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Original Article

Predictors of response and survival in patients with unresectable hepatocellular carcinoma treated with nivolumab: real-world experience

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Abstract: Real-world predictors of the treatment efficacy of immune checkpoint inhibitors for hepatocellular carcinoma (HCC) are unknown. This retrospective study enrolled 87 consecutive patients with unresectable HCC from May 2017 to December 2019 at two hospitals. Of the 87 patients, 7, 9, 60, and 11 patients had Barcelona Clinic Liver Cancer stages A, B, C, and D, respectively, and 45, 30, and 10 patients were Child-Pugh class A, B, and C, respectively. The median injection numbers of nivolumab and treatment duration were 6 (3-8) and 2.53 (1.47-4.23) months, respectively, and 64.4% of patients received combination therapy. Radiological imaging was not assessed for 25 patients. Objective response (OR) and disease control rates were 19.5% and 39.1%, respectively. A single tumor (odds ratio: 9.542, P = .015) and ≥20% decline in serum α-fetoprotein protein (AFP) levels within the first 3 months of treatment (defined as AFP response, odds ratio: 5.997, P = .042) were predictors of OR. Lack of macrovascular invasion, combination therapy, and AFP response were predictors of progression-free survival. A Cancer of the Liver Italian Program (CLIP) score of 0-2 (hazard ratio [HR]: 3.717, P = .004) and grade 1-2 immune-related adverse events (irAEs, HR: 2.217, P = .049) were predictors of overall survival (OS) in the entire cohort, and a CLIP score of 0-2 (HR: 3.257, P = .009) was a predictor of OS in evaluable patients. IrAEs ≥ grade 3 were noted in 14 patients, and three died as a result. Having a single tumor and AFP response were predictors of OR, and CLIP score was a predictor of OS.

Keywords: AFP, CLIP score, hepatocellular carcinoma, immune-related adverse event, nivolumab, survival

Introduction

Hepatocellular carcinoma (HCC) is an important global health issue [1]. HCC is usually recognized at an advanced stage, despite the recommendation of routine HCC surveillance [2]. The standard of care for advanced HCC has been sorafenib, a multitargeted tyrosine kinase inhibitor (TKI), for a decade [3, 4]. Recently, second-line therapies including regorafenib, cabozantinib, and ramucirumab have been approved in various circumstances [5-7]. Lenvatinib, another multitargeted TKI, was noninferior to sorafenib in the improvement of overall survival (OS) of advanced HCC patients without ≥50% liver occupation or main portal vein invasion [8]. However, the prolonged median survival time with these five drugs is short [3-8].

Immune checkpoint inhibitors (ICIs) are new therapeutic agents against HCC [9]. Two ICIs, antiprogrammed cell death-1 (PD-1) monoclonal antibodies (nivolumab and pembrolizumab), have been conditionally approved for patients with advanced HCC after sorafenib failure. The objective response (OR) rate (ORR) for advan-

ced HCC has been shown to be 14%-20% [9, 10], and the progression-free survival (PFS) and OS were long in a proportion of patients in the early phase I/II or phase II studies. However, further phase III trials of nivolumab as a first-line therapy (versus sorafenib) [11] and pembrolizumab [12] as a second-line therapy (versus placebo) after sorafenib failure did not demonstrate beneficial effects in prolonging survival.

Three recent real-world studies have reported the efficacy of second-line ICIs, nivolumab and pembrolizumab, in advanced HCC patients [13-15]. Real-world reports of using nivolumab for unresectable HCC after failure of TKI or locoregional therapy remain limited. This retrospective study investigated the use of nivolumab therapy in patients with unresectable HCC and considered its clinical features, application in combination therapy, immune-related adverse events (irAEs), and factors associated with therapeutic outcomes, including OR, PFS, and OS.

Patients and methods

Patients

From May 2017 to December 2019, 92 consecutive patients with unresectable HCC from China Medical University Hospital and the affiliated Asia University Hospital in central Taiwan who had received at least one dose of nivolumab therapy were enrolled in this retrospective study. The exclusion criteria were as follows: having a malignancy other than HCC, undergone liver transplantation, and human immunodeficiency virus infection. Among the enrollees, five patients who had received nivolumab therapy but not yet reached the time point of first radiological assessment were excluded from analysis. Of the 87 patients included in the final analysis, 20 died and 5 were lost to follow-up before the first radiological assessment; in total, 62 had evaluable radiological imaging (Supplementary Figure 1). Demographic data and virological features were recorded at baseline. Complete blood count and biochemical data were recorded at baseline, 4, 8, and 12 weeks and then every 2-3 months after the initiation of nivolumab therapy. This study was carried out in accordance with the 1975 Declaration of Helsinki. The Research Ethics Committee of China Medical University Hospital, Taichung, Taiwan (CMUH108-REC3-140) approved the study. Each patient's identification number was encrypted to protect their privacy; thus, the need for informed consent was waived.

Nivolumab dose, tumor assessment, and safety

The nivolumab dose was 2-3 mg/kg every 2 weeks as recommended. Tumor assessment was carried out using dynamic computerized tomography every 8-12 weeks according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) [16]. Safety assessment was performed in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03).

Laboratory tests

We performed complete blood count analyses (Sysmex HST-series, Sysmex, Kanagawa, Japan) and blood biochemistry tests (Beckman Coulter, Brea, CA, USA) in the central laboratory of the hospital. Hepatitis B virus (HBV) infection was determined by the presence of serum HBsAg for more than 6 months, and hepatitis C virus (HCV) infection was defined as the presence of serum anti-HCV antibody for more than 6 months and detectable HCV RNA (detection limit = 15 IU/mL; Roche Diagnostics, Branchburg, NJ, USA). Liver cirrhosis was diagnosed through explicit clinical, ultrasonographic, or pathological analysis.

Statistical analyses

Continuous variables are expressed as the median (first quartile-third quartile). Comparisons of continuous variables between two groups were performed using the Mann-Whitney *U* test. Logistic regression analysis was used to identify factors associated with OR, and Cox regression analysis was used to identify those associated with PFS or OS. Kaplan-Meier analysis with the log-rank test was used to compare the PFS and OS among patient subgroups. Total tumor volume (TTV) was defined as the sum of each tumor volume (formula = [4/3] × 3.14 × [radius of the tumor in cm]^3) [17]. SPSS (IBM SPSS 25.0, NY, USA) was used to perform all statistical analyses. A two-sided *P* value of <.05 was considered statistically significant.

Results

Baseline characteristics

Of the 87 patients, 79 (90.8%) were male and 67 (77%) had liver cirrhosis. In total, 23 (26.4%), 51 (58.6%), 22 (25.3%), and 25 (28.7%) patients reported drinking alcohol, having HBV infection, having HCV infection, and having diabetes mellitus, respectively. The median age was 63.4 (55.4-68.6) years. The neutrophil-lymphocyte ratio (NLR), alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, total bilirubin level, albumin level, international normalized ratio of prothrombin time (INR), and AFP level were 5.53 (3.26-10.23), 51 (29-69) U/L, 70 (43-120) U/L, 1.3 (0.8-2.0) mg/dL, 3.5 (3.0-4.0) g/dL, 1.12 (1.04-1.24), and 296.92 (15.36-7282.00) ng/mL, respectively. The median Child-Pugh score (CPS) was 6 (5-8). In total, 7 (8.0%), 9 (10.3%), 60 (69.0%), and 11 (12.6%) patients had Barcelona Clinic Liver Cancer (BCLC) stages A, B, C, and D, respectively, and the median Cancer of the Liver Italian Program (CLIP) score was 2 (1-4). Most patients (n = 50, 57.5%) had ≥4 hepatic tumors, and the maximum tumor size was 5.2 (2.7-8.3) cm. The TTV was 1032 (245-3844) cm^3. Extrahepatic metastasis (EHM) and macrovascular invasion (MVI) were observed in 52 (59.8%) and 51 (58.6%) patients, respectively, and 32 patients had both EHM and MVI. Only 14 (16.1%) patients received nivolumab as the first-line systemic therapy. The most common prior therapy was transarterial chemoembolization (TACE, n = 49, 56.3%), with a median treatment length of 4 (2-6) sessions, followed by radiotherapy (n = 44, 50.6%). Most patients received combination therapy (n = 56, 64.4%). Patients receiving concurrent nivolumab and TKI therapy (n = 48) had received TKI for more than 7 days before the addition of nivolumab therapy. The most common concurrent TKI therapy was sorafenib (n = 24, 27.6%), followed by lenvatinib (n = 19, 21.8%) (**Table 1**).

Therapeutic response

The median injection numbers of nivolumab and treatment duration were 6 (3-8) and 2.53 (1.47-4.23) months, respectively. A total of 25 patients were not assessed through radiological imaging; among them, 20 (23.0%) died before evaluation and 5 (5.7%) were lost to follow-up because of irAEs (n = 3), economic reasons (n = 1), and rapidly deteriorated bone pain (n = 1). The remaining 62 patients were assessed through radiological imaging. The number of patients with complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) was 9 (10.3%), 8 (9.2%), 17 (19.5%), and 28 (32.2%), respectively. In comparison with patients who died before evaluation, the evaluable patients had higher body mass indices and albumin levels; lower levels of NLR, ALT, AST, total bilirubin, INR, and AFP; lower platelet count, CPS, and CLIP scores; smaller maximum tumor size and TTV; a lower proportion of EHM; a higher proportion of prior or combination therapy; and a longer nivolumab treatment duration, PFS, and OS (Supplementary Table 1). The ORR (CR + PR) and disease control rate (DCR, CR + PR + SD) were 19.5% (17/87) and 39.1% (34/87), respectively. The PFS and OS were 2.67 (1.87-6.90) and 5.87 (2.43-15.93) months, respectively (**Figures 1A**, **2A**, and **Table 1**).

A total of 40 (46.0%) patients experienced at least one irAE of any grade (Supplementary Table 2). A total of 14 patients experienced ≥ grade 3 irAEs, including hepatitis (n = 5), dermatitis (n = 3), pneumonitis (n = 2), fatigue (n = 2), gastric necrosis (n = 1), and colitis (n = 1); 3 patients died from severe hepatic irAEs. The list of patients with ≥ grade 3 irAEs is presented in Supplementary Table 3. No factor was associated with ≥ grade 3 irAEs by multivariate logistic regression analysis (Supplementary Table 4).

Independent predictors of OR (CR + PR)

In 62 patients assessed using radiological imaging, univariate logistic regression analysis revealed that the significantly associated factors were TTV (≤1000 vs >1000 cm^3), tumor number (single vs multiple), MVI (no vs yes), CLIP score (0-2 vs ≥3), CPS (A vs B/C), albumin-bilirubin (ALBI) grade (1 vs 2/3), NLR (≤3.0 vs >3.0), ≥ grade 3 irAEs, and a ≥20% decrease in serum AFP level within the initial 3 months of treatment (defined as AFP response herein). Multivariate logistic regression analysis indicated that tumor number (single vs multiple, odds ratio: 9.542, 95% confidence interval [CI]: 1.537-59.225, P = .015), and AFP response (odds ratio: 5.997, 95% CI: 1.070-33.600, P =

Table 1. Patient demographics, baseline characteristics, and therapeutic response

Character (N = 87)	n (%) or median (IQR)
Age (years)	63.4 (55.4-68.6)
Sex (male), *n* (%)	79 (90.8)
Body mass index (kg/m^2)	23.26 (20.69-26.30)
NLR	5.53 (3.26-10.23)
Platelet count (10^9/L)	153 (95-231)
AST (U/L)	70 (43-120)
ALT (U/L)	51 (29-69)
Total bilirubin (mg/dL)	1.3 (0.8-2.0)
Albumin (g/dL)	3.5 (3.0-4.0)
INR	1.12 (1.04-1.24)
Etiology	
Alcohol	23 (26.4)
HBV	51 (58.6)
HCV	22 (25.3)
Diabetes mellitus	25 (28.7)
Liver cirrhosis	67 (77.0)
Child-Pugh score	6 (5-8)
Class A/B/C	45 (51.7)/30 (34.5)/10 (11.5)
ALBI grade 1/2/3	20 (23.8)/47 (56.0)/17 (20.2)
AFP (ng/mL)	296.92 (15.36-7282.00)
AFP ≥400 ng/mL	39 (44.8)
BCLC stage A/B/C/D	7 (8.0)/9 (10.3)/60 (69.0)/11 (12.6)
CLIP score	2 (1-4)
Max. tumor size (cm)	5.2 (2.7-8.3)
Tumor number	
1/2/3/≥4	20 (23.0)/8 (9.2)/9 (10.3)/50 (57.5)
Total tumor volume (cm^3)	1032 (245-3844)
MVI[a]	51 (58.6)
VP3/VP4/hepatic vein	18 (20.7)/31 (35.6)/2 (2.3)
EHM[a]	52 (59.8)
Prior therapy	
Sorafenib	43 (49.4)
Lenvatinib	7 (8.0)
TACE[c]/TARE	49 (56.3)/2 (2.3)
Radiotherapy	44 (50.6)
Surgery	16 (18.4)
RFA	15 (17.2)
PEI	4 (4.6)
Injection numbers of nivolumab/duration (months)	6 (3-8)/2.53 (1.47-4.23)
Reduction >25%	17 (19.5)
As 1st/2nd/3rd/4th-line systemic therapy	14 (16.1)/55 (63.2)/12 (13.8)/6 (6.9)
Concurrent therapy	56 (64.4)
Sorafenib[b]	24 (27.6)
Regorafenib[b]	5 (5.7)
Lenvatinib[b]	19 (21.8)
Chemotherapy	7 (8.0)
Radiotherapy	13 (14.9)

TACE	11 (12.6)
Therapeutic response	
Best Response	
Complete response	9 (10.3)
Partial response	8 (9.2)
Stable disease	17 (19.5)
Progressive disease	28 (32.2)
Not evaluable	
Death before evaluation	20 (23.0)
Lost to follow-up[d]	5 (5.7)
Objective response	17 (19.5)
Disease control	34 (39.1)
Progression-free survival (months)	2.67 (1.87-6.90)
Overall survival (months)	5.87 (2.43-15.93)

Data presented as the median (first quartile-third quartile). AFP, α-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; EHM, extrahepatic metastasis; CLIP, Cancer of the Liver Italian Program; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range; MVI, macrovascular invasion; NLR, neutrophil-lymphocyte ratio; TACE, transarterial chemoembolization; TARE, transarterial radioembolization; TKI, tyrosine kinase inhibitor; PEI, percutaneous ethanol injection; INR, international normalized ratio; RFA, radiofrequency ablation. [a]In total, 32 HCC patients had both macrovascular invasion and extrahepatic metastasis. [b]A total of 7 patients received sequential TKI therapy because of progressive disease: sorafenib→regorafenib (3), sorafenib→lenvatinib (3), and sorafenib→lenvatinib→regorafenib (1). [c]The median number of TACE sessions was 4 (2-6). [d]In total, 5 patients were lost to follow-up because of immune-related adverse events (*n* = 3), for economic reasons (*n* = 1), and because of rapidly deteriorated bone pain (*n* = 1).

.042) were independent predictors of OR (Supplementary Table 5).

Independent predictors of PFS

In the 87 enrolled patients, univariate Cox regression analysis revealed that the significantly associated factors were grade 1-2 irAEs, TTV (≤1000 vs >1000 cm^3), tumor number (single vs multiple), MVI (no vs yes), CLIP score (0-2 vs ≥3), NLR (≤3.0 vs >3.0), ALT level (≤40 vs >40 U/L), AST level (≤40 vs >40 U/L), CPS (A vs B/C), combination therapy, concurrent TKI therapy, concurrent TACE, AFP response, and OR. Multivariate Cox regression analysis indicated that lack of MVI (hazard ratio [HR] = 4.266, 95% CI: 1.822-9.988, P = .001), nivolumab monotherapy (HR = 0.107, 95% CI: 0.046-0.248, P<.001), and AFP response (HR = 3.454, 95% CI 1.631-7.317, P = .002) were independent predictors of PFS (**Table 2**). Because most of the enrolled patients had BCLC stage C (*n* = 60, 69.0%), which is a confounding variable for MVI and EHM, BCLC stage was not analyzed as a variable.

Kaplan-Meier analyses revealed that the probability of PFS differed significantly between patients with and without MVI (**Figure 1B**, P = .001), with and without combination therapy (**Figure 1C**, P<.001), and with and without AFP response (**Figure 1D**, P = .001).

Independent predictors of OS

Univariate Cox regression analysis identified the following significantly associated factors: grade 1-2 irAEs, TTV (≤1000 vs >1000 cm^3), tumor number (single vs multiple), MVI (no vs yes), CLIP score (0-2 vs ≥3), CPS (A vs B/C), ALBI grade (1 vs 2/3), NLR (≤3.0 vs >3.0), ALT level (≤40 vs >40 U/L), combination therapy, concurrent TACE, and OR. Multivariate Cox regression analysis indicated that grade 1-2 irAEs (HR: 2.217, 95% CI: 1.005-4.892, P = .049) and CLIP score (0-2 vs ≥3, HR: 3.717, 95% CI: 1.537-8.988, P = .004) were independent predictors of OS in all patients (**Table 3**). Kaplan-Meier analyses revealed that the probabilities of survival were significantly different between patients with higher (≥3) or lower (0-2) CLIP score (**Figure 2B**, P<.001) and those with or without grade 1-2 irAEs (**Figure 2C**, P = .015).

In 62 patients assessed using radiological imaging, the OS was 8.70 (4.53-20.60) months (Supplementary Figure 2A and Supplementary Table 1). Multivariate Cox regression analysis revealed that CLIP score (0-2 vs ≥3, HR: 3.257, 95% CI: 1.349-7.866, P = .009) was an inde-

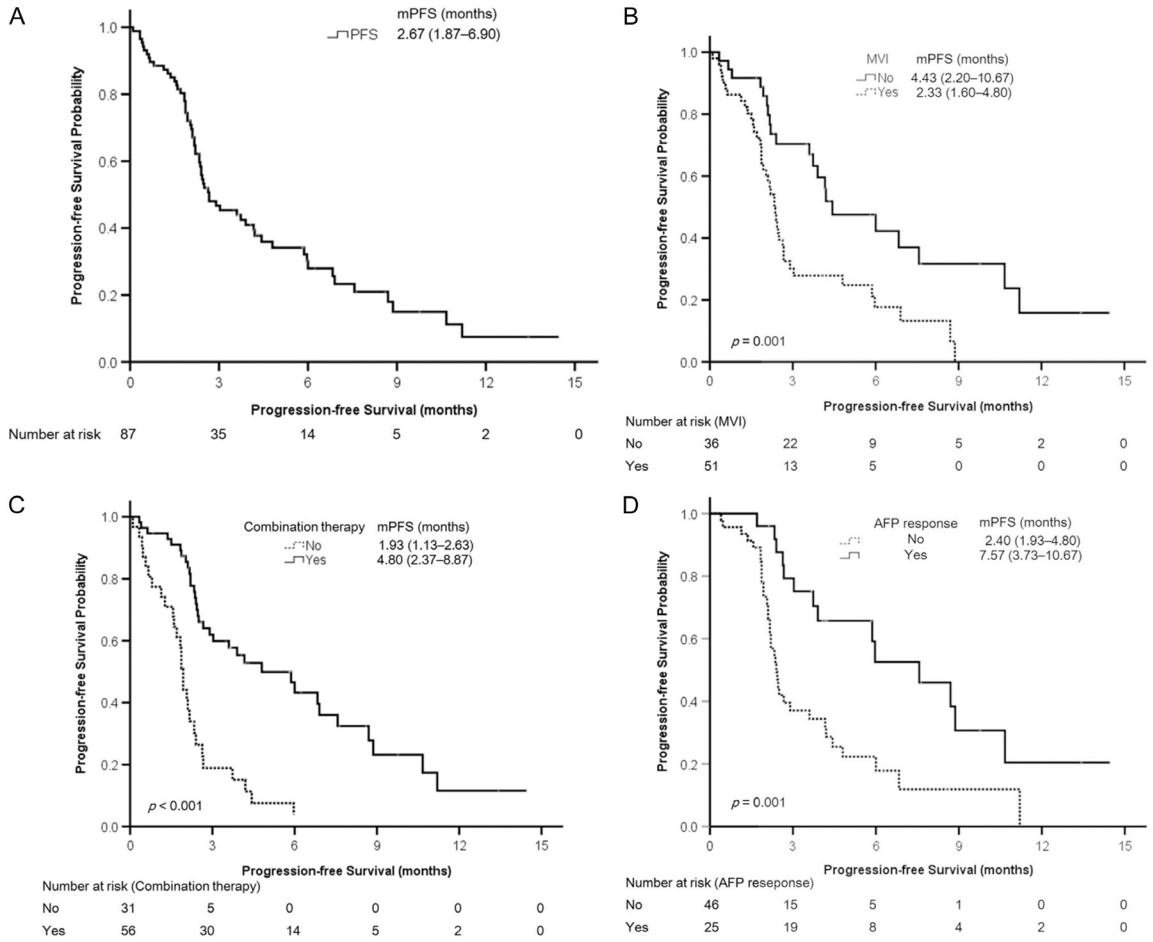

Figure 1. Kaplan-Meier analyses of progression-free survival. A. All patients. B. Patients with or without macrovascular invasion (MVI). C. Patients with or without combination therapy. D. Patients with or without an AFP response. Continuous variables are presented as the median (first quartile-third quartile). AFP, α-fetoprotein protein; mPFS, median progression-free survival.

pendent predictor of OS (**Table 4**). Patients with lower (0-2) CLIP scores had longer OS than did those with higher (≥3) CLIP scores (Supplementary Figure 2B). The results of Kaplan-Meier analyses for the probability of survival in patients with CR, PR, SD, and PD are presented in **Figure 2D**. Furthermore, we classified patients with ≥ grade 3 irAEs related to nivolumab therapy into a fifth group; the results of Kaplan-Meier analyses for the probability of survival in these patients with CR, PR, SD, PD, and ≥ grade 3 irAEs are provided in **Figure 2E**. All patients with CR or PR were alive as of December 2019. Patients with OR but without ≥ grade 3 irAEs (*n* = 11) had significantly longer OS compared with those with OR and ≥ grade 3 irAEs (*n* = 7; 21.87 [8.7-21.87] vs 5.97 [5.87-8.70] months, P = .007).

Discussion

In this real-world study of nivolumab therapy for patients with unresectable HCC, we determined an ORR and DCR of 19.5% and 39.1%, respectively, and discovered that having a single tumor and AFP response were predictors of OR. Furthermore, the median OS was 5.87 months, and a CLIP score of 0-2 and grade 1-2 irAEs were predictors of OS in the entire cohort. The median OS was 8.70 months, and a CLIP score of 0-2 was a predictor of OS in the patients with evaluable radiological imaging (*n* = 62). Patients who achieved an OR without developing ≥ grade 3 irAEs had a median survival of 21.87 months.

ICIs are a newly approved class of agents for the treatment of cancer, including HCC. Pivotal

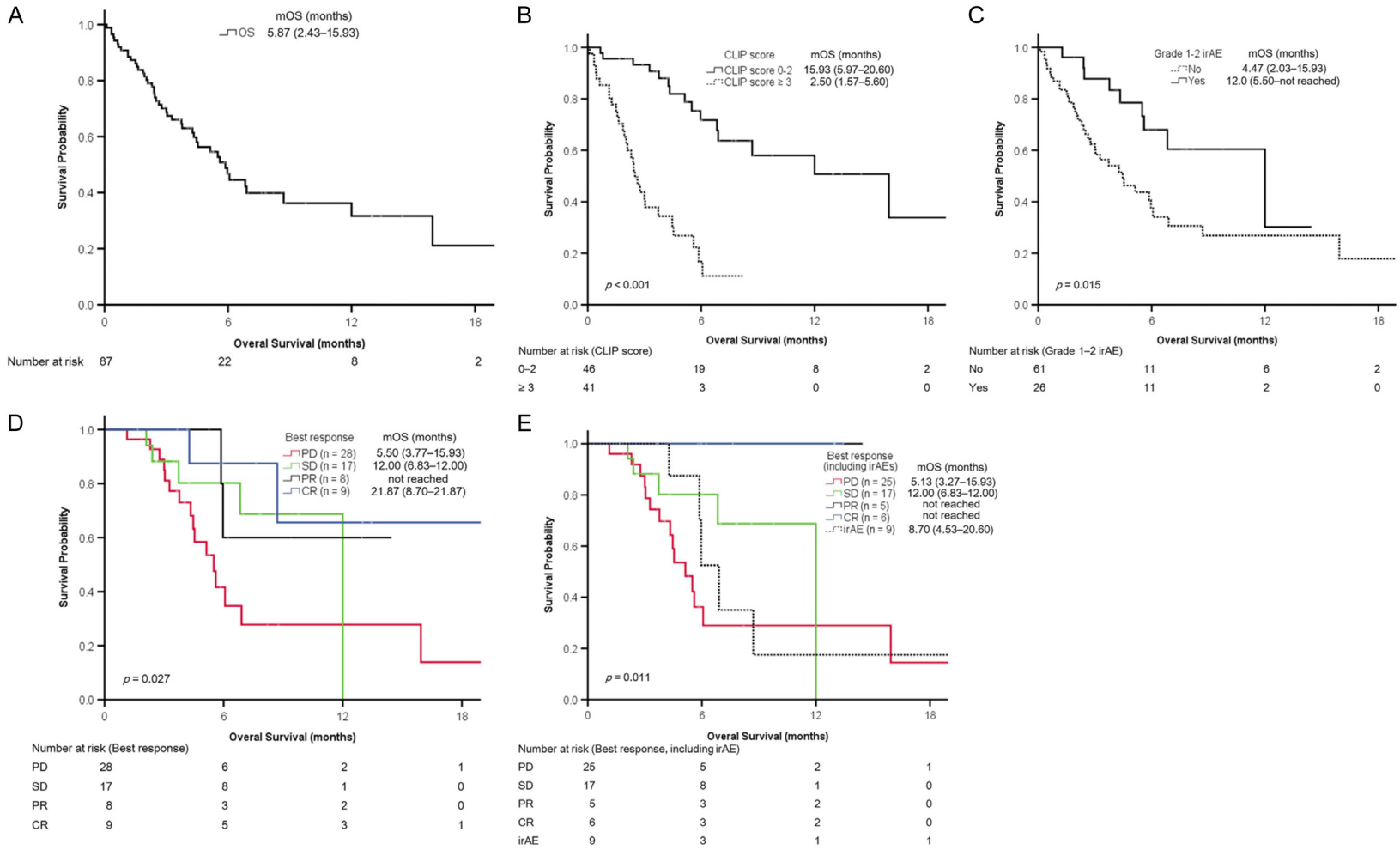

Figure 2. Kaplan-Meier analyses of overall survival. A. All patients. B. Patients with higher (≥3) or lower (0-2) CLIP score. C. Patients with or without grade 1-2 irAEs. D. Patients with CR, PR, SD, or PD. E. Patients with CR, PR, SD, PD, or ≥ grade 3 irAEs. Continuous variables are presented as the median (first quartile-third quartile). CLIP, Cancer of the Liver Italian Program; CR, complete response; irAE, immune-related adverse event; mOS, median overall survival; PD, progressive disease; PR, partial response; SD, stable disease.

Table 2. Factors associated with progression-free survival in 87 patients with hepatocellular carcinoma

Character		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (year)		0.991 (0.967-1.015)	.453		
Sex	Female vs male	0.898 (0.407-1.982)	.790		
Alcohol	No vs yes	1.174 (0.661-2.086)	.583		
HBV	No vs yes	0.916 (0.552-1.518)	.733		
HCV	No vs yes	0.830 (0.461-1.495)	.535		
Grade 1-2 irAEs	Yes vs no	2.042 (1.138-3.664)	.017		
Grade ≥3 irAEs	Yes vs no	1.032 (0.536-1.990)	.924		
TTV (cm^3)	≤1000 vs >1000	2.118 (1.274-3.523)	.004		
Tumor number	Single vs multiple	2.995 (1.462-6.135)	.003		
MVI	No vs yes	2.388 (1.3734.153)	.002	4.266 (1.822-9.988)	.001
EHM	No vs yes	1.497 (0.887-2.528)	.131		
CLIP score	0-2 vs ≥3	3.967 (2.243-7.015)	<.001		
AFP (ng/mL)	<400 vs ≥400	0.959 (0.573-1.606)	.874		
NLR	≤3.0 vs >3.0	2.530 (1.147-5.582)	.021		
AST (U/L)	≤40 vs >40	2.274 (1.020-5.066)	.045		
ALT (U/L)	≤40 vs >40	2.010 (1.155-3.500)	.014		
Child-Pugh class	A vs B/C	1.905 (1.139-3.186)	.014		
ALBI grade	1 vs 2/3	1.867 (0.966-3.607)	.063		
Prior therapy					
Sorafenib	No vs yes	0.948 (0.573-1.567)	.835		
Lenvatinib	No vs yes	1.100 (0.498-2.428)	.814		
Concurrent therapy	No vs yes	0.261 (0.151-0.451)	<.001	0.107 (0.046-0.248)	<.001
TKI	No vs yes	0.472 (0.274-0.814)	.007		
TACE	No vs yes	0.354 (0.141-0.888)	.027		
Radiotherapy	No vs yes	1.685 (0.751-3.779)	.206		
AFP response	Yes vs No	2.853 (1.502-5.422)	.001	3.454 (1.631-7.317)	.001
Best response	CR + PR vs none	4.166 (1.942-8.936)	<.001		

AFP, α-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIP, Cancer of the Liver Italian Program; CR + PR, complete response plus partial response; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; irAEs, immune-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-lymphocyte ratio; TACE, transarterial chemoembolization; TKI, tyrosine kinase inhibitor; TTV, total tumor volume.

clinical trials have reported ORRs of ICI monotherapy in advanced HCC patients in the range of 15%-20% [9, 10, 12]. Three recent real-world studies of patients with advanced or unresectable HCC reported ORRs of 11.8%, 12.3%, and 24.4% with nivolumab or pembrolizumab therapy [13-15]. Our result in an early cohort of patients with unresectable HCC is consistent with the results of the aforementioned studies. Because ICI therapy is costly and only effective in some patients, a baseline or early on-treatment biomarker of response is desirable to facilitate individualized therapy. Two recent studies revealed that early AFP response, defined as a >20% decrease in serum AFP level within the initial 4 weeks of treatment [18] or a >10% decrease in serum AFP level within the initial 4 weeks of treatment in patients with baseline AFP ≥10 ng/mL [15], could respectively predict PFS and OS [18] or OR and OS [15] in patients receiving ICI therapy. Our study demonstrated that a ≥20% decrease in serum AFP level within the initial 3 months of treatment is a predictor of PFS and OR. Because not all patients underwent monthly AFP measurements during treatment, we were unable to compare the predictive performance of AFP response at 1 or 2 months with that at 3 months. Together, these three studies highlighted the role of AFP measurement

Table 3. Factors associated with overall survival in 87 patients with hepatocellular carcinoma

Character		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (year)		0.998 (0.970-1.026)	.872		
Sex	Female vs male	2.163 (0.659-7.105)	.203		
Alcohol	No vs yes	1.347 (0.710-2.556)	.362		
HBV	No vs yes	0.614 (0.342-1.105)	.104		
HCV	No vs yes	1.053 (0.533-2.081)	.882		
Grade 1-2 irAEs	Yes vs no	2.419 (1.157-5.055)	.019	2.217 (1.005-4.892)	.049
Grade ≥3 irAEs	Yes vs no	1.290 (0.573-2.905)	.539		
TTV (cm^3)	≤1000 vs >1000	3.511 (1.858-6.633)	<.001		
Tumor number	Single vs multiple	3.144 (1.234-8.012)	.016		
MVI	No vs yes	2.806 (1.443-5.458)	.002		
EHM	No vs yes	1.614 (0.855-3.049)	.140		
CLIP score	0-2 vs ≥3	6.146 (3.042-12.418)	<.001	3.717 (1.537-8.988)	.004
AFP (ng/mL)	<400 vs ≥400	1.605 (0.878-2.935)	.124		
NLR	≤3.0 vs >3.0	14.533 (1.993-105.976)	.008		
AST (U/L)	≤40 vs >40	1.633 (0.687-3.885)	.267		
ALT (U/L)	≤40 vs >40	2.080 (1.086-3.985)	.027		
Child-Pugh class	A vs B/C	2.886 (1.556-5.355)	.001		
ALBI grade	1 vs 2/3	4.202 (1.492-11.840)	.007		
Prior therapy					
Sorafenib	No vs yes	0.852 (0.475-1.528)	.591		
Lenvatinib	No vs yes	1.170 (0.491-2.788)	.723		
Concurrent therapy	No vs yes	0.399 (0.222-0.716)	.002		
TKI	No vs yes	0.635 (0.353-1.143)	.130		
TACE	No vs yes	0.197 (0.047-0.818)	.025		
RT	No vs yes	1.383 (0.543-3.518)	.496		
AFP response	Yes vs No	1.872 (0.880-3.796)	.106		
Best response	CR + PR vs none	4.935 (1.749-13.920)	.003		

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIP, Cancer of the Liver Italian Program; CR + PR, complete response plus partial response; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; irAEs, immune-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-lymphocyte ratio; RT, radiotherapy; TACE, transarterial chemoembolization; TKI, tyrosine kinase inhibitor; TTV, total tumor volume.

in predicting the response to ICI therapy for HCC. Furthermore, we demonstrated that having a single tumor was a predictor of response to nivolumab therapy. One possible explanation is that patients with a single tumor presented with a significantly smaller tumor volume (TTV 336.5 [28.2-3646.6] cm^3 vs 1244.3 [279.3-4216.5] cm^3, P = .049) and lower proportion of MVI (30% vs 67.2%, P = .003) than did those with multiple tumors. To determine whether tumor burden and vascular invasion negatively affect response to ICI therapy, further study is necessary.

Median OS was relatively short for the entire cohort and for the subgroup of patients with evaluable radiological imaging. This finding has several possible explanations. First, we enrolled a high proportion of patients with poor liver reserve or advanced HCC (11.5% Child-Pugh class C, median tumor size 5.2 cm, 58.6% MVI, 12.6% BCLC stage D, and 47.1% CLIP score ≥3). Only 18.4% of our patients received prior surgery, and 50.6% received prior palliative radiotherapy (Supplementary Table 6) [13-15, 18]. This indicates that our patients initially presented with a less favorable liver reserve and a larger, more advanced, and unresectable HCC compared with previous study cohorts. Second, 23% of our patients died before the first radiological assessment, which accounts for the short OS of the entire cohort. These

Table 4. Factors associated with overall survival in 62 patients with hepatocellular carcinoma who underwent evaluable radiological imaging

Character		Univariate analysis		Multivariate analysis	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (year)		0.985 (0.947-1.024)	.452		
Sex	Female vs male	2.171 (0.497-9.479)	.303		
Alcohol	No vs yes	1.237 (0.505-3.032)	.642		
HBV	No vs yes	0.754 (0.338-1.679)	.489		
HCV	No vs yes	0.891 (0.333-2.380)	.817		
Grade 1-2 irAEs	No vs yes	1.786 (0.737-4.331)	.199		
Grade ≥3 irAEs	Yes vs no	1.005 (0.373-2.710)	.992		
TTV (cm^3)	≤1000 vs >1000	3.500 (1.545-7.929)	.003		
Tumor number	Single vs multiple	3.355 (0.996-11.302)	.051		
MVI	No vs yes	3.222 (1.343-7.733)	.009		
EHM	No vs yes	1.020 (0.451-2.304)	.962		
CLIP score	0-2 vs ≥3	4.333 (1.811-10.367)	.001	3.257 (1.349-7.866)	.009
AFP (ng/mL)	<400 vs ≥400	1.721 (0.779-3.800)	.179		
NLR	≤3.0 vs >3.0	9.543 (1.282-71.038)	.028		
AST (U/L)	≤40 vs >40	1.354 (0.456-4.014)	.585		
ALT (U/L)	≤40 vs >40	2.571 (1.066-6.204)	.036		
Child-Pugh class	A vs B/C	2.152 (0.960-4.826)	.063		
ALBI grade	1 vs 2/3	11.028 (1.476-82.406)	.019		
Prior therapy					
Sorafenib	No vs yes	1.476 (0.672-3.241)	.332		
Lenvatinib	No vs yes	2.059 (0.808-5.2247)	.130		
Concurrent therapy	No vs yes	0.611 (0.270-1.383)	.237		
TKI	No vs yes	0.730 (0.336-1.585)	.426		
TACE	No vs yes	0.302 (0.071-1.295)	.107		
RT	No vs yes	1.515 (0.486-4.721)	.473		
AFP response	Yes vs no	1.369 (0.600-3.124)	.455		
Best response	CR + PR vs none	3.402 (1.160-9.982)	.026		

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIP, Cancer of the Liver Italian Program; CR + PR, complete response plus partial response; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; irAEs, immune-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-lymphocyte ratio; RT, radiotherapy; TACE, transarterial chemoembolization; TKI, tyrosine kinase inhibitor; TTV, total tumor volume.

patients had significantly less favorable liver reserve (CPS and ALBI) and more advanced tumor stage (TTV, CLIP score, and BCLC stage) compared with patients who survived beyond the first radiological assessment (Supplementary Table 1). Hence, these patients might not be ideal candidates for nivolumab therapy in a clinical setting. Third, nivolumab became available to us in May 2017, and the median treatment duration was 2.53 months. A longer follow-up of the present cohort is required to demonstrate the real-world efficacy of nivolumab therapy for treating advanced HCC. Notably, all patients who achieved CR or PR without developing ≥ grade 3 irAEs during treatment were alive at the end of this study.

We identified a CLIP score of 0-2 as a predictor of OS in the entire cohort and in patients with evaluable radiological response. CLIP is a prognostic system originally used on a retrospective cohort from 16 Italian institutions (n = 435) [19]. Three parameters of the CLIP system represent tumor characteristics, namely tumor number and extent, portal vein invasion, and AFP level, and the fourth parameter is Child-Pugh class. This composite scoring system reflects both the tumor burden and the liver reserve and serves to prognosticate patients with HCC. The CLIP score is straightforward to use and is reportedly an excellent prognostic tool for patients with early to advanced HCC [20]. We demonstrated for the first time that

CLIP score is an independent predictor of survival that can stratify the probability of survival in HCC patients receiving nivolumab therapy in real-world settings. Further large-scale research is needed to validate the role of CLIP score as a prognostic tool in HCC patients receiving nivolumab therapy.

Combination therapy was identified as a predictor of PFS in this study. One interpretation of this finding is that concurrent therapy in addition to nivolumab therapy acts synergistically to inhibit tumor growth and thereby enhance the patient's response rate. Combination therapy could not be demonstrated to be a predictor of OS perhaps because of the limited number of enrolled patients (n = 87) with relatively short follow-up period. This appears to agree with the findings of several recent studies that reported an increased therapeutic efficacy of combination therapy such as pembrolizumab plus lenvatinib [21], nivolumab plus ipilimumab [22], or atezolizumab plus bevacizumab in patients with advanced HCC [23]. Notably, our patients received various modalities of concurrent therapy, including TKIs, radiation, and TACE. Whether all modalities provided synergistic effects could not be further evaluated because of the small number of patients receiving each combination therapy. The apparent beneficial effects of combination therapy could also be attributable to the possibility that patients with less advanced HCC, in the presence of better-preserved liver function, were preferentially selected for combination therapy. This may have biased the combination group toward a more favorable survival outcome. The observation that combination therapy was not a predictor of OR or OS in the evaluable patients (n = 62) in this study supports this hypothesis. Hence, the synergistic effect of concurrent therapy with ICIs in advanced HCC patients should be validated in future studies in real-world settings.

ICIs can trigger T cell immunity against cancer and self-antigens, resulting in the occurrence of various irAEs [24]. The incidence of ≥ grade 3 irAEs for nivolumab and pembrolizumab is in the range of 10%-20% in patients with HCC [25]. A tendency of a higher incidence of hepatic irAEs in HCC patients has also been reported [25]. In the current study, 14 patients (16.1%) developed hepatic irAEs of any grade; five patients (5.7%) developed ≥ grade 3 hepatitis, and three of them died. Moreover, patients who achieved OR but developed ≥ grade 3 irAEs (n = 7) had significantly shorter OS than did those who achieved OR without developing ≥ grade 3 irAEs (n = 11). This finding highlights the crucial role of ≥ grade 3 irAEs in determining the outcomes of patients with HCC receiving ICIs. However, we failed to identify any predictors of ≥ grade 3 irAEs. Previous studies revealed that melanoma or lung cancer patients receiving nivolumab therapy with irAEs (82% or more patients with grade 1-2 irAEs) had a better survival outcome [26, 27]. Our finding that grade 1-2 irAEs was a predictor of OS in all enrolled patients (n = 87) was consistent with these results. Alternatively, patients with longer OS may have higher probability of developing irAEs. IrAEs of any grade were not a predictor of OR in this study (Supplementary Table 5). Further studies are needed to clarify the underlying mechanism for their association. Thus, vigilant surveillance, early recognition, and prompt management of irAEs to prevent its progression into ≥ grade 3 severity are imperative to securing favorable patient outcomes.

There are several limitations to this study. First, this retrospective study was performed at two hospitals, and only 87 patients were enrolled. Second, the follow-up period was short, and patients who achieved OR without developing ≥ grade 3 irAEs had not yet reached the median OS time by the end of the study period. The follow-up period should be extended to reveal the beneficial effect of ICIs in patients with HCC. Third, we used mRECIST [16] instead of RECIST version 1.1 for the assessment of radiological response [28] because 56.3% and 50.6% of our patients had received prior TACE and radiotherapy, respectively, for which mRECIST serves as a better assessment tool [29]. Furthermore, it has been demonstrated that mRECIST ORR is an independent predictor of survival in advanced HCC patients treated with molecular targeted therapy [14, 29, 30]. Fourth, we did not assess the association of PD-L1 expression in the tumor or immune cells with OR. Further study is warranted to explore the predictive role of PD-L1 expression for treatment response.

In conclusion, nivolumab was effective for treating some patients with unresectable HCC. Having a single tumor and AFP response were

predictors of OR, and CLIP score was a predictor of OS in the evaluable patients. Patients who achieved OR without developing ≥ grade 3 irAEs exhibited the best survival. Appropriate selection of patients with less advanced disease status and high vigilance for irAEs during nivolumab therapy may help to improve survival in unresectable HCC patients.

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Disclosure of conflict of interest

None.

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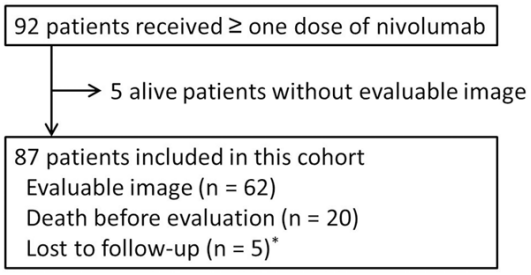


Supplementary Figure 1. Flow chart of patients enrolled in this study. * Five patients were lost to follow-up for more than 3 months.

Supplementary Table 1. Demographics of patients with evaluable radiological imaging data or death before evaluation

Character (N = 82)	Evaluable imaging (N = 62)	Death before evaluation (N = 20)	*P* value
Age (years)	62.1 (54.8-67.6)	66.8 (58.9-69.9)	.214
Sex, M/F (% male)	56 (90.3)	19 (95.0)	.518
Body mass index (kg/m^2)	24.80 (21.89-27.05)	20.83 (19.72-22.85)	<.001
NLR	4.76 (2.98-8.34)	9.96 (5.68-15.40)	.002
Platelet count (10^9/L)	141 (91-198)	175 (150-265)	.039
AST (U/L)	60 (41-100)	147 (96-274)	<.001
ALT (U/L)	50 (28-62)	61 (33-129)	.042
Total bilirubin (mg/dL)	1.1 (0.7-1.7)	1.9 (1.2-12.2)	.003
Albumin (g/dL)	3.7 (3.2-4.0)	3.0 (2.5-3.5)	.002
INR	1.09 (1.04-1.20)	1.24 (1.14-1.58)	<.001
Etiology			
Alcohol	16 (25.8)	7 (35.0)	.429
HBV	40 (64.5)	8 (40.0)	.054
HCV	14 (22.6)	6 (30.0)	.504
Diabetes mellitus	18 (29.0)	6 (30.0)	.934
Liver cirrhosis	45 (72.6)	17 (85.0)	.264
Child-Pugh score	6 (5-7)	8 (6-11)	<.001
Class A	37 (59.7)	5 (25.0)	
Class B	20 (32.3)	9 (45.0)	
Class C	3 (4.8)	6 (30.0)	
ALBI grade 1/2/3	15 (25.4)/37 (62.7)/7 (11.9)	3 (15.0)/9 (45.0)/8 (40.0)	.023
AFP (ng/mL)	337.6 (11.8-6963.0)	1651.9 (41.8-36835.5)	.261
AFP ≥400 ng/mL	28 (45.2)	10 (50.0)	.474
BCLC stage			<.001
A	6 (9.7)	0 (0)	
B	8 (12.9)	0 (0)	
C	45 (72.6)	13 (65.0)	
D	3 (4.8)	7 (35.0)	
CLIP score	2 (1-4)	4 (3-5)	<.001
Max. tumor size (cm)	4.6 (2.7-7.8)	7.3 (5.1-10.3)	.017
Tumor number			.141
1	16 (25.8)	2 (10.0)	
2	6 (9.7)	2 (10.0)	
3	7 (11.3)	2 (10.0)	
≥4	33 (53.2)	14 (70.0)	

Total tumor volume (cm^3)	667.2 (185.8-2675.2)	2794.8 (703.6-5886.8)	.011
MVI	34 (54.8)	15 (75.0)	.112
VP3	12 (19.4)	5 (25.0)	
VP4	20 (32.3)	10 (50.0)	
Hepatic vein	2 (3.2)	0 (0)	
EHM	34 (54.8)	16 (80.0)	.046
Prior therapy			.011
Sorafenib	33 (53.2)	6 (30.0)	
Lenvatinib	7 (11.3)	0 (0)	
Surgery	14 (22.6)	2 (10.0)	
TACE/TARE	41 (66.1)/2 (3.2)	5 (25.0)/0 (0)	
RFA	13 (21.0)	0 (0)	
PEI	4 (6.5)	0 (0)	
RT	41 (66.1)	7 (35.0)	
Nivolumab dose	6 (5-10)	2 (1-3)	<.001
Treatment duration	3.62 (2.39-6.03)	0.70 (0.30-1.03)	<.001
Concurrent therapy	46 (74.2)	7 (35.0)	.002
Sorafenib	20 (32.3)*	3 (15.0)	
Regorafenib	4 (6.5)*	3 (15.0)	
Lenvatinib	15 (24.2)*	1 (5.0)	
Chemotherapy	7 (11.3)	0 (0)	
Radiotherapy	11 (17.7)	1 (5.0)	
TACE	11 (17.7)	0 (0)	
irAEs	9 (14.5)	2 (10.0)	.609
Progression-free survival (months)	4.43 (2.33-8.70)	1.13 (0.43-1.93)	<.001
Overall survival (months)	8.70 (4.53-20.60)	1.27 (0.47-1.93)	<.001

Data are presented as the median (first quartile-third quartile). AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; EHM, extrahepatic metastasis; F, female; CLIP, Cancer of the Liver Italian Program; HBV, hepatitis B virus; HCV, hepatitis C virus; irAEs, immune-related adverse events; IQR, interquartile range; M, male; MVI, macrovascular invasion; RT, radiotherapy; NLR, neutrophil-lymphocyte ratio; TACE, transarterial chemoembolization; TARE, transarterial radioembolization; TKI, tyrosine kinase inhibitor; PEI, percutaneous ethanol injection; INR, international normalized ratio; RFA, radiofrequency ablation. *Seven patients received sequential TKI therapy: sorafenib→regorafenib (3), sorafenib→lenvatinib (3), and sorafenib→lenvatinib→regorafenib (1).

Supplementary Table 2. IrAEs in 87 patients with hepatocellular carcinoma

Type of irAE (N = 40)	irAE, N (%)	
	Any grade	Grade ≥3
Hepatitis	14 (16.1)	5 (5.7)
Fatigue	12 (13.8)	2 (2.3)
Dermatitis	11 (12.6)	3 (3.4)
Colitis	9 (10.3)	1 (1.1)
Hand foot syndrome	4 (4.6)	0 (0)
Fever	4 (4.6)	0 (0)
Pneumonitis	2 (2.3)	2 (2.3)
Gastric necrosis	1 (1.1)	1 (1.1)
Myalgia	1 (1.1)	0 (0)
Dizziness	1 (1.1)	0 (0)

irAE, immune-related adverse event.

Supplementary Table 3. List of patients with ≥ grade 3 irAEs

Age (years)	Sex	Nivolumab		Time of irAE (after nivolumab initiation, months)	Type of irAE	Best response	Final status[a]	COD
		Total dose	Average dosage (mg/kg)					
67.6	M	1	2.80	0.40	Gastric necrosis	DBE	E	Infection
65.4	M	6	2.96	4.17	Hepatitis	PR	S	
56.6	M	6	3.11	3.27	Hepatitis	CR	E	irAE
78.1	F	5	3.19	2.70	Colitis	LFU	C	
64.2	M	6	4.02	3.63	Dermatitis	PR	C	
58.2	M	4	1.56	2.17	Fatigue	LFU	C	
67.5	M	13	2.37	5.00	Pneumonitis	CR	E	Liver failure
78.5	M	10	3.53	5.00	Hepatitis	PR	E	Liver failure
76.4	M	2	2.67	2.30	Fatigue	LFU	C	
61.7	M	6	1.57	2.00	Dermatitis	PD	S	
56.4	F	15	3.30	8.00	Dermatitis	CR	E	Liver failure
51.0	M	6	2.94	5.00	Pneumonitis	PD	E	ICH
67.0	M	10	3.06	5.13	Hepatitis	PR	E	irAE
36.9	M	2	2.96	0.93	Hepatitis	DBE	E	irAE

C, censored (lost to follow-up before Dec 31, 2019); COD, cause of death; CR, complete response; DBE, death before evaluation; E, expired; F, female; ICH, intracranial hemorrhage; irAE, immune-related adverse event; LFU, lost to follow-up; M, male; PD, progressive disease; PR, partial response; S, survival. [a]Final status as of Dec 31, 2019.

Supplementary Table 4. Factors associated with irAEs (grade ≥3) in 87 patients with HCC

Character		Univariate analysis		Multivariate analysis	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (year)		1.012 (0.960-1.067)	.651		
Sex	Female vs male	1.861 (0.335-10.333)	.478		
Alcohol	No vs yes	0.880 (0.247-3.138)	.843		
HBV	No vs yes	0.513 (0.147-1.786)	.294		
HCV	No vs yes	1.290 (0.325-5.126)	.717		
TTV (cm^3)	≤1000 vs >1000	2.065 (0.631-6.760)	.231		
Tumor number	Single vs multiple	2.148 (0.627-7.636)	.224		
MVI	No vs yes	1.517 (0.481-4.781)	.477		
EHM	No vs yes	1.607 (0.509-5.070)	.418		
CLIP score	0-2 vs ≥3	3.981 (1.025-15.459)	.046		
AFP (ng/mL)	<400 vs ≥400	1.189 (0.374-3.785)	.769		
NLR	≤3.0 vs >3.0	1.238 (0.302-5.074)	.767		
AST (U/L)	≤40 vs >40	0.333 (0.040-2.784)	.310		
ALT (U/L)	≤40 vs >40	0.607 (0.174-2.120)	.434		
Child-Pugh class	A vs B/C	3.524 (0.895-13.874)	.072		
ALBI grade	1 vs 2/3	0.935 (0.230-3.797)	.925		
Prior therapy					
Sorafenib	No vs yes	1.370 (0.432-4.344)	.592		
Lenvatinib	No vs yes	0.441 (0.077-2.541)	.360		
Concurrent therapy	No vs yes	0.681 (0.195-2.386)	.549		
TKI	No vs yes	0.434 (0.132-1.421)	.168		
TACE	No vs yes	0.451 (0.103-1.968)	.290		
Radiotherapy	No vs yes	1.556 (0.230-10.534)	.651		
AFP response	Yes vs no	1.500 (0.455-4.946)	.505		

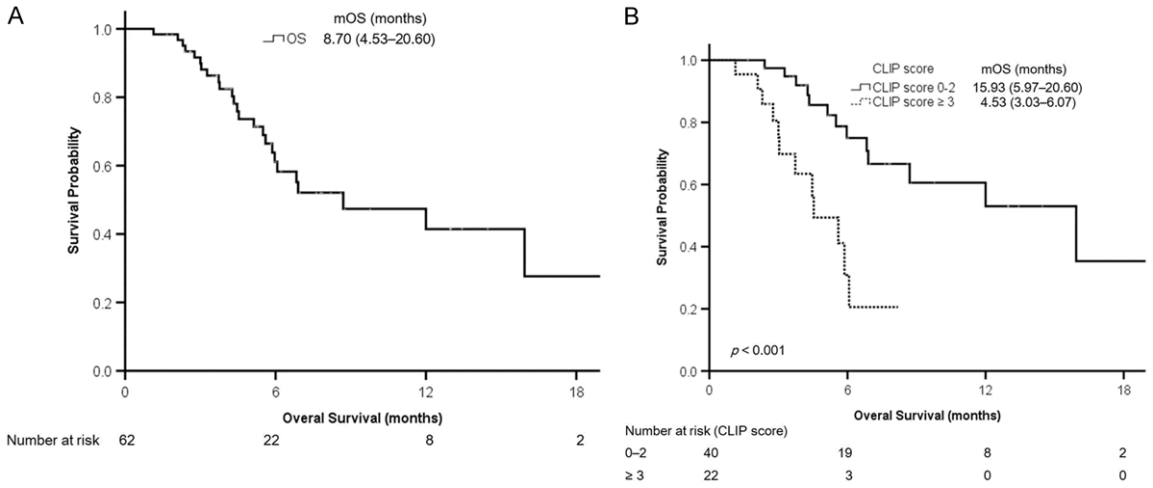
Best response	CR + PR vs none	4.227 (1.226-14.573)	.022

AFP, α-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIP, Cancer of the Liver Italian Program; CR + PR, complete response plus partial response; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; irAEs, immune-related adverse events; MVI, macroscopic vascular invasion; NLR, neutrophil-lymphocyte ratio; TACE, transarterial chemoembolization; TKI, tyrosine kinase inhibitor; TTV, total tumor volume.

Supplementary Table 5. Factors associated with objective response in 62 patients with HCC who underwent radiological assessment

Character		Univariate analysis		Multivariate analysis	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (year)		1.038 (0.982-1.097)	.192		
Sex	Female vs male	1.367 (0.226-8.247)	.733		
Alcohol	No vs yes	0.776 (0.224-2.697)	.690		
HBV	No vs yes	0.686 (0.206-2.290)	.540		
HCV	No vs yes	1.510 (0.365-6.248)	.570		
Any irAEs	Yes vs no	2.095 (0.661-6.646)	.209		
Grade 1-2 irAEs	Yes vs no	0.625 (0.188-2.079)	.443		
Grade ≥3 irAEs	Yes vs no	7.636 (1.643-35.503)	.010		
TTV (cm^3)	≤1000 vs >1000	4.879 (1.231-19.337)	.024		
Tumor number	Single vs multiple	9.286 (2.549-33.832)	.001	9.542 (1.537-59.255)	.015
MVI	No vs yes	6.500 (1.806-23.393)	.004		
EHM	No vs yes	2.143 (0.689-6.668)	.188		
CLIP score	0-2 vs ≥3	6.000 (1.226-29.371)	.027		
AFP (ng/mL)	<400 vs ≥400	0.9338 (0.305-2.879)	.910		
NLR	≤3.0 vs >3.0	6.107 (1.754-21.268)	.004		
AST (U/L)	≤40 vs >40	3.000 (0.820-10.978)	.097		
ALT (U/L)	≤40 vs >40	1.611 (0.520-4.993)	.409		
Child-Pugh class	A vs B/C	4.058 (1.017-16.186)	.047		
ALBI grade	1 vs 2/3	6.750 (1.865-24.425)	.004		
Prior therapy					
Sorafenib	No vs yes	2.750 (0.862-8.771)	.087		
Lenvatinib	No vs yes	2.2462 (0.274-22.116)	.421		
Concurrent therapy	No vs yes	0.846 (0.230-3.108)	.801		
TKI	No vs yes	0.670 (0.217-2.071)	.486		
TACE	No vs yes	0.369 (0.096-1.427)	.149		
Radiotherapy	No vs yes	2.333 (0.393-13.845)	.351		
AFP response	Yes vs no	3.808 (1.153-12.575)	.028	5.997 (1.070-33.600)	.042

AFP, α-fetoprotein; ALBI, albumin-bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLIP, Cancer of the Liver Italian Program; EHM, extrahepatic metastasis; HBV, hepatitis B virus; HCV, hepatitis C virus; MVI, macroscopic vascular invasion; NLR, neutrophil-lymphocyte ratio; TACE, transarterial chemoembolization; TKI, tyrosine kinase inhibitor; TTV, total tumor volume.



Supplementary Figure 2. Kaplan-Meier analyses of the overall survival in patients evaluable through radiological imaging. A. All patients. B. Patients with higher (≥3) or lower (0-2) CLIP score. Continuous variables are presented as the median (first quartile-third quartile). CLIP, Cancer of the Liver Italian Program; mOS, median overall survival.

Supplementary Table 6. Comparison between the present study and other studies

Status	This study	Finkelmeier *et al.* [13][a]	Scheiner *et al.* [14][b]	Shao *et al.* [18][c]	Lee *et al.* [15][d]
Number of patients	87	34	65	43	95
Age	63.4 (55.4-68.6)	65 (range 40-77)	65.2 ± 11.1	55 ± 11.9	65.5 (57.2-72.9)
Sex (male), *n* (%)	79 (90.8)	26 (76.5)	49 (75.4)	35 (81.4)	73 (76.8)
Child-Pugh class A/B/C (%)	51.7/34.5/11.5	55.9/41.2/2.9	49.2/43.1/7.7	100/0/0	72.6/24.2/3.2
AFP ≥400 ng/mL	39 (44.8)	N/A	28 (43.1)	23 (53.5)	53 (55.8)
BCLC stage A/B/C/D (%)	8.0/10.3/69.0/12.6	11.8/38.2/50/0	0/12.3/78.5/9.2	0/7.0/93.0/0	0/21.1/78.9/0
CLIP 0-2/≥3 (%)	52.9/47.1	N/A	N/A	79.1/20.9	N/A
Max. tumor size (cm)	5.2 (2.7-8.3)	N/A	N/A	N/A	5.2 (2.3-8.8)
Multiple tumors, *n* (%)	67 (77.0)	N/A	N/A	N/A	89 (93.7)
MVI, *n* (%)	51 (58.6)	19 (55.9)	24 (36.9)	17 (39.5)	51 (53.7)
EHM, *n* (%)	52 (59.8)	19 (55.9)	35 (53.8)	38 (88.4)	48 (50.5)
Prior treatment					
Sorafenib, *n* (%)	40 (46.0)	25 (73.5)	56 (86.2)	N/A	56 (58.9)
Lenvatinib, *n* (%)	7 (8.0)	N/A	N/A		N/A
TACE, *n* (%)	49 (56.3)	15 (44.1)	30 (46.2)		55 (57.9)
RT, *n* (%)	44 (50.6)				23 (24.2)
Surgery, *n* (%)	16 (18.4)	9 (26.5)	15 (23.1)		35 (36.8)
RFA, *n* (%)	15 (17.2)		9 (13.8)		31 (32.6)
Objective response, *n* (%)	17 (19.5)	4 (11.8)	8 (12.3)	15 (34.9)	22 (24.4)
Disease control, *n* (%)	34 (39.1)	12 (35.3)	32 (49.2)	25 (58.1)	33 (36.7)

AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CLIP, Cancer of the Liver Italian Program; EHM, extrahepatic metastasis; MVI, macrovascular invasion; N/A, not applicable; RT, radiotherapy; TACE, transarterial chemoembolization; RFA, radiofrequency ablation. [a]All patients received nivolumab therapy. [b]A total of 34 patients received nivolumab, and 34 patients received pembrolizumab therapy. [c]Enrolled patients from several clinical trials conducted in one hospital. In total, 27 patients received program cell death-1 (PD1) blockade, 1 patient received anticytotoxic T-lymphocyte antigen 4 (CTLA4), and 15 patients received PD1 blockade plus anti-CTLA4 therapy. [d]A total of 92 patients received nivolumab, and 3 patients received pembrolizumab therapy.