Tumor-infiltrating CD8+ T cells as a biomarker for chemotherapy efficacy in unresectable hepatocellular carcinoma

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Abstract. Atezolizumab plus bevacizumab and lenvatinib are approved frontline therapies for advanced hepatocellular carcinoma (HCC). Patients with advanced HCC continue to have a poor prognosis despite these therapeutic choices. Previous studies have reported CD8+ tumor-infiltrating lymphocytes (TILs) as a biomarker to predict responsiveness to systemic chemotherapy. The present study investigated whether evaluating CD8+ TILs by immunohistochemistry staining of liver tumor biopsy tissues could help predict the response of patients with HCC to atezolizumab plus bevacizumab and lenvatinib. In total, 39 patients with HCC who underwent liver tumor biopsy were classified into high and low CD8+ TILs groups and were then divided by therapy type. The clinical responses to treatment in both groups were evaluated for each therapy. There were 12 patients with high-level CD8+ TILs and 12 patients with low-level CD8+ TILs among those who received atezolizumab plus bevacizumab. An improved response rate was observed in the high-level group compared with the low-level group. The high-level CD8+ TILs group had a significantly longer median progression-free survival compared with the low-level group. Among the patients with HCC who received lenvatinib, five had high-level CD8+ TILs and 10 had low-level CD8+ TILs. There were no differences in response rate or progression-free survival between these groups. Although the present study included only a limited number of patients, the findings suggested that CD8+ TILs could be a biomarker for predicting response to systemic chemotherapy in HCC.

Introduction

With estimated 900,000 new cases and 830,000 deaths in 2020, hepatocellular carcinoma (HCC) is the sixth most frequent neoplasm and third leading cause of cancer-related deaths globally (1,2). Recent progress in systemic chemotherapy for advanced HCC, such as immune checkpoint inhibitors (ICIs) and molecular targeted agents, have improved patient outcomes (3-7).

The results of the IMbrave150 study indicated that a combination of atezolizumab and bevacizumab, monoclonal antibodies against programmed death ligand 1 (PD-L1) and vascular endothelial growth factor (VEGF), can extend progression-free survival (PFS) and overall survival (OS) in advanced HCC patients compared with sorafenib, a multiple-target tyrosine kinase inhibitor (TKI), through anti-angiogenesis and anti-proliferation effects (3). As a result, atezolizumab plus bevacizumab has become the first-line systemic chemotherapy regimen for advanced HCC.

Lenvatinib is an oral multi-kinase inhibitor of VEGF receptors 1-3, fibroblast growth factor (FGF) receptors 1-4, platelet-derived growth factor (PDGF) receptor α, rearranged during transfection (RET), and stem cell factor receptor (KIT) (8,9). A global, randomized, multi-center, open-label trial to evaluate the non-inferiority of sorafenib (REFLECT; NCT01761266) revealed that lenvatinib significantly improved PFS compared with sorafenib in patients with previously untreated, metastatic, or advanced HCC (4). Lenvatinib is now approved for the treatment of HCC (3).

Despite these advancements in treatment methods, patients with advanced HCC continue to have a poor prognosis. The appropriate choice of chemotherapy may further improve patient prognosis. As a result, it is critical to choose agents that are appropriate for personalized HCC treatment. Therefore, potential predictive biomarkers and an increased knowledge of the mechanisms of response or resistance to systemic chemotherapies are required.

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Abbreviations: HCC, hepatocellular carcinoma; TILs, tumor-infiltrating lymphocytes; ICI, immune checkpoint inhibitor; PD-L1, programmed death ligand 1; VEGF, vascular endothelial growth factor; PFS, progression-free survival; OS, overall survival; ALBI score, albumin-bilirubin score; CT, computed tomography; MRI, magnetic resonance imaging; CR, complete response; PR, partial response; SD, stable disease; DCR, disease control rate; ORR, objective response rate; IHC, immunohistochemistry; HFP, high-power field; BCLC, Barcelona Clinic liver cancer; AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; TMB, tumor mutation burden

However, no established biomarkers have been identified to predict responsiveness to systemic chemotherapy in HCC. Previous studies have reported CD8+ tumor-infiltrating lymphocytes (TILs) as biomarkers for systemic chemotherapy responsiveness in HCC and other cancers (10-12). Recent gene expression profiling data of liver tumor biopsy samples have shown that CD8$^+$ TILs are potentially associated with clinical response to atezolizumab plus bevacizumab, although further research is needed to confirm these findings (13). No reports have evaluated CT8$^+$ TILs as a biomarker for systemic chemotherapy in HCC using only immunostaining procedures. In this study, we investigated whether CD8$^+$ TILs identified by immunostaining of liver tumor biopsy tissues could be a useful biomarker for predicting responses to systemic chemotherapy in HCC.

Materials and methods

Patients. This single-center prospective study analyzed the efficacy of atezolizumab plus bevacizumab and lenvatinib alone in HCC patients with and without CD8$^+$ TILs at Aso Iizuka Hospital between December 2018 and September 2022. A total of 63 patients received combination therapy with atezolizumab and bevacizumab and 92 patients received lenvatinib for advanced HCC. Of these individuals, we excluded 102 patients who did not undergo liver tumor biopsy prior to chemotherapy and 14 patients who were followed up within 6 weeks before the evaluation of treatment response. In total, we evaluated 39 patients for this study. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Aso Iizuka Hospital. The study of lenvatinib was approved in approval No. 18070 and the study of atezolizumab plus bevacizumab was approved in approval No. 22008. The opt-out method was used to obtain consent for this study.

Albumin-bilirubin (ALBI) score. Liver function was assessed using the ALBI score. ALBI scores were calculated as follows: ALBI score=log10 (T-Bil [mg/dl]x17.1)x0.66 + (ALB [g/dl] x10)x-0.085, where T-Bil is total bilirubin and ALB is the serum albumin level (14).

Treatment protocol. Patients received atezolizumab (1200 mg) and bevacizumab (7.5 mg/kg) intravenously every 3 weeks. The IMbrave150 study protocol was defined by Chugai Co., Ltd. (Tokyo, Japan) (3). Treatment was continued until disease progression or intolerable side effects.

Patients received lenvatinib based on body weight (8 mg/day for those weighing less than 60 kg and 12 mg/day for those weighing ≥60 kg) (Eisai Co., Ltd., Tokyo, Japan). Dose interruption followed by dose reduction (8 mg/day, 4 mg/day, or 4 mg every other day) was allowed if a patient developed a lenvatinib-related adverse event. The protocol for the REFLECT study was provided by Eisai Co., Ltd. (4).

Evaluation of efficacy. Computed tomography (CT) or magnetic resonance imaging (MRI) was used to determine treatment response every 6 to 12 weeks after treatment initiation. Antitumor response was assessed by the treating physician on the basis of modified RECIST version 1.1 (15). The disease control rate (DCR) was defined as complete response (CR), partial response (PR), or stable disease (SD)

lasting at least 4 months. The objective response rate (ORR) was defined as PR + CR. The patient was followed up every 3 weeks and treatment was continued until disease progression or intolerable side effects occurred.

Immunohistochemistry (IHC). Liver tumor biopsy specimens fixed in 10% formalin were embedded in paraffin for 10-48 h at room temperature. Serial sections (5 μm) were cut from paraffin blocks and stained with hematoxylin and eosin. The presence of CD8$^+$ T cells was determined by IHC using the following primary antibody: mouse anti-human monoclonal CD8 (clone C8/144B; 1:50; DAKO, Agilent, Santa Clara, CA, USA). After incubation with secondary antibodies, staining reactions were performed using the Bond Polymer System (Leica Biosystems, Buffalo Grove, IL, USA).

IHC staining of CD8$^+$ cell infiltration was assessed on the basis of the number of positively stained CD8$^+$ TILs by examining high-power fields (HPFs) selected with the most confluent areas of CD8$^+$ TILs at 400x magnification. An optimal cutoff was obtained using the mean value (CD8: 15.9 cells/HPF).

Statistical analysis. JMP Pro version 11 statistical software (SAS Institute Inc. Cary, NC, USA) was used for all analyses. Data are presented as medians (interquartile ranges). Significant differences between groups were examined by the χ-test. Kaplan-Meier (KM) analysis was performed for statistical analysis of PFS. Significant differences in PFS were determined by log-rank analysis. Statistical significance was determined at P<0.05.

Results

Patient characteristics. The characteristics of the 24 patients who received atezolizumab plus bevacizumab and 15 patients who received lenvatinib are shown in Tables I and II, respectively. We classified the enrolled patients into high-level and low-level CD8$^+$ TILs groups by IHC staining of CD8$^+$ TILs in liver tumor biopsy samples.

There were 12 patients with high-level CD8$^+$ TILs and 12 with low-level CD8$^+$ TILs. In the high-level group, eight patients were categorized as Barcelona Clinic Liver Cancer (BCLC) stage B and four were BCLC stage C; in the low-level group, six patients were BCLC stage B and six were BCLC stage C (P=0.460). Age, sex, etiology, Child-Pugh grade, ALBI score, tumor size, number of intrahepatic lesions, microvascular invasion, extrahepatic spread, serum α-fetoprotein (AFP) levels, and protein induced by vitamin K absence or antagonist-II (PIVKA-II) levels were similar between the two groups.

There were five patients with high-level CD8$^+$ TILs and 10 with low-level CD8$^+$ TILs. In the high-level group, three patients were categorized as BCLC stage B and two were BCLC stage C; in the low-level group, one patient was BCLC stage A and nine were BCLC stage B (P=0.608). Age, sex, etiology, Child-Pugh grade, ALBI score, tumor size, number of intrahepatic lesions, microvascular invasion, extrahepatic spread, serum AFP levels, and PIVKA-II levels were similar between the two groups.

IHC for CD8$^+$ TILs in HCC tissues. CD8 +TIL levels were assessed by IHC prior to atezolizumab plus bevacizumab

Table I. Baseline characteristics of patients who received atezolizumab plus bevacizumab.

Characteristics	All	High-level CD8+ TILs	Low-level CD8+ TILs	P-value
Number	24	12	12	
Age, years	77.5 (21.0-85.3)	81.5 (72.3-86.8)	74.5 (66.3-79.8)	0.132
Sex, n (male/female)	19/5	8/4	11/1	0.121
MVI positive, n	6	2	4	0.480
EHS positive, n	3	2	1	0.742
Intrahepatic max tumor size, cm	5.0 (3.5-8.3)	6.0 (3.0-8.9)	4.1 (3.6-7.6)	0.568
Numbers of tumors >5	13	5	8	0.217
Etiology				0.152
HBV	3	0	3	
HCV	12	8	4	
NBNC	9	4	5	
Child-Pugh				0.614
Child-Pugh score A	19	10	9	
Child-Pugh score B/C	5	2	3	
Alb, g/dl	3.5 (3.2-3.8)	3.75 (3.2-3.8)	3.35 (3.12-3.6)	0.295
T.Bil, g/dl	0.9 (0.6-1.5)	0.80 (0.53-1.0)	1.15 (0.65-1.18)	1.000
ALBI score	-2.20 (-2.54 to -1.73)	-2.18 (-2.24 to -1.72)	-2.46 (-2.60 to -1.91)	0.322
BCLC stage				0.406
A	0	0	0	
B	14	8	6	
C	10	4	6	
Tumor marker				
AFP, ng/ml	81.1 (5.5-1563.5)	190.5 (7.2-12670)	45.3 (4.3-428.2)	0.309
PIVKA-II, mAU/ml	1,693.5 (103.8-7713.3)	3,162.0 (86.5-14698)	956.5 (103.8-5564.0)	0.680

Data are expressed as median (interquartile range). TILs, tumor-infiltrating lymphocytes; HBV, hepatitis B virus; HCV, hepatitis C virus; MVI, microvascular invasion; EHS, extrahepatic spread; Alb, albumin; T.Bil, total bilirubin; ALBI score, albumin-bilirubin score; BCLC stage, Barcelona Clinic liver cancer stage; AFP, α-fetoprotein; PIVKA-II, vitamin K absence or antagonist-II.

treatment. Typical cases are shown in Figs. 1 and 2. Case 1 was an 88-year-old man with unresectable multiple HCC related to hepatitis C virus infection (Fig. 1). IHC staining of this poorly differentiated HCC indicated high levels of CD8+ TILs. Following administration of atezolizumab plus bevacizumab, liver CT images showed a reduction in the size and enhancement of the arterial stage of the tumor, indicating a PR. The patient's serum AFP level was 1,779.7 ng/ml before treatment, which decreased to 52.5 ng/ml after four cycles. Case 2 was a 78-year-old man with hepatitis C virus-related advanced HCC (Fig. 2). The specimen was diagnosed as poorly differentiated HCC, and IHC staining showed low CD8+ TIL levels. Post-treatment CT images showed an increase in tumor size, indicating disease progression. The patient's serum AFP level was 3,337.6 ng/ml before treatment, which increased to 11,028.0 ng/ml after four cycles.

Efficacies of atezolizumab plus bevacizumab and lenvatinib in the high-level vs. low-level CD8+ TILs groups
Atezolizumab plus bevacizumab. Among the patients who received atezolizumab plus bevacizumab, the ORR (CR+PR) was 8/12 (66.6%) in the high-level CD8+ TILs group and 4/12 (33.3%) in the low-level CD8+ TILs group (P=0.012). The

DCRs (CR+PR+SD) were 10/12 (83.3%) and 6/12 (50.0%) in the high-level and low-level CD8+ TILs groups, respectively (P=0.031) (Table III). Therefore, there was a higher response rate in the high-level CD8+ TILs group compared with the low-level CD8+ TILs group following atezolizumab plus bevacizumab therapy.

Lenvatinib. Among the patients who received lenvatinib, the ORR (CR+PR) was 2/5 (40.0%) in the high-level CD8+ TILs group and 2/10 (20.0%) in the low-level CD8+ TILs group (P=0.417). The DCRs (CR+PR+SD) were 2/5 (40.0%) and 8/10 (80.0%) in the high-level and low-level CD8+ TILs groups, respectively (P=0.121) (Table IV). The CD8+ TIL levels had no effect on the efficacy of lenvatinib.

PFS

Atezolizumab plus bevacizumab. The median PFS of all patients who were given atezolizumab plus bevacizumab therapy was 6.9 months. KM analysis revealed that the median PFS in the high-level CD8+ TILs group (not reached) was increased compared with the low-level CD8+ TILs group (4.7 months) (P=0.047) (Fig. 3).

Table II. Baseline characteristics of patients who received lenvatinib.

Characteristics	All	High-level CD8⁺ TILs	Low-level CD8⁺ TILs	P-value
Number	15	5	10	
Age, years	73 (62.0-80.0)	74.5 (61.3-82.3)	71.0 (64.5-74.5)	0.759
Sex, n (male/female)	13/2	4/1	9/1	0.600
Etiology				0.069
HBV	4	2	2	
HCV	4	3	1	
NBNC	7	0	7	
MVI positive, n	3	2	1	0.182
EHS positive, n	2	1	1	0.600
Intrahepatic max tumor size, cm	3.0 (1.7-6.5)	5.3 (1.8-8.3)	3.0 (1.7-6.2)	0.389
Numbers of tumors >5	9	3	6	0.465
Child-Pugh				0.394
Child-Pugh score A	13	4	9	0.600
Child-Pugh score B/C	2	1	1	
Alb, g/dl	3.8 (3.1-4.4)	4.2 (3.4-4.4)	3.75 (3.48-4.4.1)	0.723
T.Bil, g/dl	0.9 (0.7-1.1)	1.0 (0.9-1.35)	0.75 (0.68-1.1)	0.275
ALBI score	-2.56 (-3.03-2.18)	-2.75 (-2.96-1.99)	-2.44 (-3.05-2.13	0.977
BCLC stage				0.061
A	1	0	1	
B	12	3	9	
C	2	2	0	
Tumor marker				
AFP, ng/ml	6.7 (3.3-23.7)	2,317 (6.6-6080.7)	6.2 (2.8-7.1)	0.657
PIVKA-II, mAU/ml	138 (42-1135)	77 (38.5-8650.5)	266 (38.8-2571.3)	0.575

Data are expressed as median (interquartile range). TILs, tumor-infiltrating lymphocytes; HBV, hepatitis B virus; HCV, hepatitis C virus; MVI, microvascular invasion; EHS, extrahepatic spread; Alb, albumin; T.Bil, total bilirubin; ALBI score, albumin-bilirubin score; BCLC stage, Barcelona Clinic liver cancer stage; AFP, α-fetoprotein; PIVKA-II, vitamin K absence or antagonist-II.
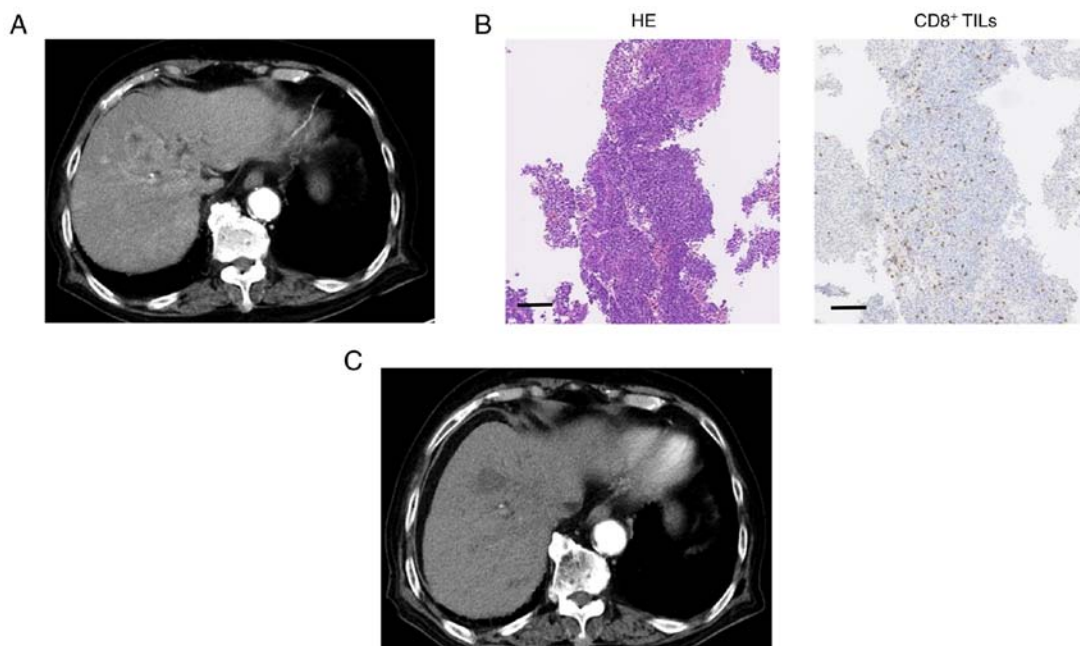

Figure 1. Typical Case 1, high-level CD8⁺ TILs. (A) CT image of the early arterial phase prior to treatment. (B) HE-staining of liver specimens (magnification, x100; scale bar, 100 μm). Immunohistochemistry staining for CD8⁺ T cells was conducted in liver specimens (magnification, x100; scale bar, 100 μm). (C) CT image of the early arterial phase to evaluate treatment efficacy. TILs, tumor-infiltrating lymphocytes; CT, computed tomography; HE, Hematoxylin and eosin.
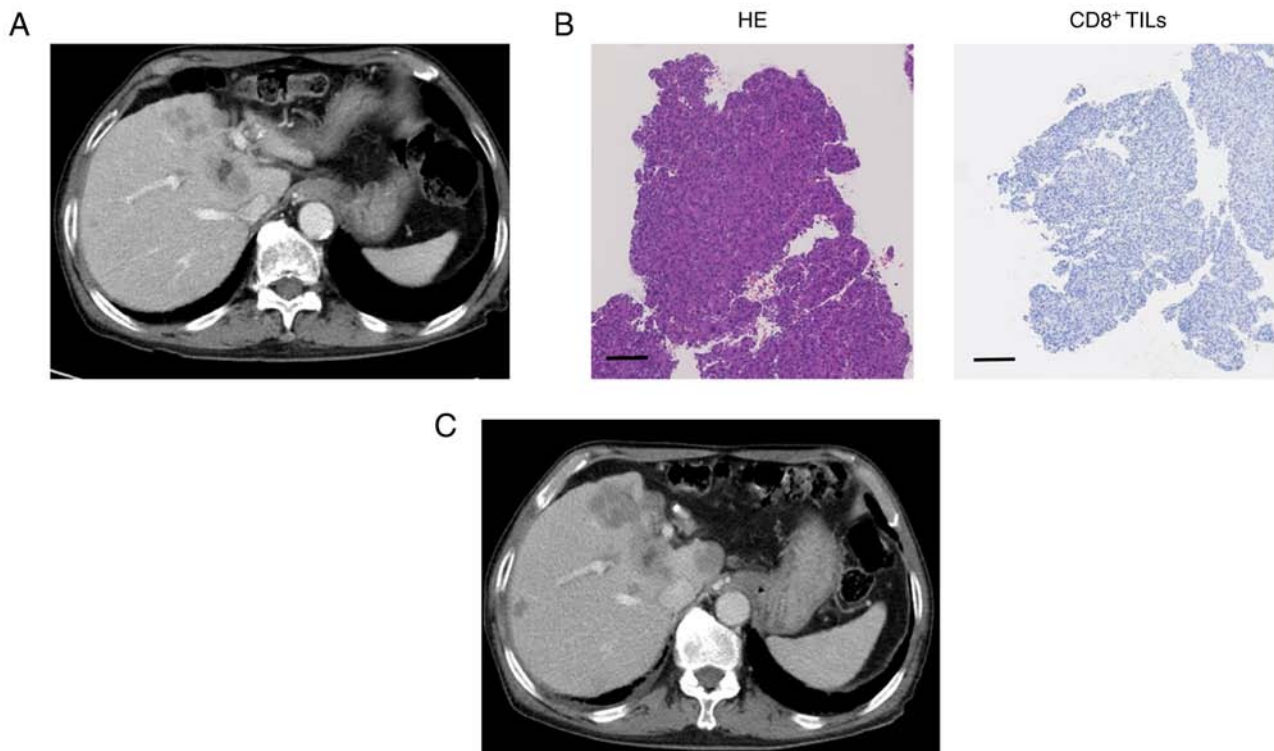
Figure 2. Typical Case 2, low-level CD8$^+$ TILs. (A) CT image of the early arterial phase prior to treatment. (B) HE-staining of liver specimens (magnification, x100; scale bar, 100 μm). Immunohistochemistry staining for CD8$^+$ T cells was conducted in liver specimens (magnification, x100; scale bar, 100 μm). (C) CT image of the arterial phase to evaluate treatment efficacy. TILs, tumor-infiltrating lymphocytes; CT, computed tomography; HE, Hematoxylin and eosin.

Lenvatinib. The median PFS of all patients who received lenvatinib therapy was 7.9 months. KM analysis showed no significant differences in median PFS between the high-level CD8$^+$ TILs group (6.3 months) and low-level CD8$^+$ TILs group (9.5 months) (P=0.315) (Fig. 4).

Discussion

The immune response potentially plays an important role in cancer progression. The most recent immunogenomic classification of HCC was published in 2022 (16). The study reported that 65% of HCC cases in the non-inflammatory group and 35% of those in the inflammatory group were more likely to respond to ICI treatment. The inflamed group can be further classified into the active, exhausted, and immune-like subclasses. The inflamed class is characterized by strong interferon signaling and cytolytic activity, upregulation of effector molecules of cytotoxic T cells, and increased levels of checkpoint molecules and CD8$^+$ T cells.

Recently, the combination of an ICI with a VEGF inhibitor using atezolizumab plus bevacizumab, as well as the multi-kinase angiogenesis inhibitor lenvatinib, were approved as systemic therapy options for patients with advanced HCC (3,4). Gene expression profiling of immune-related transcripts has recently been correlated with objective tumor response and survival in HCC patients who have received nivolumab. It was reported that non-inflamed HCC cases with immune exclusion are resistant to ICIs (17-20). Therefore, it is important to assess the immune conditions of HCC tumors prior to chemotherapy. Appropriate chemotherapy selection could further improve

prognosis. As a result, predictive biomarkers are needed for each therapy.

Recent studies across several cancer types have shown that biomarkers for response to ICI therapy include tumor mutation burden (TMB) and PD-L1 expression levels in the tumor microenvironment (TME) (21-25). However, these have proved difficult for use as HCC predictive biomarkers because of the low incidence of high TMB and high PD-L1 expression observed in HCC tumors (26,27).

In this study, we evaluated whether IHC staining of liver tumor biopsies to assess CD8$^+$ T cell infiltration in HCC tumor tissues could predict patient response to systemic chemotherapy. TILs reflect the local immune response and are potentially key for controlling tumor progression (28,29). TILs were previously characterized to be predominantly T cells, the majority of which have a cytotoxic effector phenotype (CD8$^+$) (30-32). Immune responses mediated by CD8$^+$ T cells can promote the accumulation of distinct endogenous CD8$^+$ and CD4$^+$ T cells that support antitumor activities in the TME (33-35). Correlations between CD8$^+$ T cell levels in the TME and response to ICI therapy have been reported in various cancer types (36,37). Recent studies have indicated that HCC cases with immune exclusion, which are associated with decreased CD8$^+$ T cell infiltration, show resistance to ICIs. Furthermore, gene expression profiling has suggested that CD8$^+$ TILs are potentially correlated with clinical response to atezolizumab plus bevacizumab treatment (13,18-20,38). We evaluated CD8$^+$ TIL levels using IHC staining of liver tumor biopsy tissues. In our study, HCC patients with high-level CD8$^+$ TILs had better treatment responses and longer PFS compared with those who

Table III. Comparison of responses to atezolizumab plus bevacizumab between the high-level and low-level CD8+ tumor infiltrating lymphocytes groups.

Response	All (n=24)%	High-level CD8+ TILs (n=12)%	Low-level CD8+ TILs (n=12)%	P-value
Overall Response				0.189
CR	0 (0)	0 (0)	0 (0)	
PR	12 (50)	8 (66.6)	4 (33.3)	
SD	4 (16.7)	2 (16.7)	2 (16.7)	
PD	8 (33.3)	2 (16.7)	6 (50)	
ORR (CR+PR)	12 (50)	8 (66.6)	4 (33.3)	0.012
DCR (CR+PR+SD)	16 (66.7)	10 (83.3)	6 (50)	0.031

TILs, tumor-infiltrating lymphocytes; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate.

Table IV. Comparison of responses to lenvatinib between the high-level and low-level CD8+ tumor infiltrating lymphocytes groups.

Response	All (n=15)%	High-level CD8+ TILs (n=5)%	Low-level CD8+ TILs (n=10)%	P-value
Overall Response				0.086
CR	1 (6.7)	0 (0)	1 (10)	
PR	3 (20.0)	2 (40)	1 (10)	
SD	6 (40)	0 (0)	6 (60)	
PD	5 (33.3)	3 (60)	2 (20)	
ORR (CR + PR)	4 (26.7)	2 (40)	2 (20)	0.417
DCR (CR + PR + SD)	10 (66.7)	2 (40)	8 (80)	0.121

TILs, tumor-infiltrating lymphocytes; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate.
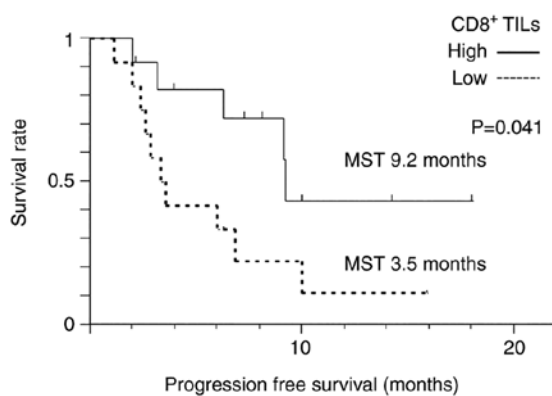

Figure 3. Kaplan-Meier analysis of PFS in patients with hepatocellular carcinoma treated with atezolizumab plus bevacizumab in the high-level and low-level CD8+ tumor-infiltrating lymphocytes groups. Significant differences in PFS were determined by the log-rank test. The time 0 was defined as the date of atezolizumab plus bevacizumab administration. PFS, progression-free survival; TILs, tumor-infiltrating lymphocytes; MST, median survival time.
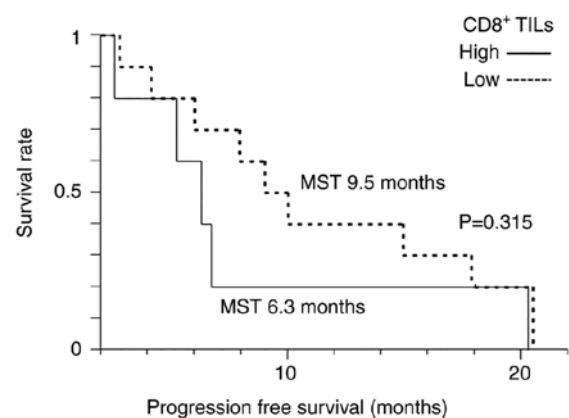

Figure 4. Kaplan-Meier analysis of PFS in patients with hepatocellular carcinoma treated with lenvatinib in the high-level and low-level CD8+ tumor-infiltrating lymphocytes groups. Significant differences in PFS were determined by the log-rank test. The time 0 was defined as the date of lenvatinib administration. PFS, progression-free survival; TILs, tumor-infiltrating lymphocytes; MST, median survival time.

had low-level CD8+ TILs. Thus, we used liver tumor biopsy tissues to identify CD8+ T cell infiltration as a predictive marker for response to atezolizumab plus bevacizumab therapy.

Conversely, we observed no significant differences between treatment responses to lenvatinib therapy between HCC patients with high-level and low-level CD8$^+$ TILs. A recent preclinical study showed that HCC cells with β-catenin activation, which indicates a non-inflammatory tumor, were more sensitive to sorafenib than those without β-catenin activation (39). Like sorafenib, lenvatinib might be effective for non-inflamed HCC cases. Further studies with more cases per subpopulation are needed to evaluate the heterogeneity of CD8$^+$ T cells.

The limitations of this study include the small number of HCC patients who underwent liver tumor biopsy because of its single-center design. This study also included advanced HCC cases of different stages. Ideally, groups could be matched according to liver function and tumor stage, but this is difficult when analyzing a small number of cases. Additionally, it is not clear whether the CD8$^+$ TILs of one tumor can reflect the status of other tumors, especially given the heterogeneous nature of HCC with multiple lesions. The difference between atezolizumab plus bevacizumab and lenvatinib treatments on the basis of the degree of CD8$^+$ TILs was not studied because of the small number of cases and varied tumor backgrounds. Additionally, we did not evaluate OS because liver function at the start of chemotherapy was not matched because of the small number of cases.

In conclusion, these findings suggest that CD8$^+$ TILs, as evaluated by IHC staining of liver tumor biopsy tissues, may be a useful biomarker for predicting HCC patient response to lenvatinib and atezolizumab plus bevacizumab treatments. Our findings indicate the recommendation of atezolizumab plus bevacizumab for HCC cases with high-level CD8$^+$ TILs by conducing IHC staining of liver tumor biopsy tissues before treatment decisions. Further studies regarding the selection of chemotherapy are needed to improve the prognosis of advanced HCC patients.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AK, MY, AM and KM designed the study. AK, KK, KT, and YM assisted with data analyses. YM and YO performed pathological examinations, including immunostaining. AK wrote the initial draft of the manuscript. MY contributed to the analysis and interpretation of the data. MY, AM, and KM assisted in the preparation and critical review of the manuscript. AK and MY confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

This research and study protocol were performed in accordance with the principles and ethical guidelines of the 1975 Declaration of Helsinki. This study received approval from the Aso Iizuka Hospital Ethics Committee (approval nos. 18070 and 22008). We applied the opt-out method to obtain consent for this study.

Patient consent for publication

Written informed consent was obtained from two patients to using their images for publication.

Competing interests

The authors declare that they have no competing interests.

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