

The presence and size of intrahepatic tumors determine the therapeutic efficacy of nivolumab in advanced hepatocellular carcinoma

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Abstract

Purpose: Inter-tumoral heterogeneity at the differential lesion level raises the possibility of distinct organ-specific responses to immune checkpoint inhibitors (ICIs). We aimed to comprehensively examine the clinicopathological factors to predict and assess the efficacy of nivolumab, programmed cell death protein 1 (PD-1) blockade at an individual tumor site-specific level in patients with advanced hepatocellular carcinoma (aHCC).

Patients and Methods: We enrolled 261 aHCC patients treated with nivolumab between 2012 and 2018. Eighty-one clinicopathological factors were comprehensively collected and analyzed. The association between all variables and survival outcomes was evaluated. According to tumor site, the organ-specific responses were assessed based on the Response Evaluation Criteria in Solid Tumors, version 1.1.

Results: The liver was the most commonly involved organ (75.1%), followed by the lungs (37.5%) and lymph nodes (LNs, 11.5%). The liver of nonresponders was more frequently the organ of progression, while the lungs of responders were more frequently the organs of response. Among the 455 individual lesions (liver, $n=248$; lung, $n=124$; LN, $n=35$; others including bone or soft tissues, $n=48$), intrahepatic tumors showed the least response (10.1%), followed by lung (24.2%) and LN tumors (37.1%), indicating the presence of distinct organ-specific responses to nivolumab. In intrahepatic tumors, the organ-specific response rate decreased as the size increased (13% for ≤ 50 mm, 8.1% for 50–100 mm, and 5.5% for >100 mm). In the subgroup analysis according to tumor location, patients with lung only metastasis (≥ 30 mm) showed the best progression-free survival (PFS) and overall survival (OS). In contrast, primary HCC (≥ 100 mm) without lung metastasis had the worst PFS and OS. Comprehensive analyses also revealed that liver function and systemic inflammatory indices, such as neutrophil-to-lymphocyte ratio (NLR), were significantly associated with PFS and OS.

Conclusion: The presence and size of liver tumors, liver function, and NLR are key factors determining the response to nivolumab in aHCC. These clinical factors should be considered when treating patients with advanced HCC with PD-1 blockade.

Keywords: clinicopathologic factors, hepatocellular carcinoma, lesion-level response, PD-1 blockade, outcome

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Introduction

Primary liver cancer is the fourth leading cause of cancer-related death, and hepatocellular carcinoma (HCC) is the most common type of

primary liver cancer, accounting for 90% of all cases.¹ Many patients with HCC are diagnosed at an advanced stage and undergo systemic therapy.² HCC is frequently accompanied by

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impairment of liver function due to chronic liver disease and cirrhosis, in addition to intrahepatic tumor burden.^{3,4} Unlike other solid tumors, liver function reserve, tumor burden, and involved organ sites determine the efficacy of systemic treatment and prognosis of HCC in a complex manner, further complicating the treatment landscape of HCC.

Recently, immunotherapy has been incorporated in systemic therapy for HCC.^{5,6} Immune checkpoint inhibitors (ICIs) elicit dramatic and durable responses in various cancer types with previously limited treatment options, opening up new paths and shifting the paradigm of solid tumor therapy.⁷⁻⁹ The concept is to reactivate dormant and exhausted T cells by transitioning to the hostile tumor microenvironment.⁹ However, the intra- and inter-tumoral heterogeneity and spatial dynamics of antitumor immune responses can alter the outcomes of ICI therapy.^{10,11} Although non-small cell lung cancer, renal cell carcinoma, melanoma, and several other malignancies have shown positive response to ICI, recent accumulating evidence suggests that cancers with liver metastasis tend to have poorer response and survival to ICI, suggesting the possibility of liver-specific immune tolerance.^{12,13} Similarly, despite the seemingly effective and successful results from phase I/II trials of ICIs,^{5,6} subsequent phase III trials failed to meet their primary endpoints.^{14,15} The failure of these phase III trials may suggest that liver function and the distinct immune microenvironment of the intrahepatic tumor determine the therapeutic efficacy of ICI in patients with advanced hepatocellular carcinoma (aHCC).

The liver is well known as the central immunomodulator responsible for maintaining an immune tolerogenic microenvironment.¹⁶⁻¹⁸ This immune-tolerogenic effect extends to other organs, thus contributing to systemic immune tolerance. Recent preclinical studies demonstrated the mechanism of liver metastasis-induced reduction in systemic antitumor immunity, in which metastatic tumor lesions in the liver promote antigen-specific immune suppression, thereby restraining the immunotherapeutic efficacy *via* macrophage-mediated T cell elimination, activation of regulatory T cells, and modulation of intratumoral CD11b+ monocytes.^{19,20} Considering that the primary site of occurrence of HCC is the liver, the naturally immune-tolerogenic microenvironment may hinder the effectiveness of immunotherapy.

Therefore, we aimed to comprehensively evaluate the clinicopathological factors to predict and assess the efficacy of nivolumab, programmed cell death protein 1 (PD-1) blockade at an individual tumor site-specific level using the data of a large, multicenter cohort of patients with advanced HCC. By comparing the efficiency of immunotherapy in HCC patients with or without tumor lesions in the liver, we demonstrated the significance of tumor location during immunotherapy.

Materials and methods

Patients

This retrospective, multicenter, observational study evaluated the efficacy and safety of the PD-1 inhibitor nivolumab in patients with incurable HCC not amenable or refractory to locoregional therapy. Patients who received nivolumab monotherapy were recruited from five referral cancer centers between June 2012 and March 2018. Patient demographics and clinical data were collected retrospectively from each participating center. Eligible patients presented with HCC confirmed by a pathologic or noninvasive assessment according to the American Association for the Study of Liver Diseases criteria in patients with cirrhosis; had measurable disease, as defined by Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1); and were unresponsive to curative or locoregional therapies or had progressed thereafter. Patients who were followed up at the clinic at least once after nivolumab administration were included in this analysis.

Treatment and assessment

Nivolumab was administered intravenously at a dose of 3 mg/kg body weight every 2 weeks. Treatment was continued until disease progression, unacceptable toxicity, or death. Dose interruptions or reductions were made according to the discretion of the attending physician. According to the local institutional guidelines, tumors were assessed by computed tomography or magnetic resonance imaging at baseline and every 6–8 weeks. Tumor responses were graded according to RECIST 1.1. A finding of complete response (CR; complete disappearance of the tumor or a nodal short-axis diameter <10 mm on follow-up), partial response (PR; $\geq 30\%$ reduction in the tumor size), or progression of disease (PD; $\geq 20\%$ increase in the tumor diameter) requires confirmation of response, a repeat

assessment for verification on two occasions ≥ 4 weeks apart. All treatment-related adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0.

Organ-specific responses

The treatment responses of primary liver, lung, lymph node (LN), and other metastatic tumors were assessed based on the organ-specific response criteria adapted from RECIST 1.1, as previously reported.²¹ Briefly, measurable lesions of up to two with a diameter ≥ 10 mm or measurable LNs with a short-axis diameter ≥ 15 mm were evaluated. The organ-specific objective response was classified as CR, PR, PD, or stable disease (SD; not CR, PR, or PD) according to each organ disease burden. The best response of the lesions in each organ was measured during the course of treatment.

Statistical analysis

Survival outcomes were estimated using the Kaplan–Meier method and compared using the log-rank test. Progression-free survival (PFS) was defined as the period from the initiation of nivolumab treatment to the date of disease progression based on RECIST v1.1 or death from any cause, whichever occurred first. Overall survival (OS) was defined as the period from the initiation of nivolumab treatment to death from any cause. In the univariate and multivariate survival analyses, a Cox proportional hazards model was used to determine the contribution of clinical factors to OS and PFS. The following variables were included in the prognostic factor analysis: (1) demographics ($n=15$), (2) baseline laboratory values ($n=20$), (3) tumor burden ($n=11$), (4) previous treatment ($n=12$), (5) treatment response ($n=5$), and (6) toxicity profiles ($n=18$). Statistical significance was defined as a two-sided p -value < 0.05 . All statistical analyses were performed using R statistical software version 3.5.0 (www.r-project.org).

Results

Patient characteristics

Between 9 June 2012 and 14 March 2018, a total of 261 patients with advanced HCC admitted to five high-volume tertiary cancer centers in Korea were included. Patient demographics,

baseline disease characteristics, and previous treatments are shown in Table 1. The median age was 59 years (range, 20–82 years). Most patients had hepatitis B virus (HBV, 75.9%) or hepatitis C virus (HCV, 6.5%) infection. The majority of patients had Child-Pugh A (79.7%) or Barcelona clinic liver cancer (BCLC) stage C (94.3%), while 29 patients (11.1%) and 15 patients (5.7%) had Child-Pugh B/C or BCLC stage B, respectively. Considering the CheckMate-040 and the KEYNOTE-240 study included patients with Child-Pugh liver A and BCLC stage B or C disease, our study cohort was comparable to the previous CheckMate-040 or KEYNOTE-240 studies. Extrahepatic metastases were observed in 217 (83.1%) patients, and the most common metastatic site was the lung ($n=137$, 52.5%), followed by the LNs ($n=59$, 22.6%). Macrovascular invasion was observed in 97 patients (37%). The patients previously received the following systemic treatments: nivolumab was administered as first-line [24 patients (9.2%)], second-line [174 patients (66.7%)], and third-line and beyond [63 patients (24.1%)] palliative treatment.

Overall efficacy of PD-1 antibody

Of the 261 patients, 255 were evaluated for treatment response (Table 2). Six patients were not evaluable due to early disease progression. Their overall objective response rate was 15.3% [95% confidence interval (CI), 10.9–19.7], including five patients who achieved CR and 35 patients who achieved PR (Table 2). The disease control rate was 46%. The median duration of treatment was 2.2 months (interquartile range, 1.4–4.6 months). The median PFS was 2.4 months (95% CI, 1.9–2.8 months), while the median OS was 6.3 months (95% CI, 4.9–8.1) (Figure 1(a)). HCC etiology, such as HBV infection, HCV infection, and alcohol, was not associated with OS nor PFS (Supplemental Table 1). Favorable liver function (Child-Pugh A) was associated with improved OS [hazard ratio (HR), 0.37; $p < 0.001$] and improved PFS (HR, 0.55; $p < 0.001$), respectively.

Next, we evaluated the OS and PFS according to primary liver disease burden and lung metastasis, respectively. Overall, patients with increased primary liver tumor burden tend to have worse OS and PFS. Regarding primary liver disease burden, patients without liver lesions had better OS and PFS than those with liver lesions [OS, 12.5 months without liver lesions *versus* 5.9 months with liver

Table 1. Baseline characteristics (n=261).

Characteristic	N (%)
Age, median (range), year	59 (20–82)
Gender	
Male	219 (83.9)
Female	42 (16.1)
ECOG performance status	
0	31 (11.8)
1	208 (79.7)
2	22 (8.4)
Child-Pugh score	
A5	138 (52.9)
A6	70 (26.8)
B7	24 (9.2)
B8–9	25 (9.6)
C10–11	4 (1.5)
BCLC stage	
B	15 (6)
C	246 (94)
Extrahepatic metastasis	218 (83.5)
Macrovascular invasion	97 (37.2)
Cause of hepatocellular carcinoma	
Hepatitis B virus	198 (75.8)
Hepatitis C virus	17 (6.5)
Substantial alcohol use	25 (9.6)
Others	31 (11.9)
Baseline AFP, median (range) (ng/mL)	179.5 (0–1,308,700)
Baseline PIVKA-II, median (range) (mAU/mL)	608 (0–100,000)
Previous therapy	
Systemic treatment	237 (90.8)
Multikinase inhibitors	236 (90.4)
Immune checkpoint inhibitors	5 (1.9)

(Continued)

Table 1. (Continued)

Characteristic	N (%)
Locoregional therapy	205 (78.5)
Surgery	93(35.6)
TACE	163 (75.5)
Radiotherapy	134 (51.3)
RFA	55 (21.1)

AFP, alpha-fetoprotein; BCLC, Barcelona clinic liver cancer; ECOG, Eastern Cooperative Oncology Group; PIVKA-II, protein induced by vitamin K absence or antagonist-II; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

Table 2. Responses to nivolumab treatment (N=261).

Response	N (%)
Overall response rate	40 (15.3)
95% CI	10.9–19.7
Best overall response	
Complete response	5 (1.9)
Partial response	35 (13.4)
Stable disease	80 (30.7)
≥24 weeks	20 (7.7)
Progressive disease	135 (51.7)
Not assessable*	6 (2.3)
Disease control rate	120 (46.0)

*Includes patients with early disease progression (n=6).

lesions <100 mm versus 3.7 months with liver lesions ≥100 mm, log-ranked $p < 0.001$, Figure 1(b); PFS, 3.2 months without liver lesions versus 2.2 months with liver lesions <100 mm versus 1.8 months with liver lesions ≥100 mm, log-ranked $p < 0.001$, Figure 1(c)]. With regard to those with metastasis in the lungs, the second most commonly involved site following the liver, patients with measurable lung metastasis ≥30 mm had better OS and PFS than patients without lung disease or measurable lung metastasis < 30 mm, suggesting that organ-specific response can be oppositely regulated in response to anti-PD-1 treatment [Figure 1(d) and (e)].

The line of nivolumab treatment was not associated with OS (median OS; 5.0 months in first-line *versus* 6.6 months in the second-line *versus* 5.9 months in third-line and beyond; log-ranked $p=0.724$; Supplemental Figure 1), implying that increased tumor burden in patients in the first-line treatment of nivolumab, compared to second-line or \geq third-line of treatment, may affect comparable survival in terms of OS and PFS. There was a trend with an increased mean PFS in the first-line of treatment (mean PFS, 8.0 months), compared to the second (mean PFS, 5.5 months) or \geq third-line of treatment (mean PFS, 4.8 months), but it was not statistically significant (log-ranked $p=0.484$). The previous history of liver surgery was associated with improved OS (Supplemental Table 1). There was no significant difference in PFS and OS according to other previous therapy histories such as prior loco-regional treatment [radiotherapy, radiofrequency ablation (RFA), or transarterial chemoembolization (TACE)], prior sorafenib treatment, or prior regorafenib treatment (Supplemental Table 1).

Organ-specific responses to anti-PD-1 antibody

Next, we measured the best percentage change over time in tumor burden according to tumor site (Figure 2) based on RECIST 1.1. A total of 455 individual lesions from 261 patients were evaluated. The measurable target organs included liver ($n=248$), lungs ($n=124$), LNs ($n=35$), and other metastatic sites (total, $n=48$; peritoneum, $n=19$; bone, $n=9$; adrenal gland, $n=9$; pleura, $n=4$; muscle, $n=4$; brain, $n=3$). Overall, the proportions of best percentage changes from baseline were 131% (95% CI, 123–139%) for primary liver tumor, 94% (95% CI, 84%–103%) for lung metastasis, 89% (95% CI, 76%–102%) for LN metastasis, and 109% for other metastases (95% CI, 97%–121%) [Figure 2(a)]. In patients who achieved CR or PR as their best response, objective response (tumor shrinkage of more than 30% based on RECIST 1.1) was mainly observed in those with lung lesions compared with those with intrahepatic lesions. In addition, progression of primary liver tumor (tumor increment of more than 20% based on RECIST 1.1) was observed in patients with PD as their best response [Figure 2(b)]. The organ-specific objective response rates (ORRs) of primary liver tumor were the least (10.1%, 25 of 248 lesions) compared with those of lung metastasis (24.2%, 30 of 124 lesions), LN metastasis (37.1%, 13 of 35 lesions), and other metastases (14.6%, 7 of 48 lesions) [Figure 2(a)].

The organ-specific ORRs of patients with primary liver tumor differed according to average liver tumor size, showing that those with a primary liver disease ≥ 100 mm had an organ-specific ORR of only 5.5% (3/55) compared with those with a liver disease < 100 mm [Figure 2(c)].

We investigated the impact of primary liver disease and lung metastasis in response to nivolumab on PFS and OS (Table 3). We categorized the patients according to the status of primary liver tumor (no liver lesions, liver lesions < 100 mm, and liver lesions ≥ 100 mm) and lung metastasis (no lung metastasis, lung lesion < 30 mm, and lung lesion ≥ 30 mm). Most patients had a primary liver disease < 100 mm without lung metastasis ($n=81$, 31%; Table 3). Patients with lung only metastasis (≥ 30 mm) showed the best PFS [odds ratio (OR), 0.36; $p=0.008$] and OS (OR, 0.52; $p=0.028$). By contrast, primary HCC (≥ 100 mm) without lung metastasis had the worst PFS (OR, 1.74; $p=0.009$) and OS (OR, 1.59; $p=0.019$), suggesting that intrahepatic tumors of advanced HCC were the least responsive to nivolumab treatment.

Prognostic factor analysis with clinicopathological features

We evaluated 81 clinical factors to determine the prognostic or predictive impact of nivolumab treatment (Supplemental Table 1). Their association with survival outcomes was evaluated, and an organ-specific response evaluation, adapted from RECIST 1.1, was conducted.

Among nivolumab-treated HCC patients, those with impaired liver function with Child-Pugh score B or C stage or ascites showed poorer survival. Among laboratory values, albumin of ≤ 3.5 g/dL, jaundice, elevated aspartate transaminase level, elevated alkaline aminotransferase level, and elevated alkaline phosphatase level were associated with poorer OS and PFS. Inflammatory markers such as elevated C-reactive protein, elevated erythrocyte sedimentation rate, elevated neutrophil-to-lymphocyte ratio (NLR ≥ 2), neutrophilia (neutrophil count $\geq 7000/\mu\text{L}$), and lymphopenia (lymphocyte count $\leq 1100/\mu\text{L}$) were also associated with poorer OS and PFS. In addition, the variables that represent the tumor burden of HCC, such as the number of intrahepatic tumors (≥ 10), largest intrahepatic tumor diameter (≥ 100 mm), and presence of tumor occupying $\geq 50\%$ of the liver, were also related to poorer survival outcomes. CR/PR/SD, AFP

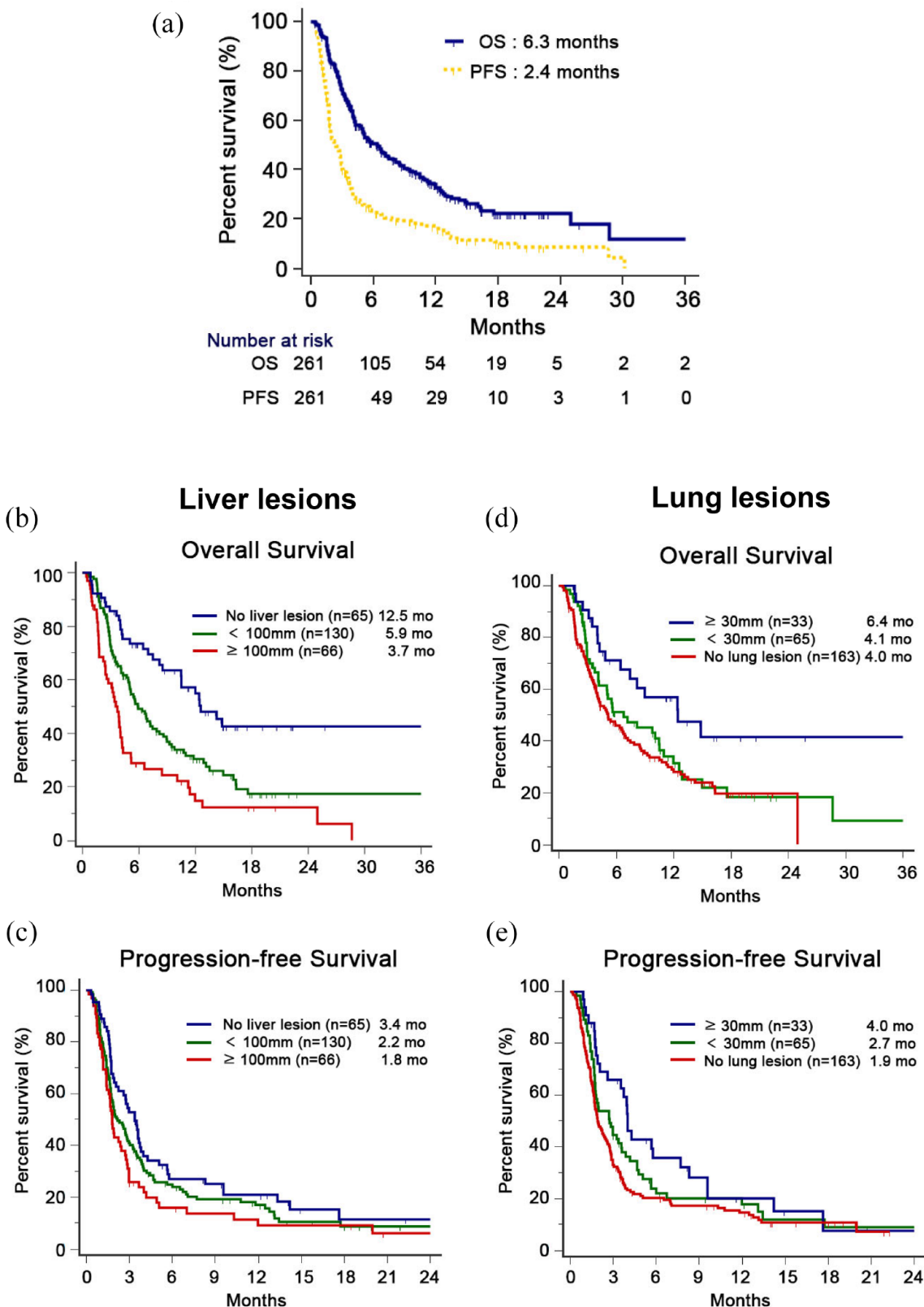


Figure 1. Kaplan-Meier estimates of the overall survival (OS) and progression-free (PFS). (a) OS and PFS in response to PD-1 blockade nivolumab. (b) OS according to the status of primary liver tumor status. (c) PFS according to the status of primary liver tumor. (d) OS according to the status of lung metastasis. (e) PFS according to the status of lung metastasis.

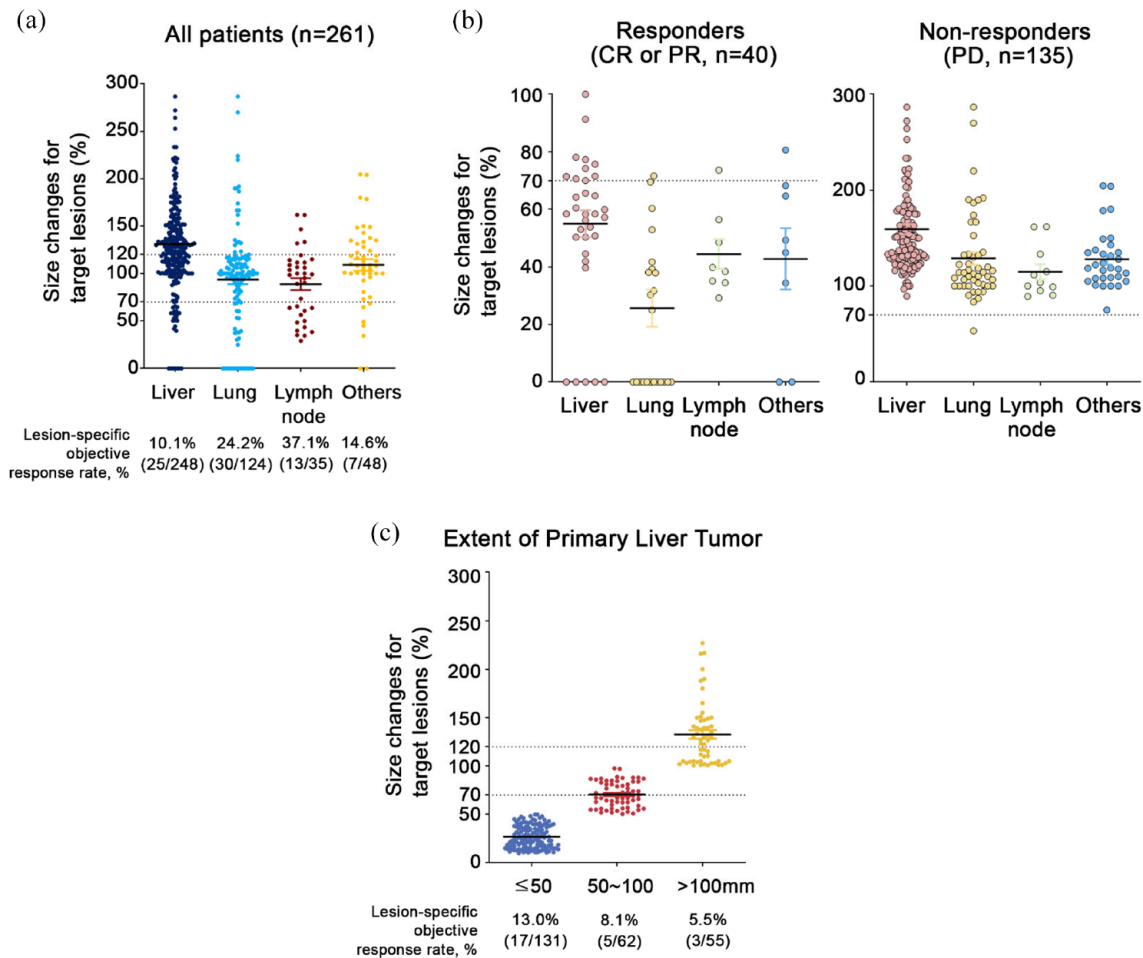


Figure 2. Best percentage changes and objective response rate (ORR) from baseline in terms of size of target lesions. (a) The best size changes and ORR in all patients according to target lesions. (b) The best size changes in responders and nonresponders. (c) Objective response rate based on extent of primary liver tumor.

decrease, and PIVKA-II decrease were significantly associated with better OS and PFS. We evaluated the prognostic significance of platelet-to-lymphocyte ratio (PLR) in terms of OS and PFS (Supplemental Figure 2). High PLR had a trend toward worse OS (9.6 *versus* 6.8 months with high PLR ≥ 100 ; log-ranked $p=0.268$) and worse PFS (2.8 *versus* 2.4 months with high PLR ≥ 100 ; log-ranked $p=0.084$), but was not statistically significant [Supplemental Figure 2(a) and (b)]. There was a linear correlation between PLR and NLR [linear regression $p < 0.001$; Supplemental Figure 2(c)].

In summary, the comprehensive association study revealed that absence of lung metastasis, decompensated liver function, primary liver lesions, and increased inflammatory markers such as NLR were associated with decreased OS and PFS (Figure 3). In the subgroup analysis of objective

responses, decompensated liver function and increased inflammatory markers such as NLR were associated with poorer objective responses to nivolumab (Supplemental Table 2). Our results suggest that the underlying liver function, inflammatory status, and location of tumor burden may be considered when prescribing PD-1 or PD-L1 inhibitor.

Safety profiles

The toxicity profile shown in this study was comparable to that of a previous study on PD-1/PD-L1 inhibitors (Table 4).^{5,15} Most AEs of any attribution were related to the underlying liver function. A total of 240 (92.0%) patients developed AEs of any grade, while 87 (33.3%) patients developed grade 3–4 AEs. The AEs of any attribution that occurred in more than 10% of patients

Table 3. Results of the univariate analysis on the impact of both liver and lung lesions in response to nivolumab treatment on PFS and OS in the (N=261).

Category	Number of patients	PFS			OS		
		OR	95% CI	p	OR	95% CI	p
Liver disease of <100mm and no lung lesion	81	1.00	-	-	1.00	-	-
Liver disease of <100mm and lung metastasis of <30mm	39	0.99	0.62-1.56	0.968	0.92	0.61-1.39	0.698
Liver disease of <100mm and lung metastasis of ≥30mm	10	0.56	0.22-1.39	0.213	1.04	0.51-2.09	0.910
No liver and no lung lesion	34	0.62	0.35-1.06	0.082	0.91	0.57-1.42	0.669
No liver lesion and lung metastasis of <30mm	12	0.46	0.18-1.15	0.099	1.08	0.55-2.11	0.818
No liver lesion and lung metastasis of ≥30mm*	19	0.36	0.17-0.76	0.008*	0.52	0.29-0.93	0.028*
Liver disease of ≥100mm and no lung lesion*	48	1.74	1.14-2.64	0.009*	1.59	1.07-2.34	0.019*
Liver disease of ≥100mm and lung metastasis of <30mm	14	1.15	0.59-2.20	0.681	0.70	0.36-1.33	0.284
Liver disease of ≥100mm and lung metastasis of ≥30mm	4	2.21	0.68-7.13	0.184	0.94	0.29-3.01	0.921

CI, confidential interval; OR, odds ratio; OS, overall survival; PFS, progression-free survival.
*p < 0.05.

were increased aspartate aminotransferase (AST) (68.6%), anemia (68.6%), hypoalbuminemia (47.5%), alanine aminotransferase (ALT) increase (41.8%), hyponatremia (37.5%), fatigue (14.6%), hyperbilirubinemia (36.8%), and pruritus (14.2%), respectively. The most frequent grade 3-4 AEs were increased AST (n=49, 18.7%), hyperbilirubinemia (n=29, 11.1%), and hyponatremia (n=27, 10.3%). Increased amylase levels were associated with improved PFS (p=0.031). Decreased liver function, such as hypoalbuminemia, hyperbilirubinemia, hyponatremia, and elevation of aspartate aminotransferase, was associated with decreased OS and PFS, respectively (Supplemental Table 1).

Discussion

We investigated a total of 455 individual lesions from 261 patients with advanced HCC who received nivolumab treatment in a large multicenter cohort. We found that tumor responses to ICIs varied significantly depending on the tumor site in advanced HCC. In particular, tumors in the liver, which is the most common site of HCC, were the least responsive to ICIs. Furthermore,

we found that the larger the hepatic tumor size, the more resistant it became to ICI. Intriguingly, patients with lung metastasis without hepatic lesions had the most favorable prognosis among patient subgroups treated with ICI. To our knowledge, this is the first study to demonstrate that the presence and size of intrahepatic tumors determine the therapeutic efficacy of PD-1 inhibitor monotherapy in patients with advanced HCC.

In advanced HCC, PD-1 inhibitors, including nivolumab and pembrolizumab, the most representative ICIs, delivered clinically meaningful response rates ranging from 17% to 20% in phase I/II trials.^{5,6} However, both the subsequent phase III CHECKMATE-459 trial and the KEYNOTE-240 trial failed to meet their primary endpoints, despite the modest efficacy of the abovementioned drugs, which was also reported in previous studies.^{14,15} Therefore, the optimal selection of patient subgroups that will most likely benefit from single-agent checkpoint blockade is of paramount importance in the treatment of advanced HCC. In the subgroup analysis of PFS in the KEYNOTE-240 study, HCC patients with only intrahepatic tumors (without extrahepatic spread) showed the least clinical

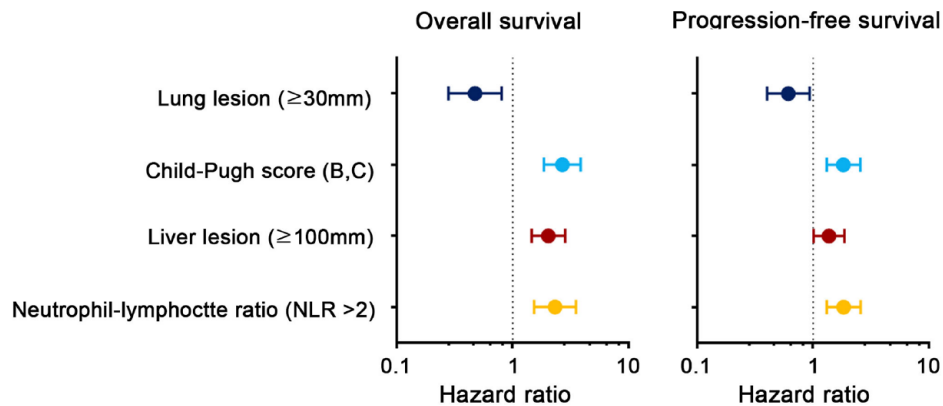


Figure 3. Clinical factors associated with survival impact in response to PD-1 inhibitor treatment. Cox-regression analysis of the overall survival and progression-free survival according to the status of liver metastasis, underlying liver function, primary liver tumor, and inflammatory markers.

benefit from pembrolizumab treatment compared with placebo (HR, 0.91; 95% CI, 0.58–1.42), whereas patients with extrahepatic spread showed meaningful clinical benefit from pembrolizumab treatment (HR, 0.67; 95% CI, 0.51–0.88).¹⁴ The results of this subgroup analysis were consistent with our findings. Recently, Lu *et al.*²¹ indicated that hepatic tumors of HCC might be less responsive to ICIs than extrahepatic lesions in a small-scale study, although those studies included patients who had received either ICI monotherapy or ICI combination treatment. Therefore, an accurate analysis of the impact of PD-1 blockade on an organ-specific basis in advanced HCC remains unaddressed.

Our study suggests that the clinical benefit of nivolumab monotherapy can be diminished in the presence of intrahepatic tumors in advanced HCC. In patients with huge intrahepatic HCC exceeding 10 cm, caution must be observed when proceeding with anti-PD-1 monotherapy. On the contrary, in patients with lung metastasis or LN recurrence without hepatic tumor due to previous locoregional treatment, PD-1 monotherapy is more preferable. Besides these organ-specific factors, this study reconfirmed that ICI may only be effective in patients with impaired liver function. In the era of immunotherapy, increasing evidence shows the importance of evaluating the systemic inflammatory markers, including NLR, PLR, and monocyte-to-lymphocyte ratio. In HCC, elevated NLR has been reported to be associated with poor prognosis in patients who underwent surgical resection, liver transplantation, RFA, chemoembolization, and systemic treatment.^{22–26} Recently, elevated NLR has been consistently

reported as a negative predictive marker even in HCC patients treated with nivolumab monotherapy.²⁷ In particular, a previous study reported that ICI monotherapy in advanced HCC can induce hyperprogressive disease in more than 10% of treated patients, which is related to the elevated NLR.²⁸ In this study, we also observed that an elevated NLR (NLR > 2) was a negative predictive factor for the response to nivolumab treatment. Taken together, our findings show that the location of the tumor, liver function, and inflammatory status of patients, including NLR, must be assessed when considering ICI monotherapy in patients with advanced HCC; therefore, a careful selection process is required to maximize the clinical benefit.

Recently, atezolizumab and bevacizumab combination immunotherapy has become the standard first-line therapy after demonstrating a survival benefit compared with sorafenib in phase III clinical trials.²⁹ Therefore, further evaluation is needed to determine whether the success of this combination immunotherapy can overcome liver-specific immune tolerance. Currently, our team is evaluating the organ-specific response to atezolizumab and bevacizumab combination therapy (ClinicalTrials.gov identifier, NCT04862949).

The main limitation of this study is its retrospective nature. Additionally, the mechanisms of organ-specific differential responses to ICIs were not explored in our study. However, our data are clinically meaningful, as our results are derived from the first large-scale data on nivolumab

Table 4. Adverse events of any attribution.

Toxicity	Grade (no. of patients)					Any grade (%)	Grade 3/4 (%)
	1	2	3	4	5		
Rash	12	3	1	0	0	6.1	0
Pruritus	28	6	3	0	0	14.2	1.1
Diarrhea	8	4	0	0	0	4.6	0
Decreased appetite	23	3	1	0	0	10.3	0
Fatigue	29	9	0	0	0	14.6	0
Asthenia	2	0	0	0	0	0.8	0
Weight decreased	8	1	0	0	0	3.4	0
Nausea	10	3	2	0	0	5.7	0.8
Hypothyroidism	9	2	0	0	0	4.2	0
Dry mouth	2	0	0	0	0	0.8	0
AST increase	98	32	42	7	0	68.6	18.8
ALT increase	74	20	14	1	0	41.8	5.7
Lipase increase	6	5	1	1	0	5.0	0.8
Amylase increase	6	3	1	1	0	4.2	0.8
Anemia	95	65	19	0	0	68.6	7.3
Hypoalbuminemia	44	77	3	0	0	47.5	1.1
Hyperbilirubinemia	42	25	21	8	0	36.8	11.1
Hyponatremia	67	4	24	3	0	37.5	10.3

monotherapy in patients with advanced HCC. We did not evaluate the hepatic tumor response after ICI treatment with modified RECIST (mRECIST). However, we believe that mRECIST is an important measure for evaluating the efficacy of anti-angiogenic agents, but RECIST is more appropriate for evaluating the efficacy of ICI. In addition, this study was performed in an HBV-endemic region; therefore, whether poor responsiveness of hepatic tumors is consistently observed in non-HBV-endemic areas needs to be investigated further. The large variance of patient age may impact a genetic predisposition, such as mutations in *TP53*, the WNT-pathway oncogene *CTNNB1*, and amplification in *MYC* oncogene, as potential selection bias in this study. A recent study showed that neither specific genetic mutation nor tumor mutational burden was not associated with ICI efficacy in

advanced HCC, suggesting that the application of predictive biomarkers from other tumor types to HCC should be interpreted with caution.³⁰ A further molecular and genomic correlative study to identify potential molecular and genetic biomarkers related to the efficacy of ICI is warranted in HCC. Lastly, most patients received nivolumab treatment in the second-line or beyond line treatment, which may affect treatment efficacy.

In conclusion, this study identified that the presence and size of tumors in the liver are key factors determining the response to nivolumab in advanced HCC. In the era of immunotherapy, liver function and liver-specific immune tolerance and systemic inflammatory markers such as NLR are important in when making treatment decisions. Therefore, these clinical factors should be

considered in a comprehensive manner when treating patients with advanced HCC with PD-1 blockade.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board (IRB) of each participating center (Yonsei Cancer Center, 4-2019-0882; Samsung Medical Center, 2019-10-063; CHA Bundang Medical Center, 2019-10-009; Haeundae Paik Hospital, 2019-10-025-001; and Ulsan University Hospital, 2019-11-031-002) and was performed in accordance with the ethical standards of the institutional research committee and the recent Declaration of Helsinki. IRBs waived the need for informed consent in this study as Korean regulations do not require consent in performing retrospective analyses.

Consent for publication

Not applicable.

Author contributions

Han Sang Kim: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Writing – original draft; Writing – review & editing.

Chang Gon Kim: Data curation; Writing – review & editing.

Jung Yong Hong: Data curation; Writing – review & editing.

Il-hwan Kim: Data curation.

Beodeul Kang: Data curation; Validation.

Sanghoon Jung: Data curation; Validation.

Chan Kim: Data curation; Resources.

Sang Joon Shin: Investigation; Resources; Supervision.

Hye Jin Choi: Investigation; Supervision; Validation.

Jaekyung Cheon: Conceptualization; Data curation; Investigation; Methodology; Writing – original draft; Writing – review & editing.

Hong Jae Chon: Conceptualization; Formal analysis; Investigation; Supervision; Writing – original draft; Writing – review & editing.

Ho Yeong Lim: Conceptualization; Formal analysis; Investigation; Writing – review & editing.

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Competing Interests

JC received research grants from Bayer and consulting and advisory roles from Roche and Eisai. HJC has received consultancy and advisory fees from Bayer, Eisai, ONO, and MSD. The other authors have no potential conflicts of interest to declare.

Availability of data and materials

All data are included in this article and supplementary materials. Further inquiries can be directed to the corresponding author.

Statement of prior presentation

Data from this study were previously presented at the 2020 American Society of Clinical Oncology (ASCO) Annual Meeting.

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Supplemental material

Supplemental material for this article is available online.

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