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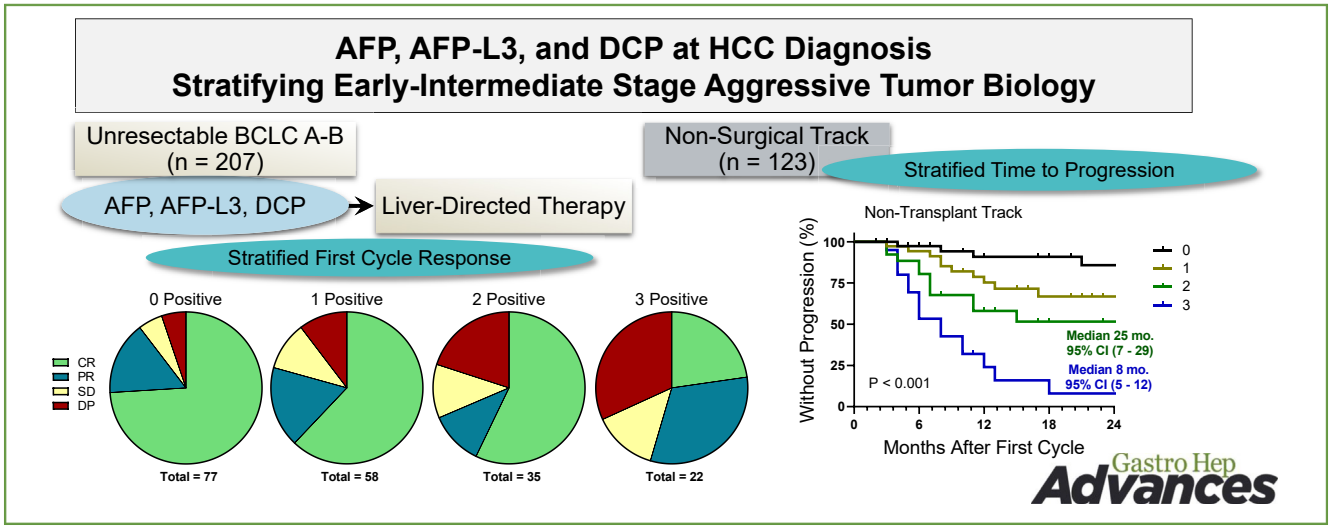
ORIGINAL RESEARCH—CLINICAL

α-Fetoprotein, α-Fetoprotein-L3, and Des-γ-Carboxy Prothrombin Stratify Hepatocellular Carcinoma Treatment Response and Progression Risk

Kelley Núñez,[1] Michael Schneider,[1] Tyler Sandow,[2] Juan Gimenez,[2] Mina Hibino,[1] Daniel Fort,[3] Ari Cohen,[1,4,5] and Paul Thevenot[1]

[1]Institute of Translational Research, Ochsner Clinic Foundation, New Orleans, Louisiana; [2]Department of Radiology, Ochsner Health, New Orleans, Louisiana; [3]Center for Outcomes Research, Ochsner Clinic Foundation, New Orleans, Louisiana; [4]Multi-Organ Transplant Institute, Ochsner Health, New Orleans, Louisiana; and [5]Faculty of Medicine, The University of Queensland, New Orleans, Louisiana



BACKGROUND AND AIMS: Assessing aggressive biology at early-stage hepatocellular carcinoma (HCC) diagnosis remains challenging. Alpha-fetoprotein (AFP) is the only clinical biomarker of aggressive HCC. In this study, AFP, *Lens culinaris* agglutinin-reactive AFP (AFP-L3), and des-γ-carboxy prothrombin (DCP) were measured at diagnosis prior to transplant evaluation and first cycle liver-directed therapy (LDT). **METHODS:** The prospective cohort included 207 patients who received LDT as a bridge/downstage to transplant or definitive treatment plan between 2016 and 2022. Plasma AFP, AFP-L3, and DCP levels were measured at diagnosis and analyzed with other factors associated with treatment response and time-to-progression. **RESULTS:** Biomarker phenotyping revealed 41% were triple negative, 30% expressed multiple biomarkers, and 12% express all 3 biomarkers. The biomarker profile was associated with target/overall response rate and time-to-progression ($P < .001$). Profiling stratified 1-year progression risk in nontransplant candidates, driven by coexpression of AFP and DCP in multivariate analysis controlling for tumor burden and staging. **CONCLUSION:** The biomarker panel at diagnosis established prognosis for LDT response and stratified 1-year HCC progression risk. AFP, AFP-L3, and DCP profiling isolated aggressive HCC biology at diagnosis and may have important implications in post-LDT surveillance and transplant wait time.

Keywords: Alpha-Fetoprotein; Carcinoma; Hepatocellular; Biomarkers; Disease Progression

Introduction

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality.[1] Liver transplantation remains the best treatment option for early- to intermediate-

Abbreviations used in this paper: **^{90}Y, ^{90}Yittrium; AFP, alpha-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive alpha-fetoprotein; ALBI, albumin-bilirubin; BCLC, barcelona clinic liver cancer; CI, 95% confidence interval; DCP, des-gamma-carboxy prothrombin; DEE-TACE, drug-eluting embolic transarterial chemoembolization; IQR, interquartile range; LDT, liver-directed therapy; LI-RADS, liver imaging reporting and data system; MELD-Na, model of end-stage liver disease; mRECIST, response evaluation criteria in solid tumors modified for HCC; MWA, microwave ablation; OR, odds ratio; PFS, progression-free survival; TARE, transarterial radioembolization; TFS, transplant-free survival; TTP, time-to-progression; UNOS-DS, United Network for Organ Sharing Down Staging.**

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stage HCC and not amendable to resection due to cirrhosis.[2] Milan criteria provides a benchmark for selecting transplant candidates and is incorporated in the Barcelona Clinic Liver Cancer (BCLC) algorithm.[3] In early- to intermediate-stage nonsurgical candidates, liver-directed therapy (LDT) can also provide a definitive treatment pathway to address HCC burden or delay systemic therapy. Although the progression risk associated with HCC size and overall burden of disease have been refined over decades to form the foundation of BCLC staging and Milan criteria, the circulating biomarker alpha-fetoprotein (AFP) is the only clinically available variable directly tied to HCC biology and biological aggressiveness.

AFP as the sole prognostic biomarker of biological aggressiveness in early- to intermediate-stage HCC has limitations. AFP levels at diagnosis continue to decline with improved HCC surveillance with recent studies reporting median values < 20 ng/mL.[4–6] AFP thresholds for risk of disease progression following LDT are as low as 8–20 ng/mL.[7,8] However, approximately 40%–60% of patients are AFP negative at diagnosis, highlighting the need for additional early-stage HCC biomarkers.[9,10] Alternative prognostic strategies, including Child-Pugh, ALBI, and Model of End Stage Liver Disease score, stratify overall survival but have tenuous links to HCC biology and risk of tumor progression.[3] The 1-year progression rates in early- to intermediate-stage HCC following LDT are 10%–20%[8] and can only be further stratified by a validated posttreatment imaging response. Multibiomarker platforms for HCC surveillance represent attractive targets to bridge the gap in defining aggressive biology in early-stage HCC prior to LDT, where they could help optimize treatment algorithms.

Lens culinaris agglutinin-reactive AFP fraction (AFP-L3 %) and des-γ-carboxy prothrombin (DCP)[11] provide the most translatable strategy for refining HCC prognosis. Elevated DCP and/or AFP-L3 levels are established independent risk factors for postsurgical tumor recurrence.[12,13] However, their prognostic implications at diagnosis for nonresectable early- to intermediate-stage HCC are unclear. The available data have largely focused on profile-independent biomarker accumulation incorporated within the framework of other prognostic indices[8,12,13] but their utility as a stand-alone baseline prognostic in early- to intermediate-stage HCC remains unclear. This study utilized a 207-patient cohort of recently diagnosed HCC staged BCLC A-B to correlate biomarker profile at diagnosis with first cycle LDT response and time-to-progression (TTP).

Patients and Methods

Study Design, Setting, and Participants

This study was conducted according to the ethical guidelines of the Declaration of Helsinki and Istanbul. This single-center, prospective study was approved by the Ochsner Health Institutional Review Board (study number 2016.131.B) for patients previously under HCC surveillance resulting in a recent diagnosis of unresectable HCC stage BCLC A-B, classified as treatment naïve, and approved to receive LDT by a multidisciplinary HCC board at a liver transplant center (Ochsner Multi-Organ Transplant Institute, New Orleans, LA, USA). HCC diagnosis adhered to the Liver Imaging Reporting and Data System (LIRADS) of LIRADS-5 or was biopsy-confirmed. Selection to receive LDT as a bridge/downstage to OLT or definitive treatment approach was made according to the American Association for the Study of Liver Disease guidelines.[14] LDT treatment approach was defined by the multidisciplinary board in patients with disease burden within Milan Criteria, United Network of Organ Sharing Downstaging criteria, or determined to be a downstaging candite beyond established criteria. HCC patients meeting the study criteria concomitantly underwent a pretransplant evaluation. The study protocol was initially powered to assess differences in 1-year progression rate following LDT using 4 possible combinations (0–3 positive biomarkers) utilizing AFP, AFP-L3, and DCP (target population rounded up to $n = 75$) with a secondary post hoc aim to examine 1-year progression rates in nonsurgical track BCLC A-B (target $n = 75$, actual $n = 123$). The transplant center is characterized as high-volume and short-wait-time. Informed consent was obtained prior to LDT, with enrollment dates between August 2016 and December 2022.

Clinical Study Variables

Variables for general patient demographics and hepatology staging, including liver-related serology, functional / metabolic, and blood chemistry, were obtained from the electronic medical record on the date of or within 30-days of the first cycle of LDT. The Child-Pugh, Model of End Stage Liver Disease modified for sodium, and ALBI scores were obtained using clinically available calculators. HCC baseline variables at diagnosis were obtained from the comprehensive multidisciplinary HCC board conference evaluation report. The conference review date was used to establish the confirmed date of HCC diagnosis. The assessment of surgical track was performed retrospectively at the time of data analysis as the transplant evaluation process concludes after the first cycle treatment and imaging follow-up. HCC patients with a liver transplant committee-approved evaluation were defined as transplant track. HCC patients without an evaluation, an incomplete evaluation, or declined evaluation were considered nontransplant track.

Response to Liver-Directed Therapy

First cycle LDT response was recorded retrospectively according to the Response Evaluation Criteria in Solid Tumors modified for HCC (mRECIST)[15] using the baseline and follow-up triple-phase computed tomography/magnetic resonance imaging. The mRECIST was calculated for research purposes and was not a factor in the assessment of the primary study endpoint. Treatment response was assessed with respect to the tumor burden targeted during the first cycle of treatment (target response) as well as the overall tumor response (overall response). Follow-up imaging was modality-dependent and targeted to 30 days for doxorubicin-eluting embolic transarterial chemoembolization and microwave ablation or 60–90 days for ^{90}Yittrium transarterial radioembolization.

Biomarker Measurements

AFP, AFP L3 %, and DCP levels were analyzed using the μTASWakoi30 (FUJIFILM Wako Diagnostics, Mountain View, CA, USA). Minimum detectable ranges were AFP > 0.3 ng/mL,

AFP-L3 > 0.5%, and DCP > 0.10 ng/mL. Biomarker values at the minimum of detection were recorded as the minimum value. Established HCC surveillance levels for the assay determined positive expression: AFP > 20 ng/mL, AFP-L3 > 15%, and DCP > 7.5 ng/mL. The cohort was then stratified by number of positive biomarkers. Patients with a DCP assay > 7.5 ng/mL were reviewed for warfarin use at the time of blood sampling and excluded from the study if actively taking warfarin due to false positive DCP.

Primary Outcome

The primary endpoint was HCC progression beyond Milan Criteria or failure to downstage within Milan Criteria as determined by the multidisciplinary HCC board. TTP was defined from diagnosis to HCC progression and censored for the following: liver transplantation, election to pursue systemic therapy without evidence of tumor progression, > 6 months without follow-up or surveillance imaging, all-cause mortality, or free of disease progression at the time of data analysis. Censoring date was defined as the most recent imaging follow-up date. Progression-free survival (PFS) was defined from diagnosis to HCC progression or death. Transplant-free survival was defined from diagnosis to death.

Statistical Analysis

Data analysis was performed using JMP 13.0 (SAS Institute Inc.) with graphical output generated using Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). Continuous variables were expressed as medians with interquartile ranges. Categorical variables were expressed as numbers and percentages of the cohort. Univariate and multivariate analyses of the factors associated with TTP were performed using the Cox proportional hazards model. Multivariate models were used to control for differences in tumor burden. Multivariate logistic regression included the component *P* value and includes the odds ratio (OR) and 95% confidence interval (CI). Differences in parameters between subgroups were analyzed using the Kruskal-Wallis test followed by the Steel-Dwass test for continuous variables and the Chi-square or Fisher's exact test for categorical variables. Kaplan–Meier curves of TTP were generated using Prism and compared using log-rank tests. The mRECIST score could not be tabulated for patients with missing follow-up data resulting in exclusion from the Chi-square and Kaplan-Meier analyses.

Results

Cohort Overview at Diagnosis

The cohort included 207 patients with early- to intermediate-stage HCC meeting institutional criteria for LDT as a bridge/downstage to liver transplant or definitive treatment plan after excluding 4 patients on warfarin at the time of LDT (Figure 1). General demographics, baseline hepatology, serum chemistry, and radiographic features of HCC are presented in Table 1. The cohort was predominantly male (157/207, 76%) with a median age of 63 years. Cirrhosis etiology was predominantly chronic HCV (116/207, 56%). The cohort had well-compensated cirrhosis with a median Model of End Stage Liver Disease modified for sodium of 9 and Child-Pugh A-B (198/207, 96%). HCC staging was predominantly BCLC-A (182/207, 88%) with 47/207 (23%) having multifocal disease. The median diameter of the largest tumor was 3.1 cm with (130/207) having a tumor size ≤ 3cm, 167/207 (81%) within Milan, and 21/207 (10%) within United Network of Organ Sharing Downstaging criteria. The median biomarker values were 10 ng/mL (AFP), 8.4% (AFP-L3), and 2.9 ng/mL (DCP), each below the recommended threshold for HCC surveillance (AFP > 20 ng/mL, AFP-L3 > 15%, and DCP > 7.5 ng/mL). At surveillance thresholds, 85/207 (41%) were triple negative for AFP, AFP-L3, and DCP, with 62/207 (30%) having 1 positive, 36/207 (17%) with 2 positive, and 24/207 (12%) positive for all 3 biomarkers.

Stratifying Post-LDT Progression Risk by Biomarker Profile at Diagnosis

The median TTP following first cycle LDT was 32 months with a 1-year progression rate of 22% (Figure 2A) and was effectively stratified ($P < .001$) by biomarker profile at diagnosis (Figure 2B). The 1-year progression rates increased from 6% (0+), 17% (1+), 38% (2+), to 64% (3+). Median TTP was reached for 1+ (31 months), 2+ (25 months), and 3+ (10 months) biomarkers. By diagnosis profile (Figure 2C), 41% (85/207) had 0+, 30% (62/207) had 1+, 17% (36/207) 2+, and 12% (3+). All possible biomarker combinations were detected in the cohort (Figure 2D), including single positives for AFP, AFP-L3, or DCP. Patients with 2 positive biomarkers predominantly expressed AFP and AFP-L3 while DCP-containing positive pairs represented the rarest phenotype. Profile at diagnosis also stratified PFS ($P < .001$) and transplant-free survival ($P = .013$) (Figure A1).

Biomarker Profile at Diagnosis and First Cycle Treatment Response

The effect of biomarker accumulation on target and overall treatment outcomes was analyzed using the first cycle mRECIST (Table 2). The target complete response rate declined from 74%, 62%, 57%, to 23% with increasing positive biomarkers accompanied and mirrored by a steady increase in the rate of progressive disease 5%, 10%, 20%, to 32%. A similar trend is observed for the overall first cycle response rate. First cycle approach to LDT was controlled across biomarker profiles ($P = .687$). Multivariate logistic regression comparing multibiomarker positive profiles and transplant criteria with first cycle objective response rate identified 0–1 positive biomarkers having a higher probability of yielding a first cycle objective target (OR 3.3 95% CI 1.6–6.7) and overall response (OR 2.9, 95% CI 1.5–5.5) compared to Milan criteria (Table 3).

Factors Associated With Early- to Intermediate-Stage Disease Progression in the Nonsurgical Track

Disease management track was next examined as a confounding factor for TTP due to early censoring for liver

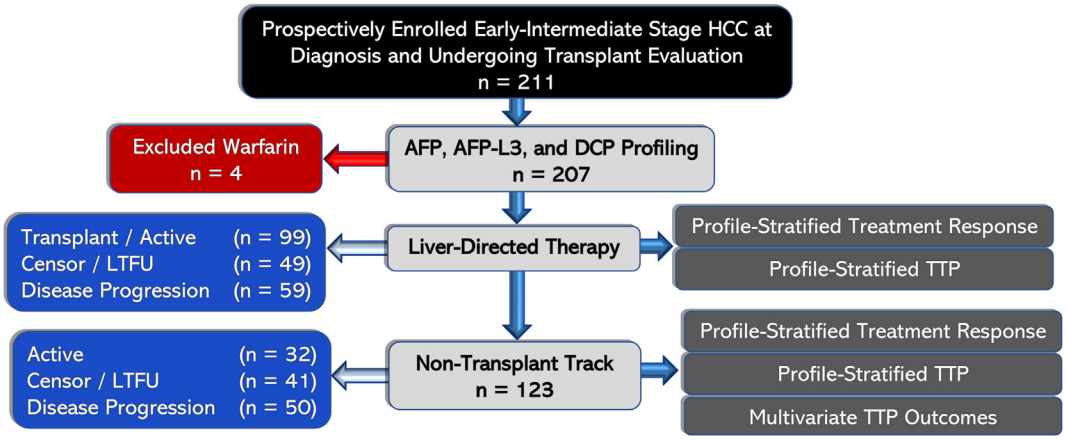

Figure 1. Cohort overview and study outline.

transplant in the surgical track. Surgical track effectively stratified TTP risk ($P = .001$) within the first 2 years after diagnosis, roughly corresponding to the bridge-downstage to transplant window for the center (Figure A2). The 1-year progression rate for transplant track was 6% (transplant track) compared to 32% (nontransplant track) normalizing to 39% (transplant) and 41% (nontransplant) at 2 years. Baseline hepatic reserve and HCC staging were similar between management groups (Table A1). However, intermediate to large index tumor size was more frequent in the nontransplant track resulting in a higher frequency of patients beyond transplant criteria (13% nontransplant track vs 4% transplant track). The median expression level for each biomarker was higher in the nontransplant track and contributed to more frequent multipositive expression profiles. This was particularly evident with the DCP biomarker and resulted in a higher percentage of double and triple positive patients in the nontransplant track ($P = .003$).

The target complete response rate was higher ($P = .014$) at 67% in transplant track compared to 58% in nontransplant track (Table A2) but with a similar overall response rate ($P = .179$). As anticipated, the overall progression rate was higher in nontransplant track (41%, 50/123) compared to transplant track (11%, 9/84). In the transplant track, 71% (60/84) were successfully bridged or downstaged to liver transplant, with a median time from diagnosis to transplant of 9 months.

Multivariate factors associated with TTP were investigated in the nontransplant track to control for the confounders identified in management track analysis. Univariate factors associated with TTP were restricted to measures of HCC burden and biomarker profile (Table 4). The BCLC was chosen for further modeling as it captures factors related to size and burden within a clinically utilized algorithm and avoided the use of transplant criteria variables in the nontransplant track ($P < .001$, HR 4.4, 95% CI 2.1–8.8). Biomarker threshold levels associated with overall risk of tumor progression were an AFP of > 21 ng/mL, AFP-L3 fraction of $> 0.5\%$, and a DCP > 7.1 ng/mL. The continuous, dichotomized, and profile-based biomarkers remained associated with TTP in multivariate analysis controlling for BCLC staging. In the 106 nontransplant track BCLC-A patients, biomarker profile at diagnosis effectively stratified 1-year progression risk with triple negative at a 6% progression rate increasing with each positive biomarker up to 74% at triple positive (Figure 3A). Progression risk was largely driven by the combined expression of AFP > 20 ng/mL and DCP > 7.5 ng/mL (Figure 3B).

Discussion

Although AFP elevation is an established risk factor for aggressive biology early-stage HCC, a level > 1000 ng/mL remains the only threshold precluding liver transplantation.[16] There is also no clear consensus on whether this is the clinically optimal threshold or how this threshold will change as early diagnosis continues to improve.[17–19] United Network of Organ Sharing policy currently requires an AFP response below 500 ng/mL.[20] Variable AFP expression at diagnosis provides additional complexity, as 35%–40% of patients have a normal AFP at diagnosis.[9,10] The expanded HCC biomarker panel, including AFP, AFP-L3, and DCP, has been approved for surveillance and recently studied in the context of posttransplant survival and recurrence risk.[4,12,13,21] This study investigated the number of positive HCC biomarkers as a prognostic factor for first cycle LDT response and means to stratify TTP risk to identify optimal candidates with favorable biology for transplant and nonsurgical candidates with excellent prognosis when managed with LDT as a definitive treatment approach.

In agreement with literature,[22] 63% of patients with early-stage HCC had an AFP < 20 ng/mL, with 47% having a normal AFP < 8 ng/mL at diagnosis. However, 41/127 (32%) of AFP negative patients express AFP-L3 and/or DCP, suggesting a more aggressive biological profile than could be ascertained by measuring AFP alone and increased 1-year HCC progression risk compared to patient truly negative for AFP, AFP-L3, and DCP. Triple positive expression at

Table 1. Transplant Candidate Early-Intermediate Stage Hepatocellular Carcinoma Cohort Demographics

Parameters	Cohort (n = 207)
General demographics	
Age at diagnosis (y), median (IQR)	63 (59–67)
Legal sex (male), number (%)	157 (76)
Declared race, number (%)	
African American/Black	46 (22)
Caucasian/White	149 (72)
Other	12 (6)
Hepatology baseline	
Cirrhosis, number (%)	203 (98)
Cirrhosis etiology, number (%)	
Alcoholic liver disease	18 (9)
Hepatitis C	116 (56)
Hepatitis C + alcoholic liver disease	25 (12)
Nonalcoholic steatohepatitis	35 (17)
Other	13 (6)
Viremic HCV at diagnosis of any HCV etiology, number (%)	75 (53)
Child-Pugh score, number (%)	
A	132 (64)
B	66 (32)
C	9 (4)
MELD-Na at diagnosis, median (IQR)	9 (7–11)
ALBI, number (%)	
Grade 1	37 (18)
Grade 2	140 (68)
Grade 3	30 (14)
Hepatocellular carcinoma baseline	
Diagnosis date, range	4/8/16–10/25/22
ECOG score, number (%)	
ECOG 0	137 (66)
ECOG 1	70 (34)
BCLC staging, number (%)	
BCLC-A	182 (88)
BCLC-B	25 (12)
Multifocal disease, number (%)	47 (23)
Index tumor diameter (cm), median (IQR)	3.1 (2.3–4)
Index tumor size group, number (%)	
Small (0–3 cm)	103 (50)
Intermediate (> 3–5 cm)	82 (39)
Large (> 5cm)	22 (11)
Cumulative tumor diameter (cm), median (IQR)	3.5 (2.5–4.7)
Transplant evaluation outcome, number (%)	
Waitlisted	84 (41)
Non-HCC related contraindication to transplantation	123 (59)
Transplant criteria, number (%)	
Milan	167 (81)
UNOS-DS	21 (10)
Beyond criteria	19 (9)
Biomarker levels at diagnosis, median (IQR)	
AFP (ng/mL)	10 (2.5–57)
AFP-L3 (%)	8.4 (0.5–21)
DCP (ng/mL)	2.9 (0.5–14)
Biomarker positive, number (%)	
AFP > 20 ng/mL	78 (38)
AFP-L3 > 15%	69 (33)
DCP > 7.5 ng/mL	59 (29)
Biomarker profile, number (%)	
0 positive	85 (41)
1 positive	62 (30)
2 positive	36 (17)
3 positive	24 (12)
Liver-directed therapy	
Treatment date, range	8/30/16–12/29/22
Diagnosis to treatment (d), median (IQR)	60 (42–80)

Table 1. Continued

Parameters	Cohort (n = 207)
First cycle treatment modality, number (%)	
DEE-TACE	56 (27)
^{90}Y-TARE	107 (52)
MWA	44 (21)

^{90}Y-TARE, ^{90}-Yittrium Transarterial Radioembolization; AFP, Alpha Fetoprotein; AFP-L3, Lectin-Reactive Fraction of Alpha Fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; DCP, Des-Gamma-Carboxy Prothrombin; DEE-TACE, Doxorubicin-Eluting Embolic Transarterial Chemoembolization; ECOG, Eastern Cooperative Oncology Group; HCC, Hepatocellular Carcinoma; HCV, Hepatitis C virus; IQR, interquartile range; MELD-Na, Model of End Stage Liver Disease modified for sodium; MWA, microwave ablation; UNOS-DS, United Network Organ Sharing Downstaging.

diagnosis, although observed at low frequency of 24/202 (12%) in recently diagnosed BCLC A-B, was one of the most common profiles among HCC expressing positive biomarkers. Consistent with previous studies, the number of positive biomarkers at diagnosis was associated with the risk of HCC progression following LDT,[8,23–26] as well PFS and TFS.

Increased expression of AFP, AFP-L3, or DCP is associated with the risk of microvascular invasion,[4,27–30] which may explain rapid progression and the higher overall risk of

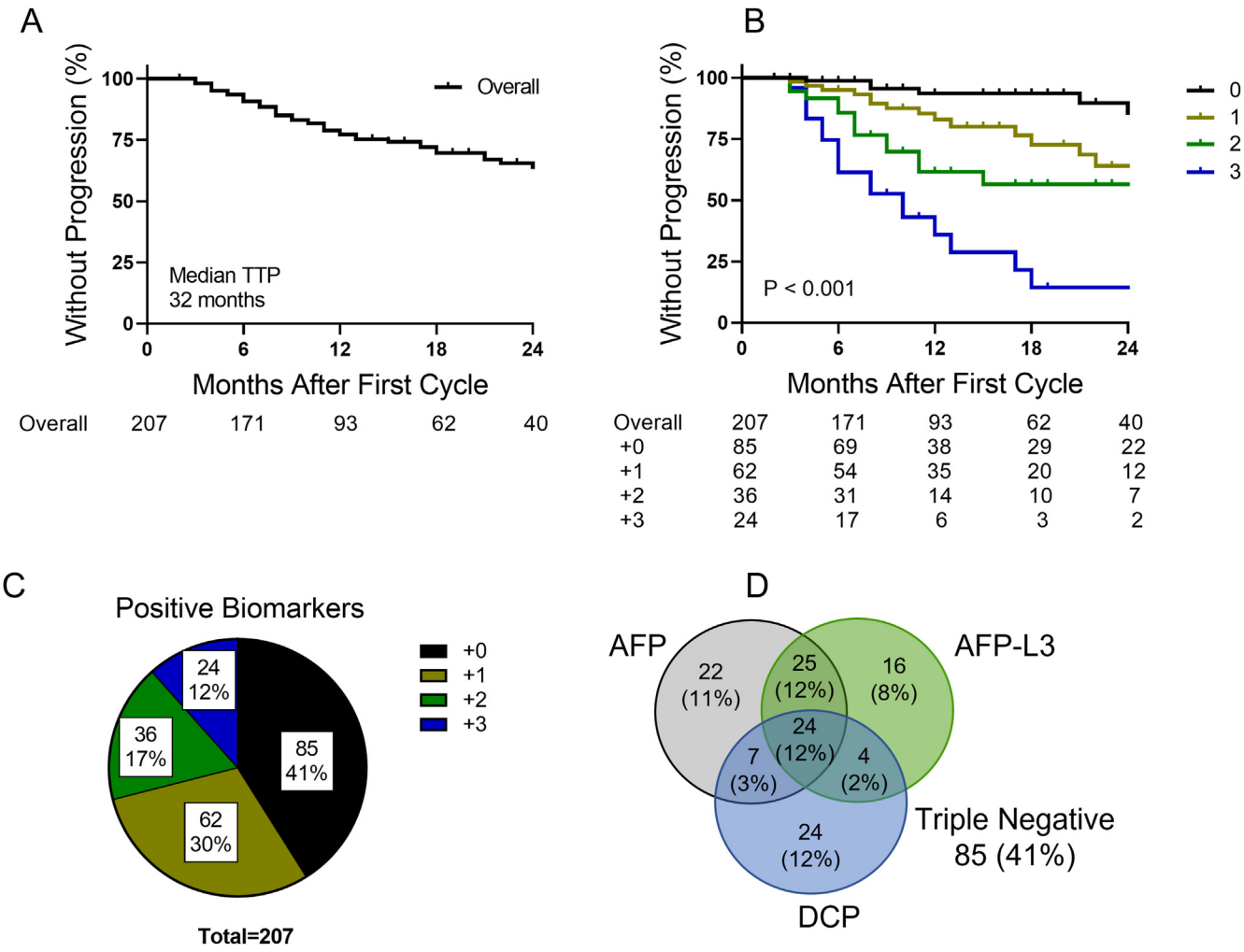

Figure 2. Biomarker Profiles at Diagnosis and Stratification of Time to HCC Progression Following Liver-Directed Therapy. (A) Kaplan Meier curve of time-to-progression for the study cohort. (B) Kaplan Meier curve of time-to-progression utilizing the number of positive biomarkers for stratification and the log-rank test to test for significant differences among the curves. (C) Biomarker breakdown utilizing the number of positive biomarkers. (D) Venn diagram showing overlapping expression of each individual biomarker. Total active patients at each time point listed below the Kaplan Meier plots.

Table 2. Associations Between Biomarker Profile and First Cycle Treatment Response Rates

Parameters	Triple Negative (n = 85)	Single Positive (n = 62)	Double Positive (n = 36)	Triple Positive (n = 24)	*P* value
Liver-directed therapy					
Missing posttreatment imaging	8 (9)	4 (6)	1 (3)	2 (8)	
First cycle target mRECIST score					<.001
Complete response	57 (74)	36 (62)	20 (57)	5 (23)	
Partial response	12 (16)	10 (17)	4 (11)	7 (32)	
Stable disease	4 (5)	6 (10)	4 (11)	3 (14)	
Disease progression	4 (5)	6 (10)	7 (20)	7 (32)	
First cycle overall mRECIST score					<.001
Complete response	54 (70)	27 (47)	16 (46)	3 (14)	
Partial response	10 (13)	9 (16)	4 (11)	5 (23)	
Stable disease	5 (6)	6 (10)	4 (11)	4 (18)	
Disease progression	8 (10)	16 (28)	11 (31)	10 (45)	
Liver-directed therapy					
Treatment date, range	11/18/16–12/29/22	1/5/17–9/23/22	1/27/17–7/1/22	8/30/16–9/29/22	
Diagnosis to treatment (d), median (IQR)	57 (38–72)	61 (44–85)	65 (42–90)	72 (35–92)	.465
First cycle treatment modality, number (%)					.687
DEE-TACE	25 (29)	16 (26)	8 (22)	7 (29)	
^{90}Y-TARE	38 (45)	36 (58)	20 (56)	13 (54)	
MWA	22 (26)	10 (16)	8 (22)	4 (17)	

P values listed are the results of the Chi-square test.
^{90}Y-TARE, ^{90}-Yittrium Transarterial Radioembolization; DEE-TACE, Doxorubicin-Eluting Embolic Transarterial Chemoembolization; IQR, interquartile range; mRECIST, Response Evaluation Criteria in Solid Tumors Modified for HCC; MWA, microwave ablation.

progression as the number of positive biomarkers at diagnosis increases. Accumulating evidence supports the hypothesis that multiple positive biomarkers or elevated expression should warrant more aggressive disease staging.[4,8,12,13] However, comparing AFP, AFP-L3, and DCP across datasets in the literature is challenging due to the wide range of biomarker thresholds and dichotomized data. The original BALAD study utilized a relatively high AFP threshold (400 ng/mL), but with AFP-L3 (10%) and DCP (1.9 ng/mL) levels more commonly associated with surveillance for very early-stage disease.[31] This study utilized thresholds that were internally validated for HCC progression risk and mapped to recommended surveillance threshold levels.[8,32,33] This approach provides continuity in assessing prognosis from surveillance to early intervention while allowing profile data at diagnosis to potentially drive multidisciplinary HCC board treatment algorithms as well as follow-up / surveillance schedules.

Notably, the cohort was well controlled for the initial LDT approach by both biomarker profile and disease management track, yet still revealed a strong association between profile and first cycle response rates. This supports the hypothesis that biomarker profiling may be the key, clinically available metric for defining aggressive biology in early- to intermediate-stage HCC. Despite efforts to eradicate tumor burden by LDT, only 56% (14/25) of patients successfully bridged to transplant showed biomarker profile improvement at the time of transplant, despite 76% (19/25) having a complete radiographic response at transplant. Accumulating evidence suggests persistent biomarker expression despite a complete radiographic response may be a key risk factor of early recurrence posttransplant.[34,35] Future studies should focus on identifying key tumor- and treatment-linked factors that distinguish responsive vs nonresponsive aggressive HCC biomarker profiles.

Table 3. Multivariate Analysis of Biomarker Profile and Transplant Criteria With Target and Overall Objective Response

	Target Objective Response		Overall Objective Response	
	Univariate	Multivariate	Univariate	Multivariate
Parameters	*P* Value	*P* Value (OR, CI)	*P* Value	*P* Value (OR, CI)
Transplant criteria				
Milan vs outside Milan	.097	.150	.044	.071
Number of positive biomarkers				
0–1 vs 2–3	<.001	.001 (3.3, 1.6–6.7)	<.001	.001 (2.9, 1.5–5.5)

CI, 95% confidence interval; OR, Odds ratio.

Table 4. Univariate and Multivariate Analysis of Variables at Diagnosis Associated With Time to Progression in Nontransplant Track Early-Intermediate Stage HCC

Parameters	Univariate *P* Value	Univariate HR (95% CI)	ROC Progression	BCLC + Biomarkers	BCLC + Profile
General demographics					
Age at diagnosis (y)	.326				
Legal sex (male)	.158				
Declared race	.955				
Hepatology baseline					
Cirrhosis	.673				
Cirrhosis etiology	.335				
Viremic HCV at diagnosis of any HCV etiology	.868				
Child-Pugh score	.342				
MELD-Na at diagnosis	.843				
ALBI	.327				
Hepatocellular carcinoma baseline					
ECOG score	.594				
BCLC staging	<.001				
BCLC-B vs BCLC-A		4.4 (2.1–8.8)		2.1 (1.1–3.8)	2.7 (1.2–5.6)
Multifocal vs Solitary disease	.001	3.0 (1.6–5.7)			
Index tumor diameter (cm), per cm increase	<.001	1.3 (1.1–1.4)			
Index tumor size group	.002				
Intermediate vs small		2.7 (1.4–5.3)			
Large vs intermediate		1.4 (0.7–2.9)			
Cumulative tumor diameter (cm), per cm increase	<.001	1.3 (1.1–1.4)			
Transplant criteria	.002				
UNOS-DS vs Milan		1.9 (0.7–4.2)			
Beyond criteria vs UNOS-DS		2.2 (0.8–6.6)			
Biomarker levels at diagnosis					
AFP (ng/mL)	.002		>21 ng/mL		
AFP-L3 (%)	<.001		>0.5%		
DCP (ng/mL)	.006		>7.1 ng/mL		
Biomarker positive					
AFP > 20 ng/mL vs ≤ 20 ng/mL	<.001	4.3 (2.4–8.1)		3.3 (1.7–6.7)	
AFP-L3 > 15% vs ≤ 15%	.003	2.4 (1.3–4.2)		1.2 (0.6–2.2)	
DCP > 7.5 ng/mL vs ≤ 7.5 ng/mL	<.001	3.1 (1.7–5.5)		2.1 (1.1–3.8)	
Biomarker profile	<.001				
1 vs 0 positive		2.5 (1.1–6.7)			2.5 (1.0–6.7)
2 vs 1 positive		2.0 (0.9–4.3)			2.0 (0.9–4.3)
3 vs 2 positive		2.1 (1.0–4.6)			1.7 (0.8–3.7)

P value corresponds to univariate Cox regression.
AFP, Alpha Fetoprotein; AFP-L3, Lectin-Reactive Fraction of Alpha Fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; DCP, Des-Gamma-Carboxy Prothrombin; ECOG, Eastern Cooperative Oncology Group; HCC, Hepatocellular Carcinoma; HCV, Hepatitis C virus; IQR, interquartile range; MELD-Na, Model of End Stage Liver Disease modified for sodium; UNOS-DS, United Network Organ Sharing Downstaging.

The study is based in a single center and limited by center-specific differences in HCC surveillance, LDT treatment algorithms, and bridge to transplant protocols including center volume and wait-time. The study site is in a region with historically high rates of chronic HCV after the introduction of direct-acting antivirals and contributing to high rates of HCV-HCC in the retrospective study period. Despite the high rates of HCV-HCC in the cohort, the data did not support a role of cirrhosis etiology on HCC biomarker profile at diagnosis.

Conclusion

Multibiomarker profiling in patients with early-stage HCC provides advanced knowledge of high-risk tumor biology that may warrant more aggressive intervention and/or surveillance posttreatment. Persistent biomarker expression after LDT may be an integral confirmation of persistent disease and an indicator of increased recurrence risk posttransplant. Biomarker-based risk stratification could have important implications for assessing aggressive tumor biology, particularly in a short wait-time transplant center setting. Given the rapid progression risk associated with double and triple positive profiles, these patients may require individualized (biomarker profile-specific wait-times) or more aggressive therapy (LDT in combination with immune checkpoint inhibitors or other systemic agents). LDT in combination with immune checkpoint inhibitors or other systemic therapies is under active investigation and may emerge as potential therapeutic approach for aggressive biology in early-stage HCC.

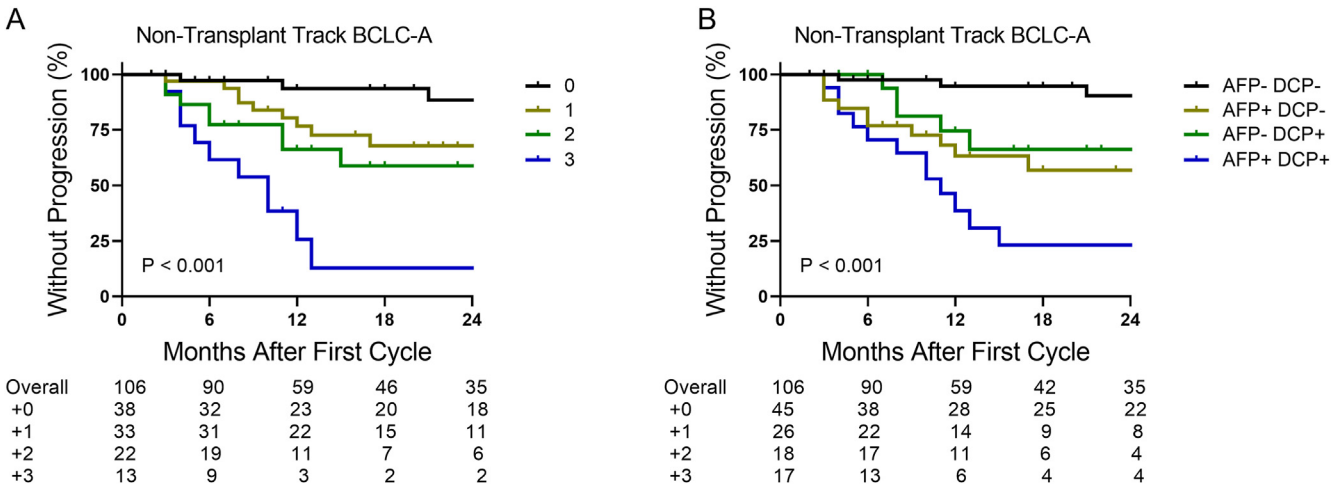

Figure 3. Time to Progression Analysis in Nontransplant Track BCLC-A Stratified by Biomarker Profile. (A) Kaplan Meier curve of time to progression after subgrouping based on biomarker profile using the log-rank test. (B) Kaplan Meier curve of time to progression after subgrouping based specific AFP and DCP expression combinations and examined for curve difference using the log-rank test. Total active patients at each time point listed below the Kaplan Meier plots.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2023.11.018.

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Correspondence:
Address correspondence to: Paul Thevenot, PhD, Institute of Translational Research, Ochsner Clinic Foundation, New Orleans, Louisiana 70121. e-mail: paul.thevenot@ochsner.org.

Authors' Contributions:
Kelley Núñez - formal analysis, investigation, visualization, writing original draft, writing review and editing; Michael Schneider - data curation, formal analysis, investigation, writing original draft; Tyler Sandow - data curation, resources, writing review and editing; Juan Gimenez – resources, writing review and editing; Mina Hibino – data curation; Daniel Fort – software, validation; Ari Cohen – funding acquisition, project administration, resources; Paul Thevenot – conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, visualization, writing original draft, writing review and editing.

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Ethical Statement:
The study was approved by the Ochsner Clinic Foundation Institutional Review Board (protocol 2016.131.B, current approval date 09/08/2023).

Data Transparency Statement:
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Reporting Guidelines:
STROBE.