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Preoperative sintilimab plus transarterial chemoembolization for hepatocellular carcinoma exceeding the Milan criteria: A phase II trial

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Abstract

Background and aims: Many patients with HCC of Barcelona Clinic Liver Cancer (BCLC) stage A exceeding the Milan criteria, or of BCLC stage B, can undergo resection after successful preoperative therapy, but an optimal approach has not been identified. We investigated preoperative drug-eluting bead transarterial chemoembolization (DEB-TACE) plus sintilimab, in this setting.

Approach and Results: In this prospective, phase II study (NCT04174781), adults with HCC of BCLC stage A exceeding the Milan criteria, or BCLC stage B, and ineligible for surgical resection, received sintilimab 200 mg and DEB-TACE. The primary endpoint was progression-free survival by modified RECIST. Secondary endpoints included objective response rate, pathologic response rate, and safety. At the data cutoff (July 2022), among 60 patients, the objective response rate was 62% (37/60) and 51 patients had undergone surgery. After a median follow-up of 26.0 months (range, 3.4–31.8), the median progression-free survival was 30.5 months (95% CI: 16.1–not reached). Among patients undergoing surgery, median progression-free survival was not reached and the 12-month progression-free survival rate was 76% (95% CI: 67–91). A pathologic complete response was achieved in 14% (7/51) of these patients. All patients experienced at least one adverse

Abbreviations: AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; ECOG, Eastern Cooperative Oncology Group; DEB-TACE, drug-eluting bead transarterial chemoembolization; IMC, imaging mass cytometry; MPR, major pathologic response; mRECIST modified response evaluation criteria in solid tumors; ORR, objective response rate; PR, partial response; TACE, transarterial chemoembolization; tSNE, t-distributed stochastic neighbor embedding; PD-1, programmed cell death protein 1; pCR, pathologic complete response; PFS, progression-free survival.

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event, but these were generally manageable. Exploratory analyses showed an association between cytokeratin, *V*-domain Ig-containing Suppressor of T-cell Activation, CD68, CD169, and cluster 13 fibroblasts and recurrence after surgery.

Conclusions. Sintilimab plus DEB-TACE before surgery showed good efficacy and safety in patients with HCC of BCLC stage A exceeding the Milan criteria or BCLC stage B.

INTRODUCTION

HCC is one of the most common malignant tumors, and ~50% of global cases occur in China.[1] The Barcelona Clinic Liver Cancer (BCLC) staging system is the most widely used staging system for HCC and provides treatment recommendations for each disease stage.[2] Selected patients with early-stage (BCLC stage A) disease are eligible for potentially curative surgical resection or liver transplantation, depending on characteristics such as the number of lesions, liver function, and overall condition. Among patients with BCLC stage A HCC that meets the Milan criteria (single tumor of diameter <5 cm or ≤3 tumor foci, each ≤3 cm; no angioinvasion; no extrahepatic involvement), liver transplantation is associated with long-term survival benefits.[3] However, in the majority of patients with early-stage HCC, the Milan criteria are exceeded at the time of diagnosis (eg, they have a single tumor >5 cm).[4] Patients with HCC exceeding the Milan criteria have a 5-year overall survival of around 10.7%.[5]

For patients with BCLC stage A HCC exceeding the Milan criteria, both Chinese and global treatment guidelines recommend surgical resection.[6,7] However, in real clinical practice, although often technically resectable, these patients have relatively large tumors and, due to factors such as a close relationship with blood vessels, insufficient liver function, and loss of capsule, are therefore difficult to directly resect, at high risk of postoperative recurrence and associated with a poor long-term prognosis.[8] For patients with BCLC stage B HCC, the treatment strategies implemented in Eastern and Western countries differ greatly.[9,10] In Western countries, transarterial chemoembolization (TACE) is a standard treatment for patients with BCLC stage B HCC, whereas Chinese treatment guidelines suggest that this subgroup of patients can achieve an opportunity for radical surgery and favorable outcomes after initial TACE treatment. However, the survival of patients treated with preoperative TACE alone remains unsatisfactory.

Programmed cell death protein 1 (PD-1) blockade has demonstrated efficacy in unresectable HCC,[11–13] and it has also been investigated in the preoperative setting.[14,15] As TACE can lead to tumor expression of neoantigens and activate the immune system,[16] the combination of an anti–PD-1 antibody and TACE may provide enhanced antitumor efficacy but has been rarely explored.

Given that TACE with drug-eluting beads (DEB-TACE) is associated with higher concentrations of drug within the target tumor and favorable safety compared with conventional TACE,[17,18] the present study was undertaken to investigate the efficacy and safety of preoperative DEB-TACE plus the selective anti–PD-1 antibody, sintilimab, for the treatment of patients with BCLC stage A/B HCC exceeding the Milan criteria.

PATIENTS AND METHODS

Study design

This prospective, open-label, phase II clinical study was conducted at the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. The study was approved by the Institutional Research Ethics Committee and performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients before enrollment. The trial was registered with ClinicalTrials.gov (NCT04174781). The trial protocol is available in Supplement 1 (http://links.lww.com/HC9/A134).

Patients

As the definition of technical resectability is very dependent on the experience and judgment of surgeons, in this study, we did not use a simple fixed classification of “resectable” or “uresectable” HCC but selected the best treatment for each patient through discussion by multidisciplinary teams. Patients aged 18 or older–75 years with histologically or clinically confirmed HCC (based on the American Association for the Study of Liver Diseases criteria[19]) that was either BCLC stage A and exceeded the Milan criteria, or BCLC stage B (ie, patients with stage Ib, IIa, and IIb

HCC according to the China Liver Cancer staging system[10]) with factors that may limit the benefit of direct surgery in patients who were technically resectable, such as large tumor load, difficult to determine boundaries, incomplete capsule or close proximity to main blood vessels/porta hepatis, therefore giving a high risk of causing disseminated metastasis during surgery, with a large number of tumors that are localized and technically resectable, or with the possibility of conversion resection (eTable 1) were eligible. Patients were also required to have Child-Pugh liver function class A; and adequate organ function. The main exclusion criterion was a hepatic tumor burden $>50\%$ of total liver volume. Patients in whom DEB-TACE or anti–PD-1 antibody therapy was contraindicated were also excluded. The full eligibility criteria are detailed in the study protocol (Supplement 1, http://links.lww.com/HC9/A134).

Interventions

DEB-TACE was conducted as described.[18] Patients received sintilimab [Innovent (Suzhou) Biopharmaceutical Co., Ltd.] 200 mg by intravenous infusion over 30 minutes at the beginning of the DEB-TACE (epirubicin 60 mg) procedure on day 1 of the first treatment cycle and every 3 weeks thereafter. A treatment cycle was defined as 1 DEB-TACE procedure plus 2 doses of sintilimab. Additional DEB-TACE procedures were carried out every 4–6 weeks based on tumor response (study protocol in Supplement 1, http://links.lww.com/HC9/A134). Dose delays or dose reductions for sintilimab were not allowed. The combination of DEB-TACE and sintilimab was continued for a maximum of 3 cycles until surgical resection, radiologic disease progression, unacceptable toxicity, or withdrawal from the study, whichever occurred first. Post-surgical adjuvant therapy was not permitted.

Outcomes and assessments

The primary endpoint was progression-free survival (PFS), defined as the time from the start of treatment to the date of progressive disease, recurrence, or death, whichever occurred first. All efficacy outcomes were assessed according to the modified response evaluation criteria in solid tumors, based on the maximum diameter change of tumor enhancement on contrast-enhanced imaging.[20] Secondary endpoints included recurrence-free survival, defined as the time from the start of treatment to the date of recurrence or death among the patients who received surgery, whichever occurred first; pathologic response, including major pathologic response (defined as $\geq 90\%$ necrosis in the resected tumor area) and pathologic complete response (pCR; defined as no viable tumor cells in the specimen); objective response rate (ORR) (defined as patients achieving a complete response or partial response); and safety. Exploratory endpoints included correlations between biomarkers and efficacy.

Tumor response was assessed using contrast-enhanced computed tomography according to modified response evaluation criteria in solid tumor at baseline, at the end of each treatment cycle, then every 3 months postoperatively for 2 years, and every 6 months thereafter.[20] A multidisciplinary liver tumor board determined each patient's eligibility for surgical resection. For patients undergoing surgery, tissue sample collection followed the process described in the Chinese Guidelines for the Diagnosis and Treatment of Primary Liver Cancer in China (2022 edition). All samples of liver tumors ≤ 3 cm are collected; tumors >3 cm were cut at 0.5 cm intervals at the largest diameter, and the most representative sections of tumor necrosis and residual tissue were selected for sampling. At the same time, samples of the tumor bed and surrounding liver tissues were taken as a control, and the pathologist determined the percentage of surviving tumor tissue by microscopic evaluation. Tumor samples taken at baseline and posttreatment from patients with early recurrence [21] and those with nonrecurrence were tested by Imaging Mass Cytometry to investigate the relationship between multiple biomarkers and efficacy (Supplement 1, http://links.lww.com/HC9/A134).

Safety was monitored continuously throughout the trial. Adverse events were assessed according to the Common Toxicity Standards of the National Cancer Institute (NCI CTCAE) version 5.0.

Statistical analyses

Based on the literature,[22] the median PFS after DEB-TACE alone for BCLC stage A HCC beyond the Milan criteria or BCLC stage B HCC is around 15 months. Considering the theoretical advantages of immunotherapy combined with interventional therapy as a preoperative treatment, this study assumed that the PFS in the study population could be extended to 24 months after DEB-TACE plus sintilimab treatment combined with surgical resection. Based on this assumption, a sample size of 61 patients would be required to provide a power of 80% with a 2-sided alpha level of 0.05 and assuming a loss to follow-up of 10%. Survival analyses were performed using the Kaplan-Meier method and log-rank test. Descriptive summaries were provided for all other efficacy and safety endpoints. All statistical analyses were conducted using R-4.0.5; a 2-sided $p < 0.05$ was considered statistically significant. The safety analysis population included all enrolled participants who received at least one treatment cycle with DEB-TACE plus sintilimab.

RESULTS

Between November 2019 and October 2020, 72 patients were screened, 61 of whom were enrolled in the study, underwent at least one treatment cycle with DEB-TACE plus sintilimab, and were included in the safety analysis population (eFigure 1, http://links.lww.com/HC9/A134). Most were male patients (52/61, 85%), and the median age was 58 years (range, 26–75). Among the patients, 52 (85%) had ≤2 lesions, and 34 (56%) had BCLC stage B HCC. The liver function of all patients was Child-Pugh A, without vascular invasion or extrahepatic metastasis, and without clinically significant portal hypertension (Table 1). The median target tumor size was 7 cm (range, 2.1–14.1). Overall, 50 of 61 (82%) patients received a single treatment cycle with sintilimab plus DEB-TACE, and 11 patients (18%) had received 2 or 3 treatment cycles. The median time on treatment was 1.4 months (range, 1.4–5.0).

One patient withdrew informed consent after one cycle of drug therapy and was not evaluated by imaging; therefore, 60 patients were evaluated for efficacy according to modified response evaluation criteria in solid tumors. At the data cutoff (July 17, 2022), the median follow-up time was 26.0 months (range, 3.4–31.8). The median PFS was 30.5 months (95% CI: 16.1–NA) (Figure 1). Twenty-eight events occurred. The estimated 12-month PFS rate was 75% (95% CI: 65–87).

TABLE 1 Patient demographics and baseline clinical characteristics

Characteristics	N = 61; n (%)
Age (y), median (range)	58 (26–75)
sex (M)	52 (85)
Etiology	
Hepatitis B infection	51 (84)
Alcohol liver disease/other	10 (16)
BCLC stage	
A	27 (44)
B	34 (56)
Child-Pugh A	61 (100)
Target tumor size (cm), median (range)	7 (2–14)
Tumor number	
1	27 (44)
2	25 (41)
3	7 (12)
5	2 (3)
Serum AFP ≥ 400 ng/mL	20 (33)
PIVKA-II (mAU/mL), median (range)	1673 (13–75000)

Abbreviations: AFP indicates α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; PIVAKA II, protein induced by vitamin K absence or antagonist-II.

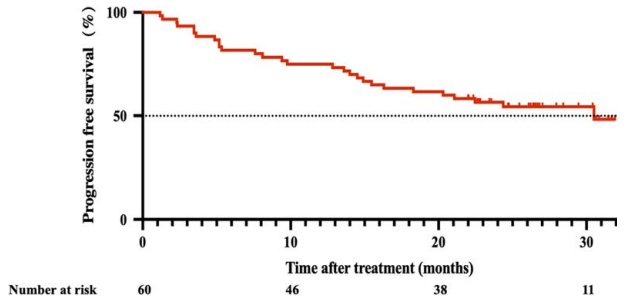

FIGURE 1 Kaplan-Meier analysis of median progression-free survival. Median follow-up time: 26.0 months (range, 3.4–31.8). Median progression-free survival: 30.5 months (95% CI: 16.1–NA). Abbreviations: NA, not Available.

Among 60 patients evaluable for efficacy, 37 (62%) achieved an objective response to DEB-TACE plus sintilimab, including 3 patients with a complete response (5%) and 34 with a partial response (57%) (Table 2). An additional 20 patients had stable disease, resulting in a disease control rate of 95% (57/60). Pseudoprogression was not observed in our study population. The percentage change in target tumor size for each patient is shown in eFigure 2 (http://links.lww.com/HC9/A134).

In 51 patients who underwent surgery, the median time between starting DEB-TACE plus sintilimab and surgical excision was 39.5 days (range, 29–151). Only one patient died in the perioperative period. Median PFS was not reached in these patients. The estimated 12-month postoperative PFS rate was 76% (95% CI: 67–91). A pCR was achieved in 14% (7/51) of these patients, with 49% (25/51) of patients classified as having a major pathologic response (Table 2). A representative case is shown in eFigure 3 (http://links.lww.com/HC9/A134). Median tumor marker levels (α-fetoprotein, or protein induced by vitamin K absence or antagonist-II in α-fetoprotein nonexpressers) declined dramatically during treatment in these patients, almost

TABLE 2 Summary of radiographic and pathologic responses

Variable	N = 60; n (%)
Best overall response (mRECIST)	
Complete response	3 (5)
PR	34 (57)
Stable disease	20 (33)
Progressive disease	3 (5)
ORR	37 (62)
Disease control rate	57 (95)
Pathologic response	N = 51[a]
pCR	7 (14)
MPR	25 (49)

[a]A total of 51 patients who underwent surgery and were evaluable for response.
Abbreviations: MPR indicates major pathologic response; mRECIST indicates modified response evaluation criteria in solid tumors; ORR, objective response rate; PR, partial response; pCR, pathologic complete response.

reaching normal levels 1 month after surgery (eFigure 4, http://links.lww.com/HC9/A134).

A total of 9 patients did not undergo surgical resection [4 patients refused surgery, 3 patients (all with BCLC stage B disease) had disease progression, and 2 patients could not undergo surgery due to an insufficient future liver remnant]. The median PFS for these patients was 14.9 months (95% CI: 13.7–16.1), with 6 events occurring. The estimated 12-month PFS rate in these patients was 67% (95% CI: 46–100). The details of subsequent treatment and survival in these 9 patients are summarized in eFigure 5 (http://links.lww.com/HC9/A134).

Adverse events were reported in all 61 (100%) patients who received DEB-TACE plus sintilimab, including grade 3 or 4 adverse events in 17 (28%) patients. Adverse events were generally tolerable. Treatment-related adverse events are summarized in Table 3 and eFigure6 (http://links.lww.com/HC9/A134). The most frequent adverse events were elevated aspartate aminotransferase (54/61, 89%), hypoalbuminemia (46/61, 75%), elevated alanine aminotransferase (45/61, 74%), anemia (34/61, 56%), weight loss (33/61, 54%), and fatigue (32/61, 52%). Grade 1/2 rash was reported in 15 patients (25%) for whom systemic steroids were unnecessary. Grade 3–4 alanine aminotransferase or aspartate aminotransferase elevations occurred in 8 (13%) and 16 (26%) of 61 patients, respectively. There were no drug-related deaths during the study. Surgery-related safety included 3 participants with bile leakage (6%), 2 with pleural effusion (4%), and 1 each with ulcerative colitis (2%) and liver failure (2%). The participant with liver failure died 2 months after surgery.

TABLE 3 Treatment-related adverse events

Treatment-related adverse events	Any grade; n (%)	Grade 1/2; n (%)	Grade 3/4; n (%)
All adverse events	61 (100)	61 (100)	17 (28)
Elevated AST level	54 (89)	39 (64)	16 (26)
Hypoalbuminemia	46 (75)	46 (75)	0
Elevated ALT level	45 (74)	37 (61)	8 (13)
Anemia	34 (56)	33 (54)	1 (2)
Weight loss	33 (54)	33 (54)	0
Fatigue	32 (52)	32 (52)	0
Pain	29 (48)	28 (46)	1 (2)
Hypokalemia	22 (36)	20 (33)	2 (3)
Nausea	22 (36)	21 (34)	1 (2)
Dry mouth	20 (33)	20 (33)	0
Fever	18 (30)	18 (30)	0
Hyperbilirubinemia	18 (30)	18 (30)	0
Rash	15 (25)	15 (25)	0
Dyspepsia	15 (25)	15 (25)	0
Oral mucositis	13 (21)	12 (20)	1 (2)
Constipation	12 (20)	12 (20)	0
Leukocytopenia	9 (15)	9 (15)	0
Thrombocytopenia	9 (15)	9 (15)	0
Dry skin	8 (13)	8 (13)	0
Infusion-related reaction	7 (11)	7 (11)	0
Neutropenia	6 (10)	5 (8)	1 (2)
Diarrhea	5 (8)	5 (8)	0
Vomiting	5 (8)	5 (8)	0

Abbreviations: ALT indicates alanine aminotransferase; AST, aspartate aminotransferase.

Tissue samples were analyzed before and after treatment for patients who had surgery and preoperative puncture specimens. Pretreatment tissue samples were provided voluntarily from 13 patients. Seven regions of interest were selected in tissue sections stained with Maxpar metal-labeled antibodies. Hyperion was used to detect the expression and location of 39 markers (Supplement 1, http://links.lww.com/HC9/A134) on preoperative puncture sections and surgical resection samples from 13 patients, 4 of whom had postoperative early recurrence (Figure 2A). In total, 182 high-dimensional pathologic tumor images were obtained. Using *t*-distributed stochastic neighbor embedding to reduce the dimension of marker expression data, 16 cell clusters were obtained and annotated (Figure 2B). A heat map was used to summarize marker expression (Figure 2C).

Compared with patients without recurrence after surgery, cluster 1 tumor cells (Pan cytokeratin$^+$), cluster 5 macrophages [*V*-domain Ig-containing suppressor of T-cell activation (VISTA)$^+$, CD68$^+$, CD169$^+$], and cluster 13 fibroblasts (Vimentin$^+$, alpha-smooth muscle actin$^+$) were highly expressed in preoperative puncture and postoperative resection specimens from patients who did have recurrence after surgery ($p < 0.05$, except cluster 5 from postoperative tissues) (Figure. 2D-6F). Baseline cluster 2 T-cell infiltration was comparable in patients with early relapse and those without relapse. After treatment with DEB-TACE plus sintilimab, the infiltration of cluster 2 T cells increased significantly in patients without recurrence ($p = 0.003$) (Figure 2G). Further statistical analysis of T cells in postoperative tissue samples revealed a significantly lower prevalence of CD8+ T cells among the relapse group versus the nonrelapse group, whereas no significant difference in the number of CD4+ or PD-1+ T cells was observed (eFigure 7, http://links.lww.com/HC9/A134).

DISCUSSION

To our knowledge, this is the first prospective study of DEB-TACE plus an anti–PD-1 antibody for preoperative

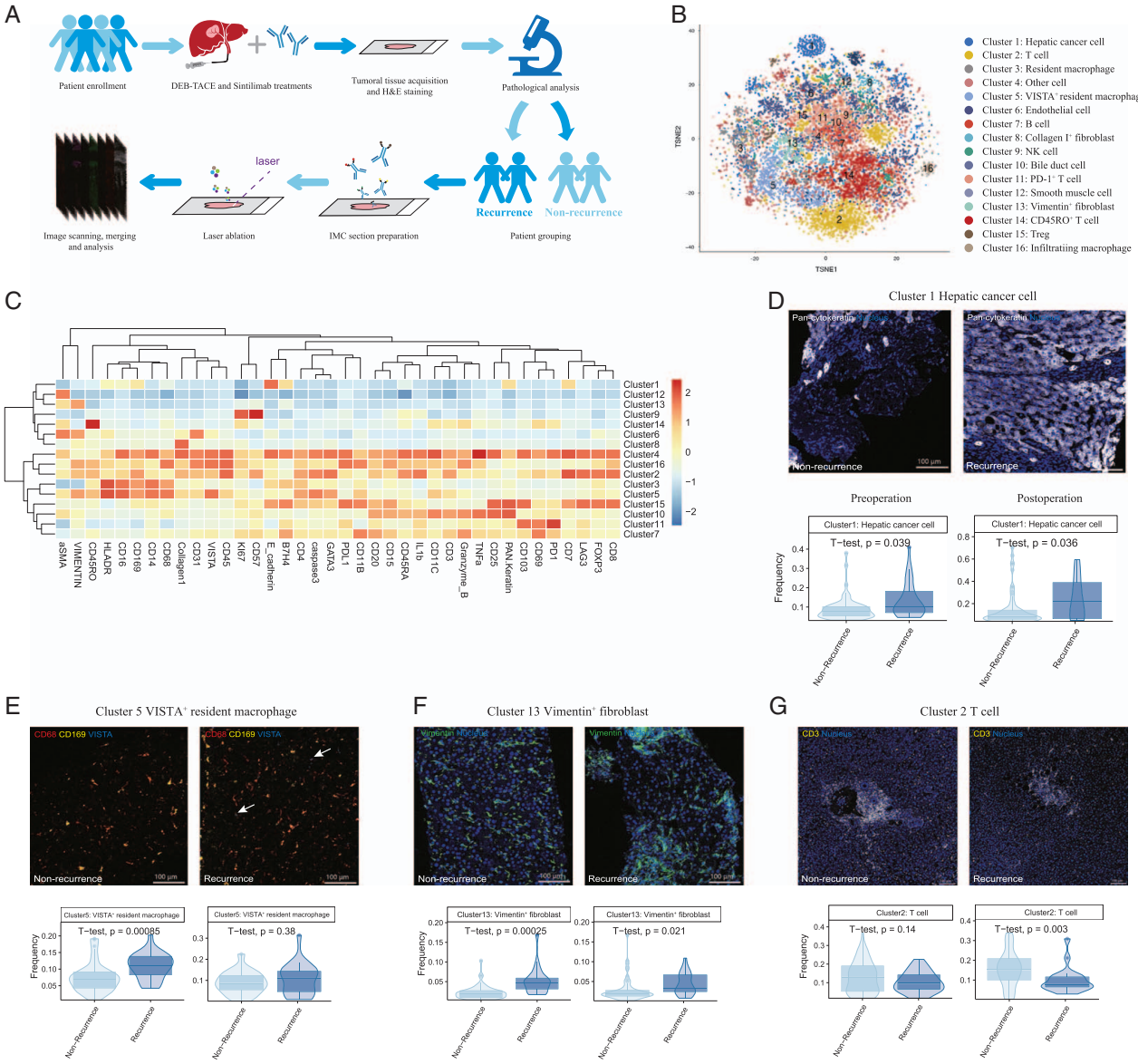

FIGURE 2 Analysis of tumor microenvironment in 2 groups of patients through IMC. (A) Patients underwent surgery after treatment with DEB-TACE and sintilimab. Tumoral tissue simples were analyzed through *H* and *E* staining and subsequently divided into 2 groups on the basis of the analytical outcome (early recurrence vs no recurrence). Tissue sections were analyzed by IMC, including section preparation, laser ablation and image scanning, merging, and analysis. (B) The map of tSNE included 16 groups of cells. (C) The heat map shows the *z*-scored mean marker expression of 16 clusters. (D–F) The upper images show the exact cell groups in preoperative samples through pseudo color map, and the cluster statistics are demonstrated in the lower graphs. (G) Upper images show T cells in postoperative sections and statistics graphs of T cells from pre and postoperative samples are presented. Abbreviation: DEB-TACE indicates drug-eluting bead transarterial chemoembolization; *H* and *E*, hematoxylin and eosin; IMC, imaging mass cytometry; tSNE, *t*-distributed stochastic neighbor embedding.

therapy in patients with HCC of BCLC stage A exceeding the Milan criteria or BCLC stage B. After a median follow-up of 26.0 months, the median PFS among all patients was 30.5 months and the 12-month PFS rate was 75%. Among the 51 patients who underwent surgery, 14% achieved a pCR and the median PFS had not been reached. DEB-TACE plus sintilimab was also well tolerated. It should be mentioned that the study population had the potential to receive surgical resection at enrollment, and the timing and scheme of operation were discussed by a multidisciplinary team. Therefore, this study shows that preoperative treatment with DEB-TACE + sintilimab not only creates better conditions for follow-up surgery but also maximizes the survival benefit of active and effective surgical resection.

Two previous studies have shown that preoperative/neoadjuvant immunotherapy for HCC is feasible and effective.[14,15] However, in contrast to these previous studies, the present study had a relatively short preoperative treatment duration and a higher proportion of patients had BCLC stage B HCC, which may

represent a worse prognosis.[23] In addition, previous studies, in which the preoperative treatment of HCC was investigated often used indicators of short-term efficacy, such as pCR or ORR and there is no evidence to support short-term efficacy as a surrogate endpoint for long-term survival. In contrast, the primary endpoint used in the current study was PFS, which represents the duration of clinical benefit, objectively reflecting the efficacy of preoperative DEB-TACE + sintilimab.

In the current study, the median follow-up was 26.0 months at data cutoff, at which time the primary endpoint, median PFS was 30.5 months, and the 12-month PFS rate was 75%. Although indirect comparisons should be undertaken with caution, in a study that enrolled similar patients to the present study (BCLC stage A/B HCC exceeding the Milan criteria), neoadjuvant hepatic arterial infusion chemotherapy was associated with a median PFS of 14.1 months and a 12-month PFS rate of 50%.[24] In another study, patients with resectable HCC who received preoperative immunotherapy with both nivolumab and ipilimumab had a median PFS of 19.53 months (2.33–NE), despite patients receiving adjuvant therapy until disease recurrence.[14] In the present study, in the absence of adjuvant treatment, a relatively short duration of preoperative DEB-TACE plus sintilimab followed by surgery was also associated with survival benefits in patients with BCLC stage A HCC exceeding the Milan criteria and stage B HCC.

Among the 51 patients who underwent surgery, 14% had a pCR and 49% had a major pathologic response. This finding is comparable to results from a previous phase II study, in which 15% of patients with resectable HCC who received preoperative immunotherapy with cemiplimab achieved a pCR, confirming preoperative treatment can induce tumor cell necrosis.[15] In addition, in a study of neoadjuvant hepatic arterial infusion chemotherapy in a similar patient population, the pCR was 10.1%,[24] consistent with the results from the present study. In the current study, the ORR to preoperative DEB-TACE plus sintilimab was 62% (37 of 60 evaluable patients). The high ORR in the current study is most likely due to the use of DEB-TACE, which has a strong liquefaction necrosis effect. Indeed, prior studies of DEB-TACE in patients with BCLC Stage B HCC have reported ORRs of 50%–60%.[17,25] It should be noted that imaging and pathologic evaluations of tumor response have their own characteristics and advantages, and the imaging evaluation in this study did not underestimate the tumor response compared with the pathologic findings, but is rather relatively conservative, while pathologic evaluation is more meticulous. In addition, due to the lasting effect of immunotherapy, tumors may continue to become necrotic in the window between preoperative tumor imaging evaluation and surgery, which is also a possible explanation for this result.

In this study, DEB-TACE plus sintilimab was safe and well tolerated. The most frequent treatment-related adverse event was an elevated aspartate aminotransferase level (89%). Abnormal liver function is often observed due to chemotherapeutic agents or postembolization syndrome; however, the majority of toxicities were attributed to both sintilimab and DEB-TACE, as they were given concomitantly. There was no apparent increase in toxicity with the combination of DEB-TACE and sintilimab compared with either therapy alone in other studies, similar to the results in previous studies using DEB-TACE in combination with antiangiogenic agents,[26,27]

Exploratory correlative assessments identified several potential biomarkers of response to combined sintilimab and DEB-TACE. High-throughput analyses and bioinformatic analysis demonstrated that cluster 1 tumor cells (Pan cytokeratin$^+$), cluster 5 macrophages (VISTA$^+$, CD68$^+$, and CD169$^+$), and cluster 13 fibroblasts (Vimentin$^+$ and alpha-smooth muscle actin$^+$) were highly expressed in the preoperative puncture specimens of patients with early disease recurrence (tumor relapse within 24 mo after surgery) compared with nonrecurrence. A series of studies have described the inhibitory effect of VISTA on tumor immunity and the ability of anti-VISTA treatment to upregulate immune response.[28,29] In the tumor microenvironment, the higher level of VISTA$^+$ resident macrophages may inhibit immune system responses in patients with disease recurrence. In this study, there was no difference in the VISTA + subpopulation between the patients with early recurrence versus those with no recurrence postsurgery, which may be due to the small sample size.

Vimentin is a type III intermediate filament, which maintains cell integrity and participates in cell migration, movement, and adhesion.[30] When overexpressed in tumors, vimentin drives the transformation of epithelial cells into stromal cells (epithelial-mesenchymal transition) eventually leading to metastasis.[31] Therefore, fibroblast (vimentin$^+$ and alpha-smooth muscle actin$^+$) overexpression in cluster 13 is conducive to the proliferation and metastasis of hepatoma cells, leading to recurrence. We also observed that, after treatment with DEB-TACE plus sintilimab, T-cell infiltration in cluster 2 increased significantly in patients who did not experience recurrence ($p = 0.003$). T-cell immunity is important for the treatment of HCC, as it has an obvious killing effect on liver cancer cells and can significantly improve the prognosis of patients.[32] T-cell infiltration in patients who had recurrence was less than in those who did not have a recurrence, which may be due to the inhibition of the immune environment caused by the resident macrophages in cluster 5 and fibroblasts in cluster 13. Therefore, these cell clusters (cluster 1 tumor cells, cluster 5 macrophages, and cluster 13 fibroblasts) could be used as biomarkers to predict the

efficacy of DEB-TACE plus sintilimab, although this needs to be verified in larger prospective studies.

LIMITATIONS

Limitations of the present study include the small sample size and the noncomparative study design. Postoperative adjuvant therapy was not permitted, and it is unclear to what extent its inclusion would have impacted the study outcomes. The role of postoperative adjuvant therapy may be examined further in future studies when its role is more clearly established.

CONCLUSION

Sintilimab in combination with DEB-TACE before surgery showed good efficacy and safety in patients with stage A/B standard BCLC exceeding the Milan criteria. A median PFS of 30.5 months was achieved; however, longer follow-up is required to determine any overall survival benefit.

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CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

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