Serum Concentration of CD137 and Tumor Infiltration by M1 Macrophages Predict the Response to Sintilimab plus Bevacizumab Biosimilar in Advanced Hepatocellular Carcinoma Patients

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ABSTRACT

Purpose: This study aimed to investigate the biomarkers of sintilimab (anti–PD-1) plus IBI305 (a bevacizumab biosimilar) in advanced hepatocellular carcinoma (HCC), as well as their safety and efficacy.

Patients and Methods: A total of 50 patients with advanced HCC received sintilimab (200 mg) plus IBI305 (7.5 or 15 mg/kg), treated every 3 weeks in a phase Ib clinical study. We performed baseline serum cytokine analysis using bead-based multiplex immunoassay and multiplex immunofluorescence on tissue specimens to discover novel biomarkers of response to VEGF/PD-1 combination therapy in HCC.

Results: The overall response rate was 34.0% (17/50). The median progression-free survival (PFS) and the median overall survival were 10.5 and 20.2 months, respectively. The incidence of grade 3 to 5 adverse events was lower in the 7.5 mg/kg (13.8%) than in the 15 mg/kg (28.6%) dose groups. Biomarker analysis showed that the serum CD137 concentration was significantly higher in patients with clinical benefit (CB) than in those without CB (median, 32.8 pg/mL vs. 19.8 pg/mL, $P = 0.034$). A markedly longer PFS was observed in patients with high CD137 concentrations compared with those with low concentrations (median, 14.2 months vs. 4.1 months, $P = 0.001$). The higher density of M1 macrophages ($CD68^{+}CD163^{-}$) in the stroma was also associated with higher efficacy ($P = 0.033$) and a longer PFS ($P = 0.024$).

Conclusions: Sintilimab plus IBI305 was well tolerated and was effective therapy for advanced HCC. Both serum concentrations of CD137 and tumor infiltration of M1 macrophages may serve as potential predictive biomarkers.

See related commentary by Cappuyns and Llovet, p. 3405

Introduction

Hepatocellular carcinoma (HCC), one of the most common malignant tumors, is the third leading cause of cancer-related deaths worldwide (1, 2). Globally, the 5-year survival rate of HCC is only 5% to 30% (3). Sorafenib, lenvatinib, regorafenib, cabozantinib, and ramucirumab are anti-angiogenic agents as the standard systemic treatment of advanced HCC, but the median overall survival (OS) is only 10.7 to 13.6 months (4–8). Nivolumab and pembrolizumab, immune checkpoint inhibitors (ICI) that block programmed cell death protein 1 (PD-1), have been approved by the FDA for advanced HCC (9, 10). However, the efficacy of mono-immunotherapy remains limited, and there was no significant improvement in the median OS with nivolumab compared with that of sorafenib therapy as first-line treatment (11).

The combination of ICIs and anti-vascular endothelial growth factor (anti-VEGF) inhibitors is a critical strategy for the treatment of advanced HCC, allowing for normalization of tumor vascularization, retraction of an immunosuppressive tumor microenvironment, and reprogramming of immune checkpoints to enhance stimulation and infiltration of immune cells into the tumor (12–14). The combined strategy of administering atezolizumab plus bevacizumab was shown to significantly improve the overall response rate (ORR), progression-free survival (PFS), and OS compared with sorafenib in the IMbrave150 study (15), and was approved as first-line treatment for advanced HCC by the FDA in May 2020. Notably, a relatively high incidence of upper gastrointestinal bleeding was observed in patients treated with atezolizumab and bevacizumab (16). We conducted a phase Ib clinical trial to evaluate the safety and efficacy of sintilimab, a specific antibody targeting PD-1, combined with different doses of IBI305, a bevacizumab biosimilar (anti-VEGF), in Chinese patients

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Clin Cancer Res 2022;28:3499–508

doi: 10.1158/1078-0432.CCR-21-3972

Translational Relevance

The combination of immune checkpoint inhibitors and anti-angiogenesis agents is an important treatment strategy for advanced hepatocellular carcinoma (HCC). However, there are currently no validated predictive biomarkers. In this phase Ib study of sintilimab (anti–PD-1) plus a bevacizumab biosimilar (anti-VEGF) in advanced HCC, we found that both high serum concentrations of CD137 and high infiltration of M1 macrophages in the tumor immune microenvironment (TiME) were significantly correlated with a better clinical benefit and prolonged survival, and thus could serve as potential predictive markers. Our findings provide new biomarkers beyond PD-L1 expression and tumor mutation burden (TMB) for screening HCC patients who are likely to benefit from combined anti–PD-1 and anti-VEGF therapy. The results also suggest that searching for predictive markers in peripheral blood and TiME is important to further enhance immunotherapy.

with advanced HCC in October 2018. An additional purpose of this study was to preliminarily determine whether low-dose IBI305 could reduce adverse events (AE) without compromising the efficacy of treatment. Preliminary results were presented at the 2020 ASCO annual meeting (17).

At present, effective biomarkers are lacking for immunotherapy of HCC, and there is no definitive evidence that programmed cell death 1-ligand 1 (PD-L1) expression and tumor mutation burden (TMB) help to screen the population of patients HCC who might benefit from immunotherapy (9, 18–20). The tumor immune microenvironment (TiME) plays an important role in antitumor activity (21). Several cell subtypes in TiME, such as Treg that infiltrate the tumor, have recently been reported to be potentially related to combination therapy with pembrolizumab and lenvatinib in patients with HCC (22, 23). Moreover, serum cytokines are potentially immune-related and can be quantified, presenting an opportunity to establish a noninvasive approach for monitoring disease status and exploring biomarkers to predict the efficacy of anticancer treatment. Therefore, in this study, the levels of cytokines in peripheral blood samples and immune cells in the TiME were simultaneously detected, and their correlation with the efficacy of the combination therapy was analyzed. Here, we report the results of biomarker analysis along with the final clinical outcomes of therapy.

Patients and Methods

Patients and study design

This was a phase Ib, single center study, conducted at the Cancer Hospital of the Chinese Academy of Medical Sciences, carried out in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines and approved by the Ethics Committee of the Cancer Hospital, Chinese Academy of Medical Sciences (Approval No. 18–126/1704). All patients provided written informed consent before enrollment. The study was registered at ClinicalTrials.gov (Registration No. NCT04072679).

Eligible patients were 18 to 75 years old and had been diagnosed with locally advanced or metastatic HCC confirmed histologically or cytologically. All patients had measurable lesions according to the RECIST v1.1, Barcelona Clinic Liver Cancer (BCLC) stage B or C, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and Child-Pugh liver function scores of ≤7.

The study included a dose-escalation part and a dose-expansion part. In the dose-escalation part, dose-limiting toxicities (DLT) were evaluated during the first 3 weeks as the DLT window. The first 6 patients received 200 mg sintilimab plus 7.5 mg/kg IBI305 every 3 weeks. If no more than one patient reported DLTs, the dose of IBI 305 was increased to 15 mg/kg. In the dose-expansion part, at least 20 patients were assigned to each tolerable dose group. Treatment was continued until disease progression, unacceptable toxicity, withdrawal of consent, or at an investigator's decision to terminate treatment, whichever occurred first. Dose reduction of sintilimab and IBI305 was not allowed during this trial. Responses were measured by investigators every 6 weeks. The frequency of assessment was allowed to be adjusted to every 12 weeks after 48 weeks.

The incidence and severity of AEs were graded in accordance with the Common Terminology Criteria for AEs version 5.0 (CTCAE 5.0). DLTs were defined as any of the following toxicities determined to be elicited by the study treatment: (i) grade 4 neutropenia lasting ≥ 7 days; (ii) grade 4 thrombocytopenia or grade 3 thrombocytopenia requiring transfusions; (iii) grade 3 or grade 4 febrile neutropenia; (iv) uncontrollable nonhematologic toxicity of grade 3 or grade 4 despite maximal supportive care; (v) toxicities that required discontinuation of the sintilimab or bevacizumab biosimilar during the DLT window and any grade 5 toxicity.

We conducted exploratory biomarker analysis of baseline serum cytokines concentrations using the bead-based multiplex immunoassay and TiME by multiplex immunofluorescence (mIF), with the aim of discovering novel biomarker of response to VEGF/PD-1 combination therapy in patients with HCC.

Bead-based multiplex immunoassay

Peripheral blood samples (about 10 mL) were collected before study treatment into a BD vacutainer blood collection tube (BD Biosciences) by venipuncture and centrifuged (1,000 × *g* for 15 minutes) to isolate the serum. A total of 59 serologic cytokines were simultaneously measured in serum samples using the ProcartaPlex Human Cytokine/Chemokine/Growth Factor Panel (Affymetrix Inc.) and the ProcartaPlexHuman Immuno-Oncology Checkpoint Panel (Affymetrix Inc.).

Multiplex immunofluorescence

Formalin-fixed paraffin-embedded tumor tissue slides at baseline were prepared. Samples were stained using an Opal automation mIF Detection Kit (Akoya). A total of 11 markers were labeled in two seven-color multiplex panels. The following antibodies were used: anti-CD163 (Abcam, Catalog no. ab182422), anti-CD8 (Abcam, Catalog no. ab178089), anti-CD68 (Abcam, Catalog no. ab213363), anti–PD-1 (Cell Signaling Technology, Catalog no. 86183S), anti-PD-L1 (Cell Signaling Technology, Catalog no. 13684S), and anti-panCK (Abcam, Catalog no. ab7753) for panel 1, as well as CD20 (Daco, Catalog no. L26 IR604), CD3 (Daco, Catalog no. A0452 IR503), CD56 (Abcam, Catalog no. ab75813), CD4 (Abcam, Catalog no. ab133616), FoxP3 (Abcam, Catalog no. ab20034), and panCK for panel 2. The labeled slides were scanned using a Vectra Polaris Automated Quantitative Pathology Imaging System (Akoya), and images from different channels were false-colored and superimposed. Tumor and stroma areas were divided according to cytokeratin (CK)-labeled tumor cells and the cell nuclei counterstained with 4′-6′-diamidino-2-phenylindole (DAPI). Results are reported as percentages (immune subset cells/total cells of

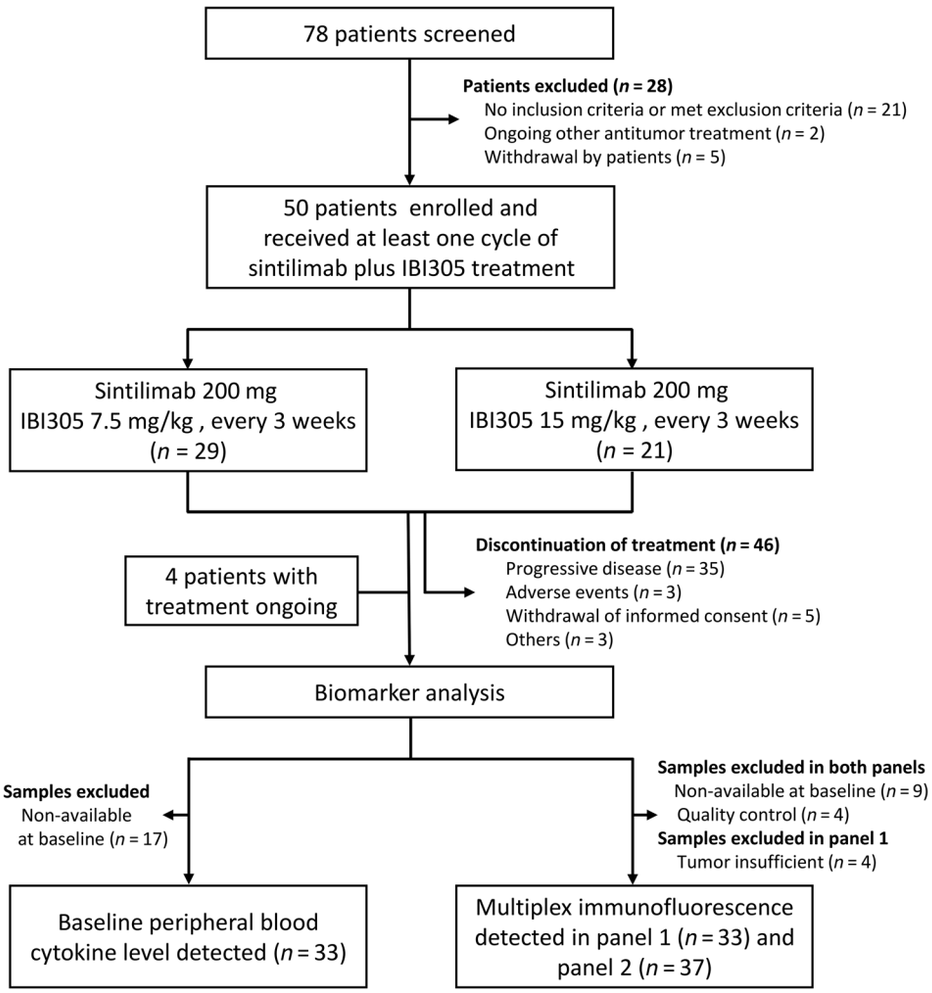

Figure 1.
Flow chart of patient selection and the study design.

DAPI) and density (cells/mm^2) from each individual cell subpopulation in the tumor or stromal area.

Endpoints and statistical analysis

The primary endpoint of the clinical study was safety, and the secondary endpoints included ORR, disease control rate (DCR), PFS, and OS. Patients with complete response (CR)/partial response (PR), or stable disease (SD) ≥ 12 weeks were defined as clinical benefit (CB), whereas those with progressive disease (PD) or SD < 12 weeks as non-clinical benefit (non-CB). Clinical benefit rate (CBR) was the proportion of patients with CB. For the biomarker analysis, PFS was designated as the primary efficacy indicator, whereas CBR and OS were considered to be secondary indicators.

Baseline levels of each serum cytokine were compared between CB and non-CB groups using Kruskal–Wallis sum-rank test. Differences in immune cell subsets between these two groups were analyzed using the Mann–Whitney *U* test. Cytokines and immune cell subsets with *P* values < 0.05 intergroup analysis were selected for further analysis. For each of the selected factors, the ROC curve was created and the optimal cut-off value, which maximized the Youden index, was identified. Survival curves (PFS and OS) were generated using the Kaplan–Meier method and compared between patients with high and low levels (i.e., above/below the optimal cut-off) of the factors investigated using the log-rank test. In addition, Fisher exact test was used to analyze CBR between patients with high/low levels. Multivariate analysis was performed by Cox regression to evaluate independent predictive factors. All statistical tests were double-sided; $P \leq 0.05$ was considered to represent a statistically significant difference.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the corresponding author (A.Z.).

Results

Patient demographic and clinical characteristics

From October 11, 2018 to January 30, 2020, 78 eligible patients were screened (**Fig. 1**). A total of 50 patients were enrolled (42 males vs. 8 females) with a median age of 56 (range, 33–75) years. Among them, 41 (82.0%) patients were systemic therapy naive (**Table 1**). In the dose-escalation part of the study, no DLTs were reported in any of 6 patients receiving doses of 7.5 or 15 mg/kg of IBI 305. Thus, both doses were considered to be tolerable. In the dose-expansion part of the study, the sample size of both dose groups was expanded (7.5 mg/kg, $n = 29$; 15 mg/kg, $n = 21$). All patients received at least one cycle of IBI305 plus sintilimab treatment. By the cut-off date of May 13, 2021, 4 patients (8.0%) remained on treatment, and the median cycle of treatment was

Table 1. Baseline characteristics and survival analysis.

Characteristics	Dose group, *n* (%) 7.5 mg/kg (*n* = 29)	15 mg/kg (*n* = 21)	All patients (*n* = 50), *n* (%)	Median PFS, months (95% CI)	*P* value (log rank)[a]
Sex					0.227
Male	23 (46)	19 (38)	42 (84)	9.705 (7.327–12.083)	
Female	6 (12)	2 (4)	8 (16)	15.869 (4.901–26.837)	
Age					0.816
<60	21 (42)	13 (26)	34 (68)	11.148 (8.507–13.788)	
≥60	8 (16)	8 (16)	16 (32)	10.295 (4.796–15.794)	
ECOG performance status					0.505
0	14 (28)	12 (24)	26 (52)	11.148 (9.897–12.398)	
1	15 (30)	9 (18)	24 (48)	9.410 (5.639–13.181)	
Barcelona Clinic Liver Cancer stage					0.690
B	7 (14)	5 (10)	12 (24)	7.148 (0.000–18.890)	
C	22 (44)	16 (32)	38 (76)	10.525 (8.195–12.854)	
Treatment lines					0.103
First-line	21 (42)	20 (40)	41 (82)	11.410 (10.147–12.673)	
Second- or third-line[b]	8 (16)	1 (2)	9 (18)	4.328 (0.378–8.278)	
Dose group (IBI305)					0.499
7.5 mg/kg	29 (58)	0 (0)	29 (58)	9.705 (5.579–13.831)	
15 mg/kg	0 (0)	21 (42)	21 (42)	11.475 (7.281–15.670)	
Viral status[c]					0.784
Hepatitis B virus	26 (52)	21 (42)	47 (94)	10.295 (7.497–13.094)	
Uninfected	3 (6)	0 (0)	3 (6)		
Baseline AFP					0.144
<200 ng/mL	13 (26)	12 (24)	25 (50)	12.623 (8.577–16.668)	
≥200 ng/mL	16 (32)	9 (18)	25 (50)	8.295 (1.363–15.227)	
Macrovascular invasion					0.827
Yes	7 (14)	2 (4)	9 (18)	11.410 (7.331–15.489)	
No	22 (44)	19 (38)	41 (82)	10.525 (7.959–13.091)	
Extrahepatic disease					0.152
Yes	22 (44)	15 (30)	37 (74)	11.148 (8.437–13.858)	
No	7 (14)	6 (12)	13 (26)	10.295 (0.446–20.144)	
Liver cirrhosis					0.249
Yes	21 (42)	12 (24)	33 (66)	10.295 (7.563–13.027)	
No	8 (16)	9 (18)	17 (34)	11.410 (5.591–17.229)	
Stroma M1 macrophage level					**0.024**
Low	7 (14)	9 (18)	16 (32)	3.016 (0.000–10.583)	
High	13 (26)	4 (8)	17 (34)	11.410 (10.969–11.851)	
NA	9 (18)	8 (16)	17 (34)	-	
Serum CD137 level					<**0.001**
Low	10 (20)	8 (16)	18 (36)	4.131 (0.000–9.122)	
High	10 (20)	5 (10)	15 (30)	14.197 (11.352–17.041)	
NA	9 (18)	8 (16)	17 (34)	-	

[a]Biomarker analysis was performed in patients with cytokines or TiME data available ($n = 33$).
[b]Including 7 patients in second-line and 2 patients in third-line treatment.
[c]HBV infection was defined as hepatitis B surface antigen positive and/or detectable HBV DNA.

8 (range, 1–29). Reasons for discontinuation of treatment were mainly disease progression ($n = 35$, 70.0%) and AEs ($n = 3$, 6.0%). Further details are given in **Fig. 1**.

Safety and efficacy

The most common treatment-related AEs were hypertension (32.0%), proteinuria (26.0%), and fever (26.0%) (**Table 2**). The incidence of grade 3 to 5 AEs was 20.0% among all patients; it was 13.8% and 28.6% in 7.5 and 15 mg/kg groups, respectively. Two (4.0%) patients developed esophageal and gastric varices and bleeding in the 15 mg/kg group, of whom one died. The most common probably immune-related AEs (irAE) were fever (26.0%), hypothyroidism (24.0%), myoarthropathy (20.0%), and rash (18.0%). Probably irAEs of grade 3 were observed in 4 patients (8.0%), including rash, elevated aspartate aminotransferase, hyperglycemia, and pneumonia. No grade 4 to 5 irAEs occurred.

As of May 13, 2021, the median follow-up time was 17.8 months (IQR, 9.1–22.9). PR was observed in 17 (34.0%) patients, with SD in 22 (44.0%) and PD in 11 (22.0%). The ORR was 34.0% [95% confidence interval (CI), 20.0–48.0], and disease control rate (DCR) was 78.0% (95% CI, 66.0–90.0; **Fig. 2A**; Supplementary Table S1). The ORR of the 7.5 mg/kg dose ($n = 29$) was 31.0% (95% CI, 13.0–49.0), and the DCR was 76.0% (95% CI, 59.0–92.0). For the 15 mg/kg dose group ($n = 21$), the ORR was 38.0% (95% CI, 15.0–61.0) and the DCR was 81.0% (95% CI, 63.0–99.0). The median PFS time of all patients was 10.5 months (95% CI, 8.3–12.7), and the median OS time was 20.2 months (95% CI, 16.1–24.3; **Fig. 2B** and **C**). The median PFS times in the 7.5 and 15 mg/kg groups were

Table 2. Summary of AEs.

	7.5 mg/kg ($n = 29$)		15 mg/kg ($n = 21$)		All patients	
Treatment-related AEs[a]	**Any grade**	**Grade 3–5**	**Any grade**	**Grade 3–5**	**Any grade**	**Grade 3–5**
All, *n* (%)	29 (100)	4 (14)	20 (95)	6 (29)	49 (98)	10 (20)
Most common, *n* (%)						
Hypertension	8 (28)	0 (0)	8 (38)	1 (5)	16 (32)	1 (2)
Proteinuria	7 (24)	0 (0)	6 (29)	2 (10)	13 (26)	2 (4)
Fever	9 (31)	0 (0)	4 (19)	0 (0)	13 (26)	0 (0)
Hypothyroidism	6 (21)	0 (0)	6 (29)	0 (0)	12 (24)	0 (0)
Elevated aspartate aminotransferase	6 (21)	1 (3)	4 (19)	0 (0)	10 (20)	1 (2)
Myoarthropathy	6 (21)	0 (0)	4 (19)	0 (0)	10 (20)	0 (0)
Hypertriglyceridemia	5 (17)	1 (3)	4 (19)	0 (0)	9 (18)	1 (2)
Rash	5 (17)	0 (0)	4 (19)	1 (5)	9 (18)	1 (2)
Hypercholesterolemia	4 (14)	0 (0)	5 (24)	0 (0)	9 (18)	0 (0)
Hyperglycemia	6 (21)	1 (3)	1 (5)	0 (0)	7 (14)	1 (2)
Fatigue	5 (17)	0 (0)	2 (10)	0 (0)	7 (14)	0 (0)
Neutropenia	4 (14)	0 (0)	3 (14)	0 (0)	7 (14)	0 (0)
Thrombocytopenia	4 (14)	0 (0)	3 (14)	0 (0)	7 (14)	0 (0)
Hyperbilirubinemia	2 (7)	1 (3)	4 (19)	1 (5)	6 (12)	2 (4)
Hyperthyroidism	3 (10)	0 (0)	2 (10)	0 (0)	5 (10)	0 (0)
Elevated amylase	3 (10)	0 (0)	1 (5)	0 (0)	4 (8)	0 (0)
Diarrhea	1 (3)	0 (0)	2 (10)	1 (5)	3 (6)	1 (2)
Nausea	3 (10)	0 (0)	0 (0)	0 (0)	3 (6)	0 (0)
Esophageal varicosity hemorrhage	0 (0)	0 (0)	2 (10)	1 (5)	2 (4)	1 (2)

[a]Including treatment-related AEs occurring in ≥4% of patients.

9.7 months (95% CI, 5.6–13.8) and 11.5 months (95% CI, 7.3–15.7), respectively. The median OS times in the 7.5 and 15 mg/kg groups were 17.5 months (95% CI, 12.4–22.7) and 21.2 (95% CI, NE–NE) months, respectively. In terms of PFS (**Fig. 2D**, $P = 0.499$) and OS (**Fig. 2E**, $P = 0.365$), no significant difference was observed between the two groups. The median duration of response (DOR) of all patients was 13.2 months (95% CI, 5.9–20.4), and a DOR of at least 12 months was found in 9 of 17 patients.

Peripheral CD137 as a predictive biomarker of VEGF/PD-1 combination for HCC

We performed exploratory biomarker analysis of 59 serum cytokines to look for novel biomarkers of response to VEGF/PD-1 combination therapy in patients with HCC. A total of 33 baseline blood samples were available and passed quality control (**Fig. 1**). Each serum cytokine was firstly compared between CB ($n = 25$) and non-CB groups ($n = 8$). The results showed that the CB group had significantly higher serum CD137 concentrations than the non-CB group (32.8 pg/mL vs. 19.8 pg/mL, $P = 0.034$; **Fig. 3A**). In addition, serum expression levels of IFNγ-inducible protein-10 (IP-10; median, 15.9 pg/mL vs. 10.8 pg/mL, $P = 0.015$), stem cell factor (SCF; median, 7.0 pg/mL vs. 4.1 pg/mL, $P = 0.044$), and monocyte chemoattractant protein-1 (MCP-1; median, 30.6 pg/mL vs. 18.4 pg/mL, $P = 0.048$) were also higher in the CB group than in the non-CB group (Supplementary Table S2). However, no significantly different *P* values were found by applying the Bonferroni correction. Considering that the Bonferroni method might be overly conservative and lead to irrelevant null hypotheses, we selected the factors with uncorrected *P* values < 0.05 for further survival analysis, in which serum CD137 was the most significant cytokine associated with longer survival (Supplementary Table S3). On the basis of the optimal threshold concentration of CD137 (high ≥ 31.8 pg/mL, $n = 15$ and low < 31.8 pg/mL, $n = 18$), PFS was significantly longer in the high-concentration group compared with the low-concentration group (median PFS: 14.2 months vs. 4.1 months, $P < 0.001$; **Fig. 3B**). Similarly, the CD137 high group was associated with a significantly longer OS (median OS: NE vs. 15.6 months, $P = 0.023$; **Fig. 3C**). In addition, CBR was 100% (15/15) in patients with high concentrations of CD137, which was significantly greater than those with the low concentrations (55.6%, 10/18; $P = 0.004$, **Fig. 3D**).

High infiltration of M1 macrophages associated with better efficacy of VEGF/PD-1 combination therapy

To explore whether immune cell subsets in TiME may predict the efficacy of sintilimab plus IbI305, mIF was performed on baseline tumor tissues. Thirty-three samples in panel 1 (12 from biopsy, 21 from surgery) and 37 samples in panel 2 (15 from biopsy, 22 from surgery) were available and passed quality control for the final analysis (**Fig. 1**). We first assessed the predictive value of PD-L1 expression, but no significant difference in PD-L1 expression between the CB and non-CB groups was found (Supplementary Fig. S1). Interestingly, infiltration of M1 macrophages ($CD68^+CD163^-$) in the stroma area in the CB group was significantly greater than in the non-CB group (density, $P = 0.032$ and percentage, $P = 0.047$, **Fig. 4A** and Supplementary Table S4). The total number of macrophages ($CD68^+$) in the stroma area (density, $P = 0.039$), and the natural killer (NK) cells ($CD56^+$) in the tumor area (density, $P = 0.028$ and percentage, $P = 0.021$) in the CB group were noticeably greater than in the non-CB group. Differences of other immune cell subsets in TiME for patients in two groups were shown in Supplementary Table S4. Further analysis of the subsets with *P*-values < 0.05 (uncorrected) in the intergroup test demonstrated that only the density of M1 macrophages in the stroma area was associated with survival. According to the optimal threshold (118.1 cells/mm^2), patients were divided into a M1 macrophages high infiltration group (≥118.1 cells/mm^2, $n = 17$) and a low

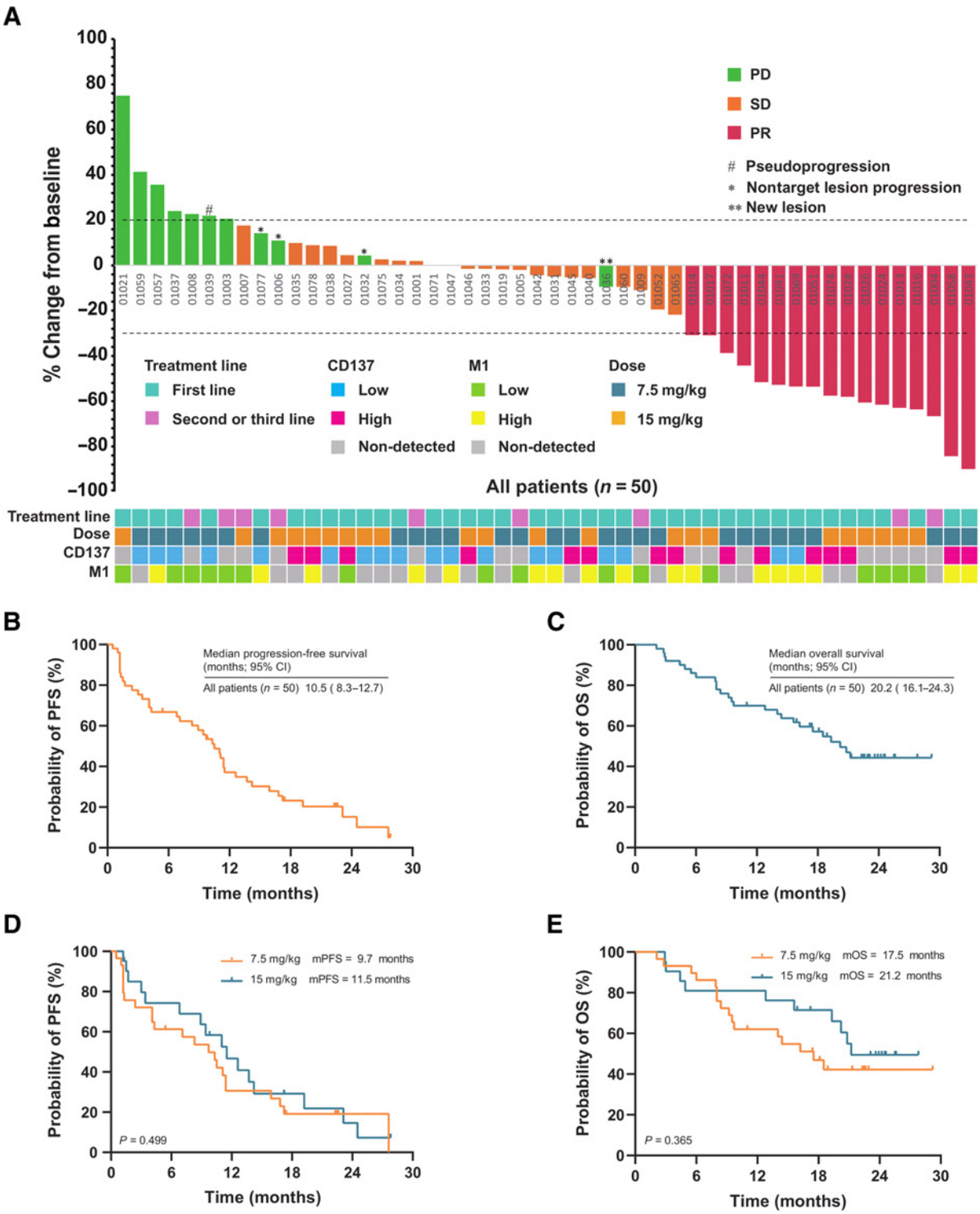

Figure 2.

Efficacy and survival of the overall population ($n = 50$). **A,** Waterfall plot of best percentage change in tumor size from baseline by RECIST V.1.1. **B,** Kaplan–Meier estimate of PFS in all patients. **C,** Kaplan–Meier estimate of OS in all patients. **D,** Kaplan–Meier estimate of PFS between 7.5 mg/kg and 15 mg/kg IBI305 dose groups. **E,** Kaplan–Meier estimate of OS between 7.5 mg/kg and 15 mg/kg IBI305 dose groups.

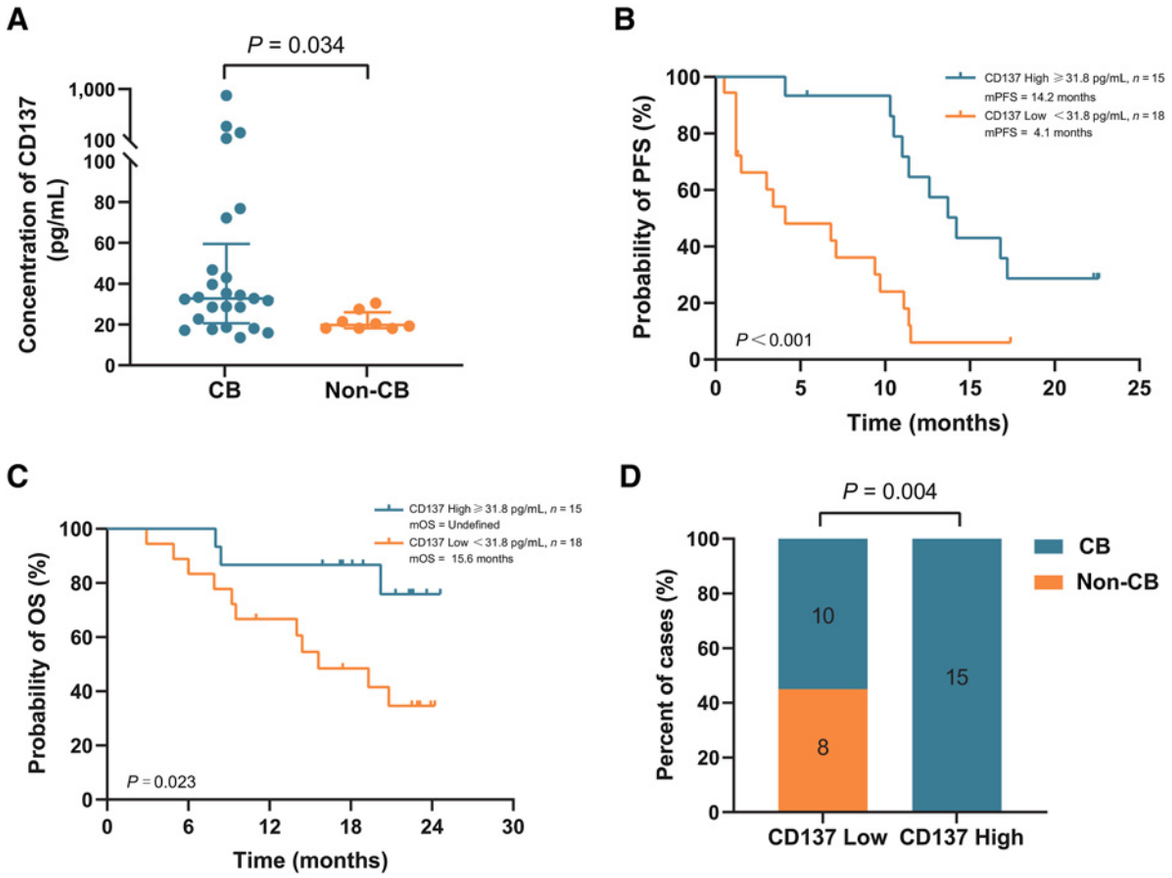

Figure 3.

Relationship between peripheral blood cytokines and efficacy of combined therapy in HCC ($n = 33$). **A,** Serum levels of CD137 in patients with CB compared to those with non-CB. **B,** Kaplan–Meier estimate of PFS based on serum levels of CD137. **C,** Kaplan–Meier estimate of OS based on serum levels of CD137. **D,** Difference of clinical benefit rate between high and low serum concentrations of CD137.

infiltration group (<118.1 cells/mm^2, $n = 16$). The median PFS in the high infiltration group was significantly longer than in the low infiltration group (11.4 months vs. 3.0 months, $P = 0.024$, **Fig. 4B**). Moreover, an increased density of M1 macrophages ($CD68^+CD163^-$) was also significantly correlated with a longer OS (median OS: NA vs. 17.5 months, $P = 0.046$, **Fig. 4C**). In addition, The CBR of the M1 high group was 88.2% (15/17), which was significantly higher than for the M1 low group (50.0%, 8/16) ($P = 0.026$; **Fig. 4D**).

Univariate and multivariate analysis

Univariate analysis was carried out to determine whether CD137, M1 macrophages, or various clinicopathological factors, including age, sex, tumor stage, dose group, AFP, etc., correlated with PFS. The results revealed no significant correlation between clinicopathologic factors and PFS except for CD137 and M1 (**Table 1**; Supplementary Table S5). Next, parameters with P values < 0.1 in the univariate analysis and potentially relevant clinicopathologic factors were put into the Cox proportional hazards model. Results showed that the high concentration of serum CD137 ($P = 0.047$; HR, 0.245; 95% CI, 0.061–0.984) and the high density of M1 macrophages ($P = 0.004$; HR, 0.020, 95% CI, 0.001–0.286) were independent positive predictors for PFS.

Combined analysis of serum CD137 and tumor M1 macrophages for predicting efficacy

Subsequently, we tried to combine the results for serum CD137 and tumor M1 macrophages to evaluate therapeutic efficacy. A total of 20 patients with both cytokine and TiME data available were divided into three groups: dual-factor high expression ($CD137^{high}M1^{high}$) [$n = 7$ (35%)], one-factor high expression ($CD137^{high}M1^{low}$/$CD137^{low}M1^{high}$) [$n = 9$ (45%)], and dual-factor low expression ($CD137^{low}M1^{low}$) [$n = 4$ (20%)]. Results showed that the median PFS times in the dual-factor high expression, one-factor high expression, and dual-factor low expression groups were 16.8, 11.0, and 1.2 months, respectively. Patients with dual-factor high expression ($CD137^{high}M1^{high}$) had the longest median OS. In addition, 100% (7/7) of patients with $CD137^{high}M1^{high}$ and 78% (7/9) with $CD137^{high}M1^{low}$/$CD137^{low}M1^{high}$ achieved CB, whereas only 25% (1/4) of patients in the $CD137^{low}M1^{low}$ group exhibited CB (Supplementary Fig. S2).

Discussion

To our knowledge, no effective predictive biomarkers have been clearly identified for immunotherapy plus anti-VEGF agents for HCC therapy, although a recent study indicated a correlation between PD-L1 expression at the RNA level and the efficacy of combination

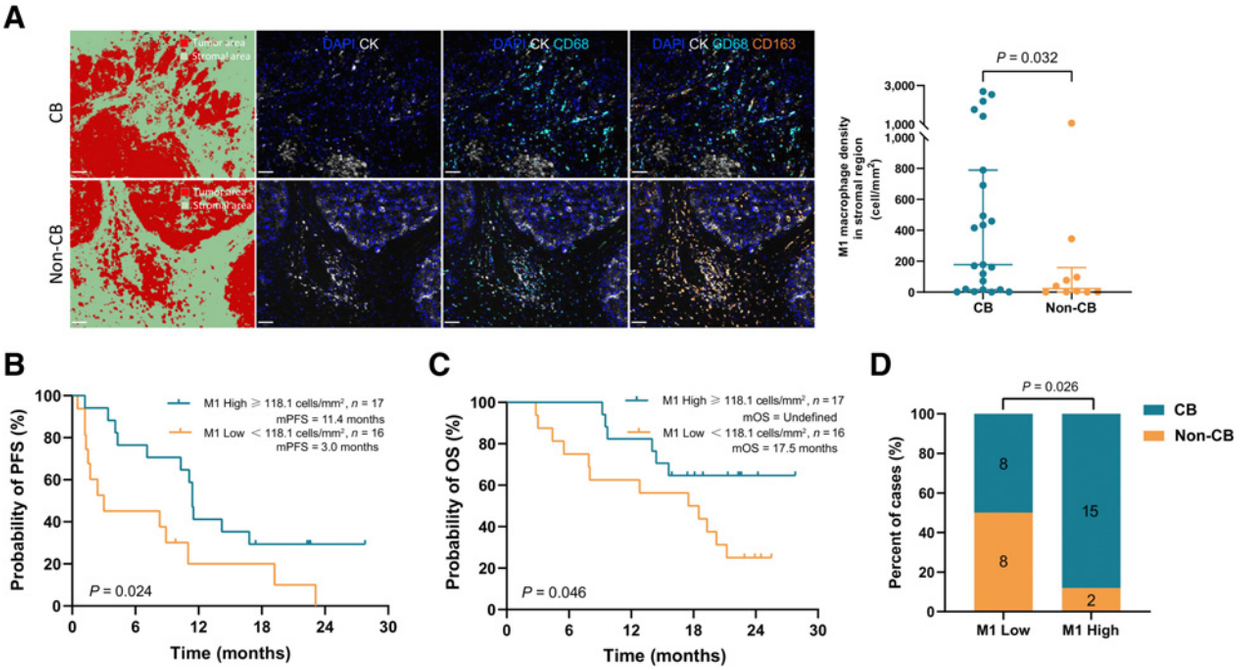

Figure 4.

Relationship between immune cell subsets in TiME and the outcomes of combined therapy. **A,** Images of representative mIF results (left, scale: 50 μm) and M1 macrophages (right, $CD68^+CD163^-$) in patients with CB compared with non-CB patients. **B,** Kaplan–Meier estimate of PFS based on infiltration levels of M1 macrophages ($CD68^+CD163^-$). **C,** Kaplan–Meier estimate of OS based on infiltration levels of M1 macrophages ($CD68^+CD163^-$). **D,** Differences in the clinical benefit rate between high and low levels of M1 macrophages.

treatment with atezolizumab and bevacizumab (24). However, neither PD-L1 expression at the protein level nor TMB was verified to predict the response to immunotherapy in HCC (9, 18–20, 25). Importantly, our study showed that a high serum level of CD137 at baseline was associated with a greater clinical benefit rate and longer survival. These results suggest that the serum concentration of CD137 is a potential predictive biomarker for the effectiveness of anti–PD-1 plus bevacizumab biosimilar therapy for HCC.

CD137, also known as 4–1BB or TNF Receptor Superfamily Member 9 (TNFRSF9), is a member of tumor necrosis factor family and an important costimulatory molecule in the process of T-cell activation, which can enhance the antitumor effects of T cells (26, 27). CD137 is mainly expressed by activated $CD4^+$T and $CD8^+$T cells, though it can also be found on the surface of NK cells, neutrophils, dendritic cells, and monocytes (28, 29). The expression of CD137 in HCC tissues was higher than that in tissues affected by other types of cancer (e.g., small cell lung cancer and colorectal cancer) and CD137 was found to be expressed predominantly in exhausted $PD\text{-}1^{high}CD8^+$T cells (30). Moreover, activated T cells in peripheral blood samples may also express CD137 (26, 31, 32), and the increased number of $CD137^+CD8^+$ T cells in peripheral blood samples was correlated with longer DFS in patients with melanoma who were treated with ipilimumab plus nivolumab (33). Preclinical studies have also shown that there is synergistic antitumor activity between PD-1/PD-L1 inhibitors and activation of the CD137 signaling pathway (34). In addition, CD137 is also a potential target of immunotherapy (35, 36). At present, several agonist anti-CD137 antibodies are undergoing clinical trial. Potentially higher antitumor activity has been reported when using the strategy of agonist anti-CD137 antibody plus nivolumab therapy (37).

M1 macrophages may inhibit the progression of tumors by affecting the immune microenvironment (38), and numerous studies have shown that a high infiltration of M1 macrophages is largely associated with a good prognosis (39–42). Reprogramming tumor-associated macrophages (TAM) from pro-tumor (M2) to the anti-tumor (M1) phenotype may induce immune effects (43). We found that patients with a high infiltration of M1 macrophages had not only a significantly longer OS as previously reported, but also a significantly improved CBR and PFS. These results suggest that the high infiltration of M1 macrophages in HCC might be a potential positive predictor for the efficacy of anti–PD-1 plus anti-VEGF therapy. Ho and colleagues also found that certain macrophage subtypes were associated with treatment efficacy and further analysis revealed the proximity between lymphoid and macrophage subtypes as the key determinant of response to a combination of cabozantinib and nivolumab (44). Similar results were also observed in patients with lung cancer who were treated with ICIs (45). Interestingly, in our study, 2 patients with PR had low concentrations of serum CD137, but high infiltration of M1 macrophages (**Fig. 2A**). These findings suggested that CD137 plus M1 might be complementary and together provide a better predictive value. The results remain to be verified further by investigations involving a larger cohort of patients.

In this study, IBI305 plus sintilimab produced promising clinical efficacy in the treatment of advanced HCC, with an ORR and a DCR of 34% and 78%, respectively, and PFS of 10.5 months. Similar to the results of two extensive phase III studies, IMbrave150 and ORIENT-32 (15, 46), our findings reconfirmed the effectiveness of ICIs plus anti-VEGF antibody for the treatment of patients with advanced HCC. Moreover, the lower dose group of IBI305

at 7.5 mg/kg seemed to have a more favorable safety profile. Whether a lower dose of bevacizumab or its biosimilar is also efficacious remains a concern in clinical practice. In our study, the PFS of IBI305 at a dose of 7.5 mg/kg reached 9.7 months along with numerically similar ORR and DCR values compared with the regular dose of the 15 mg/kg. Serum CD137 concentrations ($P = 0.392$, Supplementary Fig. S3) at baseline in the two dose groups were similar, and there was no statistical difference in survival between the two groups, although the OS were numerically slightly shorter in the low-dose group. These results suggested that the 7.5 mg/kg IBI305 dose plus sintilimab showed promising antitumor activity, which might be an option with acceptable efficacy for some patients who cannot tolerate high dose bevacizumab, especially those at a higher risk of gastrointestinal bleeding, who have cardiovascular and cerebrovascular diseases, or are elderly. The efficacy and safety of low-dose IBI305 at 7.5 mg/kg remains to be further verified by subsequent clinical studies.

There are several limitations to our study. First, the sample size was relatively small. The exact efficacy of a 7.5 mg/kg dose needs to be further evaluated by studies with a larger sample size. Second, previous treatment of each patient was not uniform and a minority of patients received the therapy as second-line or third-line treatment, which could potentially introduce bias to the comparison of results between 7.5 and 15 mg/kg groups. Third, biomarker analysis was exploratory with insufficient specimens, which might result in bias. Fourth, the correlation of serum CD137 concentrations and CD137 expression levels in tumor tissues was not clear, and further investigation is required to identify the specific immune cell subsets that express CD137.

In conclusion, sintilimab plus IBI305 exhibited prominent antitumor activity in patients with advanced HCC. The safety profile of a low dose of IBI305 (7.5 mg/kg) seemed more favorable with acceptable efficacy and could be used as an alternative dose for patients who are not eligible for intensive treatment. Both a high serum concentration of CD137 and a high density of M1 macrophage infiltration in the tumor stroma were significantly correlated with better efficacy, prolonged PFS and OS. These factors may serve as potential predictive biomarkers for the effectiveness of combination therapy of PD-1 inhibitor and VEGF antibody.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

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Acknowledgments

The authors are grateful to the patients for participating in this study and to their families for their understanding. In addition, the authors wish to thank our colleagues Kaitai Zhang and Wen Zhang from the Department of Immunology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital for their helpful discussions and suggestions. We want to present our thanks to Mr. Junyan Zhang from Bothwin Clinical Study Consultant, Shanghai, China, who gave us the valuable suggestions on statistical modifications. We would also like to thank Mr. Youhui Tian from the Medical Department, 3D Medicines Inc., for partial data processing of immunofluorescence results. This work was partly supported by the Non-profit Central Research Institution Fund of Chinese Academy of Medical Sciences (2019PT310026, to H. Zhao).

The publication costs of this article were defrayed in part by the payment of publication fees. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Received November 9, 2021; revised January 5, 2022; accepted March 9, 2022; published first March 11, 2022.

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