ORIGINAL RESEARCH

Programmed Cell Death Protein-1 (PD-1)-Targeted Immunotherapy for Advanced Hepatocellular Carcinoma in Real World

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Background: Hepatocellular carcinoma (HCC) is one of the most common malignant solid tumors. Its incidence is increasing worldwide due to the dissemination of hepatitis B infection, HCV infection and nonalcoholic steatohepatitis-related HCC. For patients with advanced HCC, the available treatments are extremely limited and the prognosis is very poor. Therefore, it is urgent to discover new innovative approaches. Programmed cell death protein-1-targeted immunotherapy has shown promising results in multicenter clinical trials.

Aim: To evaluate the effectiveness and safety of anti-PD-1 agent in patients with advanced primary hepatocellular carcinoma.

Methods: A retrospective analysis of 55 patients with advanced primary hepatocellular carcinoma who had been administered anti-PD-1 agent. Tumor response was assessed according to the modified Response Evaluation Criteria in Solid Tumors and any adverse events were recorded.

Results: The median overall survival (OS) was 15 months. The median progression-free survival (PFS) was 10 months. No patient had complete response (CR) and 12 (22%) participants achieved partial response (PR), resulting in an overall response rate (ORR) of 22%. Thirty-seven (67%) patients showed stable disease (SD) and 6 (11%) subjects had progressive disease (PD) at first radiological evaluation. The disease control rate (DCR) was 89%. The total side effect rate was 61.8% and most were relieved after treatment.

Conclusion: Programmed cell death protein-1-targeted immunotherapy is a safe and effective treatment for advanced primary hepatocellular carcinoma.

Keywords: hepatocellular carcinoma, programmed cell death protein-1-targeted immunotherapy, immune checkpoint inhibitor, adverse events

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant solid tumors, which is also mainly counted for the death of patients with liver cirrhosis. It is the fifth most common cancer[1] and is the third leading cause of cancer-related death worldwide. On a global scale, liver cancer accounts for more than 850,000 new cancer cases annually, and approximately 90% of these are HCC.[2] Its incidence is increasing worldwide due to the dissemination of hepatitis B infection in Africa and Middle East[3] and increased incidence of HCV infection and nonalcoholic steatohepatitis-related HCC in Europe, and North America.[4] About 80% of victims are distributed in Asian countries. Among them, over 50% of the patients are distributed in China. Most of them are diagnosed at advanced stages with poor

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prognosis and only to receive palliative treatments or best supportive care. The curative treatments can be applied to only 30% of patients. Despite those curative efforts, the survival benefits are still limited, and the majority of patients ultimately experience a recurrence or disease progression. Therefore, there is an urgent need to develop an effective treatment for the patients with HCC. For the last decade, a multi-tyrosine kinase inhibitor, sorafenib, was the only effective drug available for HCC.[5] After the development of sorafenib,[6,7] 4 agents have demonstrated improved outcome data: lenvatinib[8] in the first-line and regorafenib,[9] cabozantinib,[10] and ramucirumab[11] after first-line disease progression. However, their efficacy is limited and lack predictive factors of response.

Immunotherapy has shown promising survival outcome in patients with HCC. Immune checkpoint inhibitor (ICI) demonstrated encouraging efficacy in certain cancer types, particularly in melanoma and lung cancer.[12] Programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated protein-4 (CTLA-4) are the main targets of ICIs.[13] Following by the approval of ipilimumab for malignant melanoma in 2011, a number of ICIs, including three anti-PD-1 antibodies (nivolumab, pembrolizumab, and cemiplimab) and three anti-PD-L1 antibodies (atezolizumab, durvalumab, and avelumab), have been approved by the Food and Drug Administration (FDA) for different types of cancers.[14] In the present clinical trials, Nivolumab and pembrolizumab have shown promising efficacy and safety results in non-comparative, open-label Phase II studies of advanced HCC.[15,16] Based on these studies, the United States Food and Drug Administration (FDA) already granted accelerated conditional approval to both agents for sorafenib-experienced patients with HCC. Both nivolumab and pembrolizumab are currently being investigated in ongoing Phase III trials.

In this study, to evaluate the effectiveness and safety of anti-PD-1 targeted therapy in advanced HCC, we retrospectively analyzed the data of advanced HCC patients treated with nivolumab or pembrolizumab in our hospital.

Materials and Methods
Study Design and Participants
This was a retrospective study of advanced HCC patients treated with anti-PD-1 agent in Chinese People's Liberation Army General Hospital (Beijing, China). Patients with histologically or radiologically confirmed advanced HCC who received PD-1-targeted immunotherapy with nivolumab or pembrolizumab were eligible. We confirmed the written informed consent from patients and patient data confidentiality. The retrospective analysis was approved by Chinese PLA General Hospital Ethics Committees. The recommendations of the Declaration of Helsinki for biomedical research involving human subjects were also followed. Fifty-six patients were recruited in this study.

Dosing of Nivolumab and Pembrolizumab
Nivolumab was administered at 1–3 mg/kg body weight or at a fixed dose of 240 mg every 2 weeks intravenously. Pembrolizumab was given at 2 mg/kg body weight or at a fixed dose of 200 mg every 3 weeks intravenously. AK105, a kind of home-made anti-PD-1 agents in clinical trials, was given at a fixed dose of 200 mg every 3 weeks intravenously. Dose delays were made based on toxicity.

Assessments
Patients had to have measurable disease. Tumor response was assessed according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST).[17] Hyperprogression was defined as a progressive disease (RECIST version 1.1[18]) on the first radiological evaluation during immunotherapy with a delta tumor growth rate of >50%, corresponding to an absolute increase in tumor growth rate exceeding 50% per month.[19]

Statistical Analysis
The primary endpoint was included overall survival (OS), progression-free survival (PFS), the secondary endpoints was included disease control rate (DCR), defined as the percentage of patients achieving a best overall response of either a complete response (CR), partial response (PR), or stable disease (SD) (maintained for at least 8 weeks), objective response rate (ORR), duration of response and toxicity of anti-PD-1 agents.

The Kaplan–Meier method was used to estimate the rates of PFS and OS. PFS was measured from the start date of treatment to the date of documented disease progression or death from any cause, whichever occurred first. The DCR and ORR were estimated by the percentage of patients achieving these criteria. These statistical analyses were performed using SPSS statistical software (version 13.0). A P value <0.05 was considered to be statistically significant.

Results

General Data

This study included 46 male and 9 female patients with a median age of 56 years old (range from 40 to 83 years old). All patients were diagnosed with HCC by pathology or radiology. The clinical-pathological features of the patients, including age, gender, ECOG performance status, liver cirrhosis, extrahepatic metastasis, vascular invasion, Child-Pugh grade, serum Alpha-fetoprotein (AFP) level, hepatitis B surface antigen (HBsAg) status, the name of anti-PD-1 agents and previous treatment are summarized in Table 1.

Twenty patients (36.3%) had Child-Pugh stage B/C. At data cut-off, 2 (6%) and 9 (29%) patients were still on treatment with anti-PD-1 agents. Immunotherapy was discontinued mainly due to radiological or clinical disease progression and adverse events.

Clinical Survival

Thirty-six subjects were treated with nivolumab,13 patients with pembrolizumab and 6 with AK105, a kind of home-made anti-PD-1 agent. As of 1st April 2016, the median follow-up period was 13 months (3–34 months) and the one-year survival rate was 29.9%. The date of data cut-off was August 31, 2019. There were 30 (55%) surviving cases and still under follow-up, with a median overall survival (OS) of 15 months (95% CI: 8.4–21.6, Figure 1A). The median progression-free survival (PFS) was 10 months (95% CI: 6.0–14.0, Figure 1B). Median OS for patients with partial response or stable disease was 19 months (95% CI: 8.6–29.4) and was significantly longer compared to that of patients with progressive disease, which was 2 (95% CI,0.4–3.6; $P = 0.000$) months (Figure 2A). Median PFS for patients with partial response or stable disease was 11 months (95% CI,7.2–14.8) and was significantly longer compared to that of patients with progressive disease, which was 1 (95% CI,6.0–14.0; $P = 0.000$) month (Figure 2B). Patients with Child-Pugh A or B had significantly better survival than those with Child-Pugh C. Median OS for patients with Child-Pugh A, Child-Pugh B, and Child-Pugh C were 19 months, 10 months and 3 months, respectively ($P = 0.01$) (Figure 3A). Median PFS for patients with Child-Pugh A, Child-Pugh B, Child-Pugh C were 11 months, 7 months and 2 months, respectively ($P = 0.026$) (Figure 3B). ECOG performance status and Child-Pugh grade were influence factors of OS and PFS by univariate analysis (Table 3).

Table 1 Clinicopathologic Features in 55 Patients with HCC

Patient Characteristics	N = 55
Median age (range)	56
Female/male	9/46
ECOG performance status	
0	37
1	16
2	2
Hepatocirrhosis	
Yes	34
No	21
Extrahepatic metastasis	
Yes	34
No	21
Vascular invasion	
Yes	22
No	33
Child-Pugh grade	
A	35
B	18
C	2
AFP >400μg/L	18
Hepatitis	
HBV	43
HCV	2
Other	10
Anti-PD-1 agents	
Nivolumab	36
Pembrolizumab	13
AK105	6
TACE	40
Sorafenib	14
Other therapy	
Radiofrequency ablation	18
Ethanol injection	3
Radiotherapy	15
Hepatic transplantation	1

Efficacy

No patient had complete response (CR) and 12 (22%) participants achieved partial response (PR), resulting in an overall response rate (ORR) of 22%. Thirty-seven (67%) patients showed stable disease (SD) and 6 (11%) subjects had progressive disease (PD) at first radiological evaluation. The disease control rate (DCR) was 89%. Among all the patients, only one patient (2%) administered pembrolizumab was evaluable for hyperprogression. Among the six patients
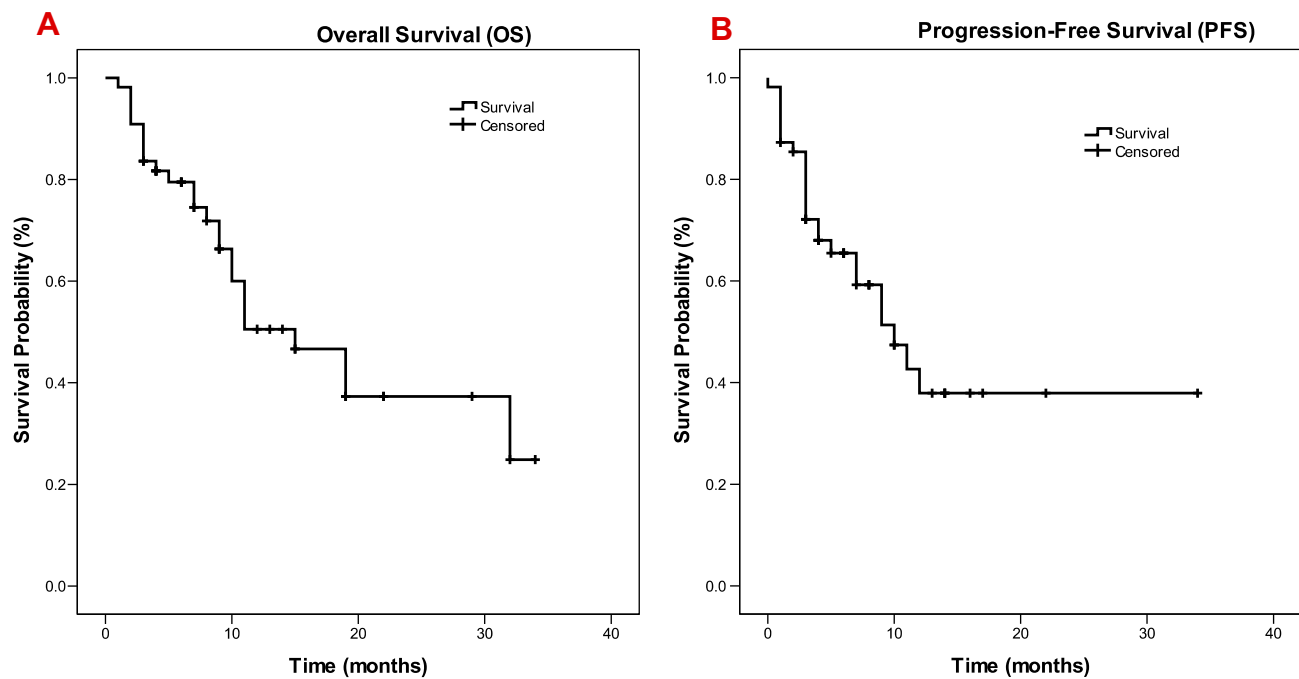
A

B

Figure 1 Kaplan–Meier curve showing OS and PFS for the whole cohort of patients treated with programmed cell death protein-1 (PD-1)-targeted immunotherapy. (**A**) OS rates in patients with HCC receiving anti-PD-1 agents. (**B**) PFS rates in patients with HCC receiving anti-PD-1 agents.
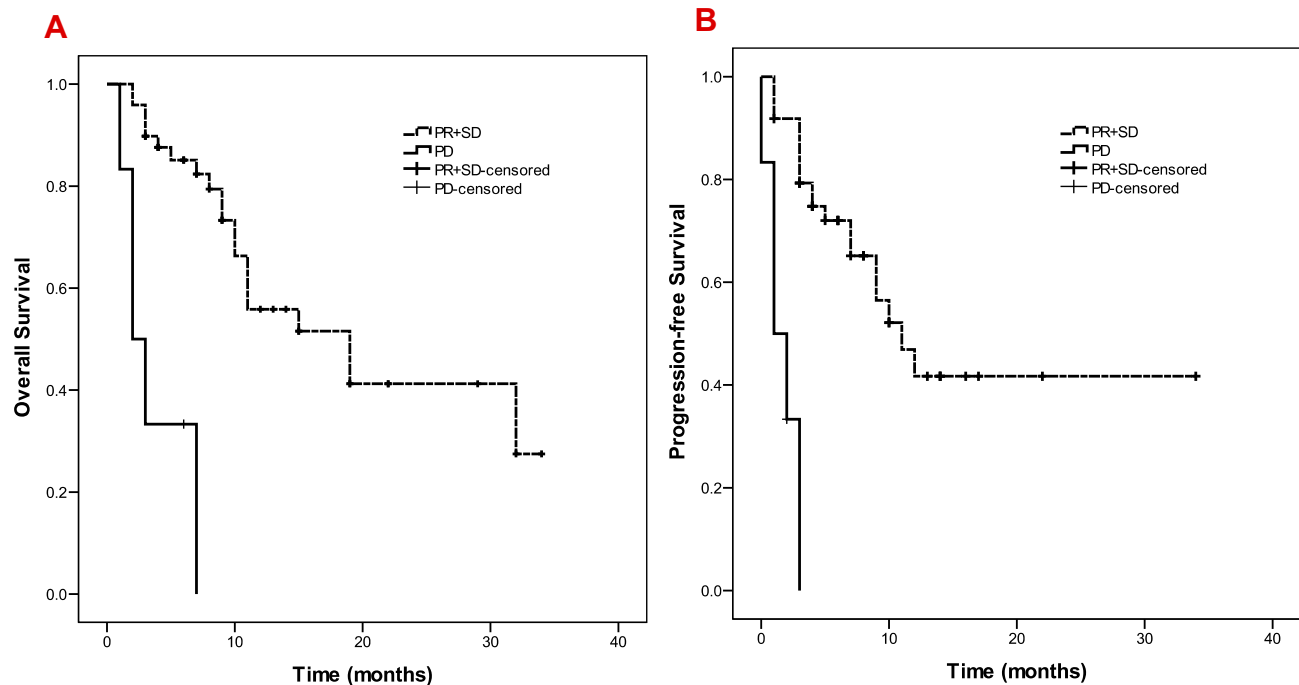
A

B

Figure 2 Kaplan–Meier curves showing OS and PFS for patients treated with PD-1-targeted immunotherapy according to radiological tumor response (partial response (PR)/stable disease (SD) vs progressive disease (PD)). (**A**) OS rates in patients with or without disease progression. (**B**) PFS rates in patients with or without disease progression.

evaluated PD, two of them were given following treatment, one was administered lenvatinib, one was given TACE, the other four patients died of disease progression, including one hyperprogression case.

Toxicity

Toxicity data are shown in Table 2. The major treatment-related AEs (AEs using CTCAE-4 criteria) observed included fatigue, nausea, abdominal distention, with
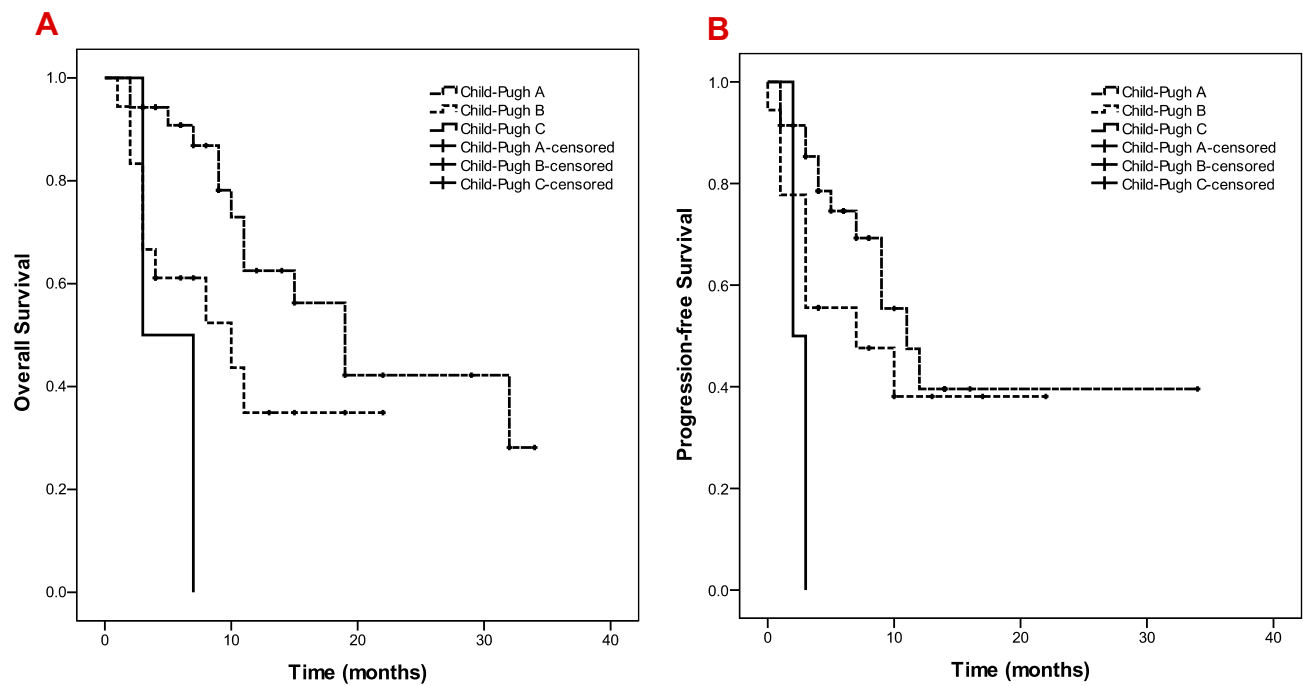
Figure 3 Kaplan–Meier curves showing OS and PFS for patients with different Child-Pugh Grade. (**A**) OS rates in patients with Child-Pugh A, B, C, respectively. (**B**) PFS rates in patients with Child-Pugh A, B, C, respectively.

a total side effect rate of 61.8% (in 34 of 55 patients). The treatment-related AEs in most patients were relieved after symptomatic treatment. Treatment-related serious AEs

Table 2 Toxicity in 55 Patients with HCC

	No. of Patients (%)	
	Any Grade	**Grade 3/4**
Treatment-related SAEs	4(7)	4(7)
Infection	2(4)	2(4)
Rash	2(4)	2(4)
Pruritus	1(2)	—
Diarrhea	1(2)	—
Appetite decreased	1(2)	—
Fatigue	9(16)	—
Nausea	3(5)	—
Arthralgia	1(2)	—
Fever	1(2)	—
Myalgia	1(2)	—
Abdominal pain	1(2)	—
Abdominal distention	3(5)	—
Pain	1(2)	—
Hypothyroidism	1(2)	—
WBC decrease	2(4)	—
Platelet count decrease	2(4)	—
Intestinal perforation	1(2)	—
Edema	1(2)	—
Hypertension	1(2)	—
Encephalorrhagia	1(2)	—

occurred in 4 patients (7%) including immunoassociated pneumonia in two patients, encephalorrhagia in one patient and disease hyperprogression in one patient.

Among of them, one died of rupture and hemorrhage of liver cancer. In addition, a patient died of bleeding during hepatectomy operation.

Discussion

In this retrospective research, we demonstrate that PD-1-targeted immunotherapy showed promising efficacy and mild toxicity in a real-world cohort of patients with advanced stage HCC. There was no CR case in this research. Overall survival of patients with PR or SD was significantly longer than that of subjects with PD (19 vs 2 months). For most patients, the side effects were manageable. Only four cases suffered serious AEs.

To the best of our knowledge, one of the earliest immunotherapy trials that established promising activity was a Phase 2 study of the anti-CTLA-4 monoclonal antibody tremelimumab in patients with advanced HCC and HCV-related cirrhosis who developed disease progression while receiving sorafenib.[4] Two phase II clinical trials proved the efficacy of nivolumab and pembrolizumab in patients with intermediate-advanced stage HCC. One is the CheckMate 040 study, an open-label, noncomparative, phase I/II trial, tested nivolumab in 262 patients with HCC and Child-Pugh

Table 3 Univariate Analysis for Overall Survival and Progression-Free Survival

	N	Overall Survival		Progression-Free Survival	
		X^2	p value	X^2	p value
Gender					
Male	46	0.402	0.526	2.506	0.113
Female	9				
ECOG performance status					
0	37	6.875	0.032	7.693	0.021
1	16				
2	2				
Hepatocirrhosis					
Yes	34	0.305	0.581	0.093	0.761
No	21				
Extrahepatic metastasis					
Yes	34	0.037	0.847	0.15	0.699
No	21				
Vascular invasion					
Yes	22	1.557	0.212	2.035	0.154
No	33				
Child-Pugh grade					
A	35	9.191	0.010	7.334	0.026
B	18				
C	2				
AFP					
<400 μg/L	37	0.117	0.732	0.03	0.863
>400 μg/L	18				
Hepatitis					
HBV	43	0.836	0.658	0.614	0.736
HCV	2				
Other	10				
Anti-PD-1 agents					
Nivolumab	36	1.043	0.594	0.799	0.671
Pembrolizumab	13				
AK105	6				

class A.[15] Overall response rate was 23% in sorafenib-naive and 19% in sorafenib-pretreated patients. A median overall survival was 28.6 months in sorafenib-naive and around 15 months in sorafenib-experienced patients. Based on these promising results, nivolumab, a fully human immunoglobulin G4 monoclonal antibody to PD-1, was conditionally approved as second-line treatment of patients with advanced and metastatic HCC. Another is the KEYNOTE-224 trial,[16] a nonrandomised, open-label phase II study investigating pembrolizumab in sorafenib-pretreated patients (n = 104)

with Child-Pugh stage A. Again, it showed encouraging results with an overall response rate of 17%, a median PFS of 4.9 months, and a median OS of 12.9 months, which making pembrolizumab receive FDA approval in the United States. In our study, most patients received combination therapy, such as anti-PD-1 agents with TACE, target therapy, radiofrequency ablation and radiotherapy. Therefore, the median OS and PFS were 15 months and 10 months, respectively, significantly better than the two trials above. As we know, TACE and some anti-angiogenic agents, such as sorafenib and regorafenib could intensify tumor hypoxia, which promotes an immunosuppressive tumor microenvironment,[20,21] increasing tumoral PD-L1 expression in experimental models in HCC.[22,23] Therefore, immunotherapy maybe has a synergistic effect when combined with anti-angiogenic agents or TACE. A Phase 1/2 trial currently is recruiting patients for a comparative study of durvalumab plus tremelimumab in combination with TACE, radiofrequency ablation, or cryoablation (ClinicalTrials.gov identifier NCT02821754). Similarly, a phase ⅠB trial of lenvatinib with pembrolizumab currently is underway (ClinicalTrials.gov identifier NCT03006926).[4]

The CheckMate-040 and the KEYNOTE-224 study only included Child-Pugh A patients. However, in our study, nearly 40% of patients were Child-Pugh B or C. There was no significant increase in the number of patients with any grade and high-grade adverse events, suggesting that immunotherapy is safe enough even in patients with advanced liver function impairment. As we know, monoclonal antibodies are not metabolized by the liver, which could make immune checkpoint inhibitors adaptable to patients with advanced liver cirrhosis.[24] Moreover, it was shown that Child-Pugh grade was an influential factor of OS and PFS by univariate analysis in our study. The OS and PFS in patients with Child-Pugh A or B were better than those with Child-Pugh C significantly.

In conclusion, the advent of immunotherapy using ICIs has a great impact on many malignant tumors, including HCC. Anti-PD-1 agents are safe, effective and provide another treatment option for advanced HCC patients. However, there is no clear biomarker to predict the efficacy and sensitivity. It is generally known that HCC can be diagnosed by radiology, which means difficult to get pathological specimen. Therefore, it is necessary to find blood examination indicators and biomarkers to select patients who are most likely to benefit from immunotherapy. In addition, the combination of immunotherapy and other therapy (e.g. target therapy, TACE) may have the potential to further improve the outcome, which needs to

be verified by clinical research. Further studies need to be performed to benefit advanced HCC patients.

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Disclosure

The authors report no conflicts of interest in this work.

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