFP-producing recurrent tumors compared with patients with AFP-producing recurrent tumors, irrespective of the AFP status prior to the LT.

Perhaps most importantly, AFP status was able to further discriminate outcomes among patients who had tumors within or outside the radiologic Milan criteria, indicating that size criteria alone fail to capture the tumor heterogeneity in these patients. For example, patients with non-AFP-producing tumors within the Milan criteria had an excellent 5-year recurrence-free survival of 71%, with only a 6% adjusted risk

of recurrence, compared with a 55% recurrence-free survival and a 15% recurrence rate at 5 years for patients with AFP-producing tumors within the Milan criteria (Figure 2). This stratification by AFP status appeared even more important in the subset of patients with tumors outside the Milan criteria, allowing for identification of a group of patients with non-AFP-producing tumors outside of the Milan criteria who demonstrated an acceptable 5-year recurrence rate of 19% compared with an unacceptably high 5-year recurrence rate of 42% for patients with AFP-producing tumors outside the Milan criteria (Figure 2B).

Similar to prior reports,[15,37-39] the ability to downstage tumors to meet the Milan criteria was an important requirement to achieve acceptable cancer outcomes in the subset of

Table 2. Bivariate Analysis of Factors Associated With HCC Recurrence Stratified by AFP Status

Factor	457 Patients With AFP-Producing Tumors		208 Patients With Non-AFP-Producing Tumors		P Value for Interaction[a]
	HR (95% CI)	P Value	HR (95% CI)	P Value	
LT recipient					
Age, per SD	0.77 (0.62-0.97)	.02	0.78 (0.57-1.06)	.11	.75
Male	1.72 (1.03-2.88)	.04	0.80 (0.28-2.30)	.68	.12
Diabetes	0.92 (0.55-1.54)	.75	0.33 (0.07-1.44)	.14	.18
Hypertension	0.92 (0.55-1.52)	.74	0.71 (0.20-2.50)	.60	.62
BMI, per SD	0.93 (0.71-1.22)	.62	1.07 (0.75-1.51)	.73	.69
MELD, per SD	1.15 (0.93-1.42)	.19	1.03 (0.72-1.46)	.89	.54
NLR, per log SD	1.42 (1.19-1.69)	<.001	1.12 (0.59-2.14)	.72	.48
Total cholesterol, per SD	1.19 (0.99-1.44)	.07	0.99 (0.98-1.00)	.20	.09
Radiologic factor					
No. of lesions					
2 vs 1	2.16 (1.27-3.68)	.004	1.00 (0.21-4.70)	.10	.28
≥3 vs 1	2.94 (1.75-4.94)	<.001	4.98 (1.72-14.4)	.003	
Diameter of lesion, per log SD					
Maximum	1.67 (1.36-2.04)	<.001	1.70 (1.16-2.50)	.01	.91
Cumulative	1.93 (1.57-2.37)	<.001	1.70 (1.12-2.59)	<.001	.64
Outside Milan criteria	6.25 (3.44-8.33)	<.001	10 (3.7-33.3)	<.001	.33
No. of pretransplant locoregional therapies					
1 vs 0	0.58 (0.34-0.99)	.05	0.81 (0.25-2.63)	.73	.35
2 vs 0	0.58 (0.40-1.09)	.10	1.20 (0.32-4.53)	.79	
≥3 vs 0	1.87 (1.04-3.34)	.04	0.55 (0.06-4.74)	.58	
Modality					
Transarterial therapy	0.66 (0.40-1.09)	.10	0.14 (0.02-1.07)	.06	.33
Thermal ablation	0.44 (0.24-0.83)	.01	0.20 (0.05-0.93)	.04	
Both	1.00 (0.53-1.91)	.99	0.69 (0.19-2.50)	.57	
Pathologic factor					
No. of tumors					
2 vs 0-1	1.65 (0.88-3.10)	.12	2.01 (0.53-7.63)	.30	.76
≥3 vs 0-1	2.65 (1.62-4.33)	<.001	4.39 (1.32-14.6)	.02	
Diameter, per log SD					
Maximum	2.06 (1.66-2.56)	<.001	1.97 (1.23-3.15)	.01	.83
Cumulative	2.35 (1.86-2.98)	<.001	2.32 (1.43-3.77)	.001	.94
Vascular invasion					
Microvascular vs none	2.35 (1.37-4.03)	.002	3.07 (1.02-9.24)	.05	.73
Macrovascular vs none	11.1 (6.87-18.0)	<.001	8.75 (2.15-35.6)	.002	
Differentiation					
Moderate vs well	2.46 (1.11-5.45)	.03	2.44 (0.53-11.3)	.25	.70
Poor vs well	3.73 (1.64-8.47)	.002	1.97 (0.28-14.1)	.50	

Abbreviations: AFP, α-fetoprotein; BMI, body mass index; HCC, hepatocellular carcinoma; LT, liver transplant; MELD, Model for End-Stage Liver Disease; NLR, neutrophil to lymphocyte ratio.

[a] Indicates whether effect of any given covariate on HCC recurrence differs significantly among patients with AFP-producing tumors and patients with non-AFP–producing tumors.

patients initially outside the Milan criteria (Figure 3). Inclusion of serum AFP level in addition to radiologic size criteria for transplant candidate selection has previously been proposed. Duvoux et al[22] proposed a model incorporating serum AFP level, tumor size, and number of tumors that significantly improved on the ability of size criteria alone to determine posttransplant recurrence. Similarly, Hameed et al[23] demonstrated that exclusion of patients with an AFP level of higher than 1000 ng/mL who were within the radiologic Milan

criteria would exclude only 5% of recipients from an LT but would eliminate 20% of posttransplant recurrences.

One of the main objectives of the present study was to identify important predictors of HCC recurrence in patients with non-AFP-producing tumors and to compare these predictors with those in patients with AFP-producing tumors. As expected, some of the most important predictors for HCC recurrence in patients with non-AFP-producing tumors included radiologic size, number of tumors, adherence to size criteria,

pathologic size, and presence of vascular invasion. These same factors were equally important in predicting HCC recurrence in patients with AFP-producing tumors, with no evidence that any covariate was differentially significant between the 2 groups ($P > .05$ for interaction).

As important as it is to have identified factors predicting posttransplant recurrence, differential prioritization of LT recipients with HCC also requires the determination of the factors that affect waitlist dropout. While we have not yet examined the effect of AFP status on waitlist dropout at our center, fortuitously, several studies have shown that the factors affecting waitlist dropout due to tumor progression similarly influence posttransplant outcomes.[40,41] In a recent study,[23] more than 40% of patients with an initial AFP level of higher than 1000 ng/mL experienced waitlist dropout due to tumor progression; not surprisingly, patients with an AFP level of higher than 1000 ng/mL undergoing an LT had a 5-year disease-free survival of 52.7%, significantly inferior to the 80.3% survival observed among patients with an AFP level of lower than 1000 ng/mL. The determination of the "optimal" AFP cutoff remains to be determined. However, along with previously reported experiences, our findings support the concept that prioritization of LT recipients by AFP status in addition to radiologic size criteria will align the goals of minimizing waitlist dropout and optimizing posttransplant cancer outcomes.

In addition to the inherent flaws in a retrospective analysis, several limitations to the present study warrant discussion. At an AFP threshold of 10 ng/mL, it is likely that some patients were categorized as having AFP-producing tumors, even though an underlying hepatitis, and not their tumor, per se, was the source of an elevated AFP level of higher than 10 ng/mL. Furthermore, the longer median waitlist times in our region[42] may have resulted in some patients with initially non-AFP-producing tumors beginning to produce AFP and, hence, to be categorized as AFP-producing tumors, which may limit the generalizability of our findings to other centers. Despite this, it is important to emphasize that we have not proposed an AFP threshold of 10 ng/mL as a prognostic cutoff, but rather we have identified important radiologic and pathologic characteristics of tumors that do not produce AFP even over a prolonged period of time. Ultimately, a change in the HCC allocation policy will require a prospective assessment of dynamic changes in both the serum AFP and radiologic parameters to pretransplant locoregional therapy to minimize the risk of waitlist dropout while preserving posttransplant oncologic outcomes.

Conclusions

In conclusion, despite significant improvements in posttransplant outcomes for LT recipients with HCC with the institution of the Milan criteria, there is now overwhelming evidence that radiologic size criteria alone do not optimally stratify patients with HCC for LT. Serum AFP is an important biomarker of tumor behavior that reliably indicates a more aggressive tumor phenotype, and we show that a higher AFP expression is associated with a significantly greater frequency of poorly differentiated tumors and microvascular and macrovascular invasion. The inclusion of AFP status to the existing radiologic Milan criteria allows for improved discrimination of tumor biology for LT recipients within or outside the Milan criteria. We propose that the inclusion of serum AFP status to the current radiologic size criteria will improve transplant candidate selection and prioritization.

REFERENCES

1. Duffy JP, Vardanian A, Benjamin E, et al. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg.* 2007;246(3):502-509.

2. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med.* 1996;334(11):693-699.

3. Yao FY, Ferrell L, Bass NM, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology.* 2001;33(6):1394-1403.

4. Brown RS, Rush SH, Rosen HR, et al. Liver and intestine transplantation. *Am J Transplant.* 2004;4(suppl 9):81-92.

5. Ioannou GN, Perkins JD, Carithers RL Jr. Liver transplantation for hepatocellular carcinoma: impact of the MELD allocation system and predictors of survival. *Gastroenterology.* 2008;134(5):1342-1351.

6. Wiesner R, Edwards E, Freeman R, et al; United Network for Organ Sharing Liver Disease Severity Score Committee. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology.* 2003;124(1):91-96.

7. Kim WR, Lake JR, Smith JM, et al. Liver. *Am J Transplant.* 2016;16(suppl 2):69-98.

8. Kim WR, Stock PG, Smith JM, et al. OPTN/SRTR 2011 annual data report: liver. *Am J Transplant.* 2013;13(suppl 1):73-102.

9. Massie AB, Caffo B, Gentry SE, et al. MELD exceptions and rates of waiting list outcomes. *Am J Transplant.* 2011;11(11):2362-2371.

10. Washburn K, Edwards E, Harper A, Freeman R. Hepatocellular carcinoma patients are advantaged in the current liver transplant allocation system. *Am J Transplant.* 2010;10(7):1643-1648.

11. Massie AB, Chow EK, Wickliffe CE, et al. Early changes in liver distribution following implementation of Share 35. *Am J Transplant.* 2015;15(3):659-667.

12. Organ Procurement and Transplantation Network. Learn about policy changes approved at the November 2014 board meeting. Capping the HCC Exception Score at 34. US Dept of Health and Human Services/Health Resources and Services Administration website. https://optn.transplant.hrsa.gov/news/learn-about-policy-changes-approved-at-the-november-2014-board-meeting/. Accessed August 31, 2016.

13. DuBay D, Sandroussi C, Sandhu L, et al. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann Surg.* 2011;253 (1):166-172.

14. Lu DS, Siripongsakun S, Kyong Lee J, et al. Complete tumor encapsulation on magnetic resonance imaging: a potentially useful imaging biomarker for better survival in solitary large hepatocellular carcinoma. *Liver Transpl.* 2013;19(3): 283-291.

15. Agopian VG, Harlander-Locke M, Zarrinpar A, et al. A novel prognostic nomogram accurately predicts hepatocellular carcinoma recurrence after liver transplantation: analysis of 865 consecutive liver transplant recipients. *J Am Coll Surg.* 2015;220 (4):416-427.

16. Agopian VG, Morshedi MM, McWilliams J, et al. Complete pathologic response to pretransplant locoregional therapy for hepatocellular carcinoma defines cancer cure after liver transplantation: analysis of 501 consecutively treated patients. *Ann Surg.* 2015;262(3):536-545; discussion 543-545.

17. Cucchetti A, Cescon M, Bigonzi E, et al. Priority of candidates with hepatocellular carcinoma awaiting liver transplantation can be reduced after successful bridge therapy. *Liver Transpl.* 2011;17(11): 1344-1354.

18. Mehta N, Dodge JL, Goel A, Roberts JP, Hirose R, Yao FY. Identification of liver transplant candidates with hepatocellular carcinoma and a very low dropout risk: implications for the current organ allocation policy. *Liver Transpl.* 2013;19(12): 1343-1353.

19. Otto G, Herber S, Heise M, et al. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transpl.* 2006;12 (8):1260-1267.

20. Vitale A, D'Amico F, Frigo AC, et al. Response to therapy as a criterion for awarding priority to patients with hepatocellular carcinoma awaiting liver transplantation. *Ann Surg Oncol.* 2010;17(9): 2290-2302.

21. Berry K, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl.* 2013;19(6):634-645.

22. Duvoux C, Roudot-Thoraval F, Decaens T, et al; Liver Transplantation French Study Group. Liver transplantation for hepatocellular carcinoma: a model including α-fetoprotein improves the performance of Milan criteria. *Gastroenterology.* 2012;143(4):986-994.

23. Hameed B, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level > 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl.* 2014;20(8):945-951.

24. Lai Q, Avolio AW, Manzia TM, et al. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. *Clin Transplant.* 2012;26(2):E125-E131.

25. Mailey B, Artinyan A, Khalili J, et al. Evaluation of absolute serum α-fetoprotein levels in liver transplant for hepatocellular cancer. *Arch Surg.* 2011;146(1):26-33.

26. Sotiropoulos GC, Molmenti EP, Lösch C, Beckebaum S, Broelsch CE, Lang H. Meta-analysis of tumor recurrence after liver transplantation for hepatocellular carcinoma based on 1,198 cases. *Eur J Med Res.* 2007;12(10):527-534.

27. Toso C, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology.* 2009; 49(3):832-838.

28. Edmondson HA, Steiner PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer.* 1954;7(3):462-503.

29. American Joint Committee on Cancer (AJCC). *Cancer Staging Handbook.* 7th ed. New York, NY: Springer; 2010.

30. Agopian VG, Petrowsky H, Kaldas FM, et al. The evolution of liver transplantation during 3 decades: analysis of 5347 consecutive liver transplants at a single center. *Ann Surg.* 2013;258 (3):409-421.

31. Merani S, Majno P, Kneteman NM, et al. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol.* 2011;55(4):814-819.

32. Soriano A, Varona A, Gianchandani R, et al. Selection of patients with hepatocellular carcinoma for liver transplantation: Past and future. *World J Hepatol.* 2016;8(1):58-68.

33. Todo S, Furukawa H; Japanese Study Group on Organ Transplantation. Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. *Ann Surg.* 2004;240(3):451-459.

34. Gouw AS, Balabaud C, Kusano H, Todo S, Ichida T, Kojiro M. Markers for microvascular invasion in hepatocellular carcinoma: where do we stand? *Liver Transpl.* 2011;17(suppl 2):S72-S80.

35. Pawlik TM, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy: implications for transplant eligibility. *Ann Surg.* 2007;245(3):435-442.

36. Fujiki M, Takada Y, Ogura Y, et al. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant.* 2009;9 (10):2362-2371.

37. Chapman WC, Majella Doyle MB, Stuart JE, et al. Outcomes of neoadjuvant transarterial chemoembolization to downstage hepatocellular carcinoma before liver transplantation. *Ann Surg.* 2008;248(4):617-625.

38. Yao FY, Hirose R, LaBerge JM, et al. A prospective study on downstaging of hepatocellular carcinoma prior to liver transplantation. *Liver Transpl.* 2005;11(12):1505-1514.

39. Yao FY, Kerlan RK Jr, Hirose R, et al. Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. *Hepatology.* 2008;48 (3):819-827.

40. Allard MA, Sebagh M, Ruiz A, et al. Does pathological response after transarterial chemoembolization for hepatocellular carcinoma in cirrhotic patients with cirrhosis predict outcome after liver resection or transplantation? *J Hepatol.* 2015;63(1):83-92.

41. Cucchetti A, Cescon M, Bertuzzo V, et al. Can the dropout risk of candidates with hepatocellular carcinoma predict survival after liver transplantation? *Am J Transplant.* 2011;11(8):1696-1704.

42. Halazun KJ, Patzer RE, Rana AA, et al. Standing the test of time: outcomes of a decade of prioritizing patients with hepatocellular carcinoma, results of the UNOS natural geographic experiment. *Hepatology.* 2014;60(6):1957-1962.