Blood MALT1 serves as a potential biomarker reflecting the response and survival of immune-checkpoint-inhibitor therapy in advanced hepatocellular carcinoma

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Abstract. Treatment modalities involving an immune-checkpoint-inhibitor (ICI) have emerged as therapeutic options in advanced hepatocellular carcinoma (HCC). Nonetheless, auxiliary biomarkers are required to evaluate their efficacy. The present study aimed to assess the potential of blood mucosa-associated lymphoid tissue 1 (MALT1) in reflecting clinical response and prognosis in patients with advanced HCC who received ICI therapy. Peripheral blood was collected from 51 patients with advanced HCC who were about to receive ICI or ICI-based treatment. Blood MALT1 levels were determined using reverse transcription-quantitative PCR, and the blood MALT1 levels in 50 healthy controls (HCs) were also assessed. Besides, the treatment response and survival data were collected. The Wilcoxon rank-sum test was used for comparison analysis and the Spearman's rank correlation coefficient test was used for correlation analysis. The prognostic value of MALT1 was determined by Kaplan-Meier curve analysis with the log-rank test. Univariate and multivariate Cox regression models were used to identify factors associated with progression-free survival (PFS) and overall survival (OS). The results demonstrated that blood MALT1 levels were significantly increased in patients with advanced HCC compared with that in HCs (P<0.001). Blood MALT1 levels were increased in patients with portal vein invasion (vs. without portal vein invasion; P=0.010), extrahepatic disease (vs. without extrahepatic disease; P=0.026) and α-fetoprotein (AFP) ≥200 ng/ml (vs. AFP <200 ng/ml; P=0.040). After 4 cycles of ICI therapy, the objective response rate (ORR) and disease control rate (DCR) was 29.4 and 68.6%, respectively. Blood MALT1 levels were also significantly and negatively associated with the ORR (P=0.043) and DCR (P=0.004). Furthermore, PFS and OS were shortened in patients with high blood MALT1 levels (cut-off by the median) compared to those with low blood MALT1 levels. After adjusting using multivariate Cox regression models, high blood MALT1 levels were demonstrated to be a significant independent risk factor for shortened PFS [hazard ratio (HR)=2.419; P=0.009] and OS (HR=2.706, P=0.018) in patients with advanced HCC who received ICI therapy. In summary, blood MALT1 levels serve as a potential biomarker to reflect treatment response and survival in patients with advanced HCC who receive ICI therapy.

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Introduction

Hepatocellular carcinoma (HCC) ranks sixth in cancer morbidity and fourth in cancer-related mortality globally. It is a global disease burden, especially in Asia, where it accounts for ~72% of HCC cases (1,2). Owing to the late presentation of symptoms, >50% of patients with HCC are diagnosed at an advanced stage (3). Aside from the traditional molecular classification, HCC has recently begun to be classified according to the immunological environment, including active immune phenotypes (with enriched T cell response effectors), exhausted immune phenotypes [featured by T cell exhaustion, immunosuppressive macrophages and transforming growth factor β (TGFβ) signaling], and excluded immune phenotypes (immunosuppressive signatures in the surrounding tissues of the tumor but with little immune gene expression in the tumor core) (4). Moreover, the aforementioned immunological classification of advanced HCC is associated with different survival rates, which attracts the attention of clinicians to HCC immunity (5).

Mucosa-associated lymphoid tissue 1 (MALT1) is an intracellular signaling gene with both protease activity and scaffold function. It facilitates tumorigenesis by modulating cancer cell proliferation, migration and stemness in several solid cancers (6-10). A previous study reported that MALT1 serves as an oncogene by enhancing tumor cell proliferation and invasion in prostate carcinoma (8), and another study demonstrated that the MALT1 gene potentiates the crosstalk

between TGFβ and nuclear factor κB (NF-κB) to participate in tumor progression (10). Notably, one study reported that MALT1 paracaspase was upregulated and facilitated cancer growth in an HCC cell line (9).

In addition to the direct oncogenic role, MALT1 also activates NF-κB signaling to regulate cytotoxic T lymphocytes and immune escape (9,11,12). For instance, a previous study reported that MALT1 restrained antitumor immunity by facilitating cluster of differentiation (CD)8^+ T cell exhaustion (9). Another study reported that MALT1 decreased the activity of tumor-infiltrating $CD8^+$ T cells and elevated the immunosuppressive effects of regulatory T cells (Tregs) in malignant melanoma (11). Notably, a previous study reported that MALT1 induced adaptive immune resistance and thereby weakened the response of tumor cells to immune-checkpoint inhibitor (ICI) treatment (12). Furthermore, the ICI-involved systemic treatment modality emerges with the evolving therapeutic landscape of advanced HCC and brings certain survival benefits (13,14). For instance, a phase III clinical trial (KEYNOTE-240) found that pembrolizumab following sorafenib plus best supportive care prolonged the survival of patients with advanced HCC compared to those with placebo plus best supportive care [hazard ratio (HR)=0.781, P=0.0238] (15). Another study showed that atezolizumab plus bevacizumab resulted in a better progression-free survival (PFS) compared to sorafenib in patients with unresectable HCC (median PFS, 6.8 vs. 4.3 months) (16). However, the ICI efficacy is varied among each patient with advanced HCC and, the treatment response of ICI is still unmet in certain patients (17).

Therefore, the present study aimed to assess the clinical significance of MALT1 for estimating ICI treatment outcomes in patients with advanced HCC, which, to the best of our knowledge, has not been reported yet.

Materials and methods

Subjects. A total of 51 patients with advanced HCC who were treated with an ICI or ICI-based therapy in Handan Central Hospital (Handan, China) between February 2020 and November 2022 were consecutively enrolled in the present study. The inclusion criteria were as follows: i) Diagnosis with primary HCC using a pathological method; ii) Barcelona Clinic Liver Cancer stage C (18) [also recognized as China liver cancer staging (CNLC) stage III (19)]; iii) age ≥18 years old; iv) Eastern Cooperative Oncology Group Performance Status (ECOG PS) score ≤2 (20); v) Child-Pugh stage A or B (21); and vi) scheduled to receive ICI or ICI-based treatment. The exclusion criteria were as follows: i) Additional malignant diseases; ii) absence of measurable lesion to be assessed using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (22); iii) refusal to provide peripheral blood (PB) sample for use in the present study; and iv) pregnancy or lactation. Furthermore, 50 healthy participants were enrolled as healthy controls (HCs), whose eligibility criteria were as follows: i) No signs of abnormalities in recent physical examinations; ii) age and sex-matched with patients with advanced HCC; and iii) willingness to cooperate with this study. The Ethics Committee of Handan Central Hospital approved the present study and all subjects gave their written informed consent to participate.

Data and samples. Clinical characteristics were collected from patients with advanced HCC, including demographics and disease-related characteristics. PB samples were obtained from patients with advanced HCC before treatment initiation, whilst samples from HCs were obtained at enrollment.

After PB sample collection, PB mononuclear cells (PBMCs) were isolated using the Ficoll-Paque® centrifugation machine (GE Healthcare). Subsequently, the levels of MALT1 in PBMCs were detected using reverse transcription (RT)-quantitative (q)PCR. The RNeasy® Protect Mini Kit (Qiagen GmbH) was used for total RNA extraction, and then the PrimeScript™ RT Reagent Kit (Takara Biotechnology Co., Ltd.) was used for RT (37˚C for 15 min, 85˚C for 5 sec). Subsequently, qPCR (1 cycle of 95˚C for 30 sec, 40 cycles of 95˚C for 5 sec and 60˚C for 10 sec) was performed using the TB Green® Fast qPCR Mix (Takara Biotechnology Co., Ltd.). GAPDH was set as an internal reference. The quantitation of MALT1 was calculated using the $2^{-\Delta\Delta Cq}$ method (23). The sequences of primer for MALT1 and GAPDH were the same as in a previous study (24).

Treatment regimen. The present study was an observational study and the authors did not intervene in the treatment of the enrolled patients. ICI monotherapy or ICI-based treatments were administered according to the disease status of the patients, physician consultations and willingness of the patients to undergo the treatments. The regimens included: i) Camrelizumab + apatinib (25); ii) pembrolizumab + lenvatinib (26); iii) sintilimab + lenvatinib (27); iv) atezolizumab + bevacizumab (28); v) sintilimab monotherapy (29); vi) camrelizumab monotherapy (30); vii) atezolizumab monotherapy (31); and viii) nivolumab monotherapy (32). In detail, the dosage was as follows: 200 mg camrelizumab was administered intravenously every 2 weeks; 250 mg apatinib was given orally on day 1 of a 21-day cycle; 200 mg pembrolizumab was administered intravenously on day 1 of a 21-day cycle; 8 mg lenvatinib for bodyweight <60 kg and 12 mg for bodyweight ≥60 kg was administered orally once daily; 200 mg sintilimab was given intravenously on day 1 of a 21-day cycle; 1,200 mg atezolizumab was administered intravenously on day 1 of a 21-day cycle; 15 mg/kg bevacizumab was given intravenously on day 1 over a 21-day cycle; and 3 mg/kg nivolumab was administered intravenously every 2 weeks. The drug treatment was continued until disease progression, intolerable toxicity or voluntarily withdrawal from the treatment.

Follow-up and evaluation. Patients with advanced HCC underwent routine follow-ups, with a median follow-up of 13.3 months (range, 1.4-29.4 months). The last follow-up was performed in March 2023. During the follow-up, patients received imaging examinations every 2 cycles (~42 days). Based on treatment response data after 4 cycles (~3 months), the objective response rate (ORR) and disease control rate (DCR) were calculated, which was assessed according to RECIST version 1.1 (33). The ORR was defined as the sum of complete response (CR) and partial response (PR) rates, whereas the DCR was defined as the sum of CR, PR and stable disease (SD) rates. In addition, the PFS and overall survival (OS) were calculated according to the disease status or death of a patient.

Statistical analysis. SPSS 26.0 (IBM Corp.) and GraphPad Prism 7.01 (Dotmatics) were used for analyzing data and plotting figures, respectively. The Wilcoxon rank-sum test was used for comparison analysis and the Spearman's rank correlation coefficient test was used for correlation analysis. The receiver operating characteristics curve demonstrated the ability of MALT1 to differentiate patients with advanced HCC from HCs. To estimate the effect of MALT1 on prognosis in patients with advanced HCC, MALT1 was divided into high and low levels by its median value. The Kaplan-Meier curve was used to assess the PFS and OS, in which the log-rank test was used for comparing PFS and OS between patients with high and low MALT1. Univariate and multivariate Cox regression models were used to identify factors associated with PFS and OS, in which the forward stepwise method was performed in the multivariate model. All factors included in the univariate model were put into the forward stepwise-multivariate model. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of patients with advanced HCC. Among the 51 patients with advanced HCC, there were 7 (13.7%) females and 44 (86.3%) males, whose mean age was 59.0±8.3 years. A total of 13 (25.5%), 36 (70.6%) and 2 (3.9%) patients had ECOG PS scores of 0, 1, and 2, respectively. Moreover, 44 (86.3%), 21 (41.2%) and 32 (62.7%) patients had portal vein invasion, hepatic vein invasion and extrahepatic disease, respectively. A total of 19 (37.3%) and 32 (62.7%) patients were diagnosed as CNLC stage IIIa and IIIb, respectively. Detailed information regarding the patients is presented in Table I.

Treatment information of patients with advanced HCC. A total of 19 (37.3%) patients received ICI therapy as a first-line treatment, whilst 32 (62.7%) patients were treated with ICI therapy as a second-line treatment. Furthermore, 11 (21.6%), 5 (9.8%), 5 (9.8%), 4 (7.8%), 9 (17.6%), 8 (15.7%), 6 (11.8%) and 3 (5.9%) patients received camrelizumab + apatinib, pembrolizumab + lenvatinib, sintilimab + lenvatinib, atezolizumab + bevacizumab, sintilimab monotherapy, camrelizumab monotherapy, atezolizumab monotherapy and nivolumab monotherapy, respectively (Table II).

Blood MALT1 levels in patients with advanced HCC and HCs. Blood MALT1 levels were significantly increased in patients with advanced HCC compared with HCs ($P<0.001$; Fig. 1A) and it possessed a good ability to distinguish patients with advanced HCC from HCs (area under the curve, 0.895; 95% confidence interval, 0.836-0.954; Fig. 1B).

Relationship between blood MALT1 levels and tumor features in patients with advanced HCC. Blood MALT1 levels were significantly increased in patients with portal vein invasion (vs. without portal vein invasion; $P=0.010$), extrahepatic disease (vs. without extrahepatic disease; $P=0.026$) and α-fetoprotein (AFP) ≥200 ng/ml (vs. AFP <200 ng/ml; $P=0.040$). However, blood MALT1 levels were not significantly correlated with ECOG PS score ($r=0.193$, $P=0.175$) or significantly varied in patients with Child-Pugh stage A (vs. stage B; $P=0.145$), largest tumor size >10 cm (vs. ≤10 cm; $P=0.053$), hepatic vein invasion (vs. without; $P=0.157$) or programmed cell death 1

Table I. Characteristics of patients with advanced hepatocellular carcinoma (n=51).

Characteristic	Value
Age, years	59.0±8.3
Sex	
Female	7 (13.7)
Male	44 (86.3)
History of drinking	
Yes	30 (58.8)
No	21 (41.2)
HBV	
Yes	40 (78.4)
No	11 (21.6)
Liver cirrhosis	
Yes	28 (54.9)
No	23 (45.1)
ECOG PS score	
0	13 (25.5)
1	36 (70.6)
2	2 (3.9)
Child-Pugh stage	
A	33 (64.7)
B	18 (35.3)
Largest tumor size, cm	8.8 (6.7-11.2)
Portal vein invasion	
Yes	44 (86.3)
No	7 (13.7)
Hepatic vein invasion	
Yes	21 (41.2)
No	30 (58.8)
Extrahepatic disease	
Yes	32 (62.7)
No	19 (37.3)
BCLC stage C	51 (100.0)
CNLC stage	
IIIa	19 (37.3)
IIIb	32 (62.7)
AFP[a], ng/ml	226.3 (26.8-2219.6)
PD-L1 CPS	
≥1	37 (72.5)
<1	14 (27.5)

Data are presented as mean ± standard deviation, n (%) or median (interquartile range). [a]Normal range of AFP is 0-10 ng/ml. HBV, hepatitis B virus; ECOG PS, Eastern Cooperative Oncology Group Performance Status; BCLC, Barcelona Clinic Liver Cancer; CNLC, China liver cancer staging; AFP, α-fetoprotein; PD-L1, programmed cell death 1 ligand 1; CPS, combined positive score.

Table II. Treatment information of patients with advanced hepatocellular carcinoma (n=51).

Item	n (%)
Treatment line	
1	19 (37.3)
2	32 (62.7)
Regimen	
Camrelizumab + apatinib	11 (21.6)
Pembrolizumab + lenvatinib	5 (9.8)
Sintilimab + lenvatinib	5 (9.8)
Atezolizumab + bevacizumab	4 (7.8)
Sintilimab monotherapy	9 (17.6)
Camrelizumab monotherapy	8 (15.7)
Atezolizumab monotherapy	6 (11.8)
Nivolumab monotherapy	3 (5.9)

ligand 1 combined positive score (PD-L1 CPS) ≥1 (vs. PD-L1 CPS<1; P=0.095) (Table III).

Association between blood MALT1 levels and clinical response in patients with advanced HCC who received ICI therapy. After 4 cycles of ICI therapy, 0 (0.0%), 15 (29.4%), 20 (39.2%) and 16 (31.4%) patients with advanced HCC had CR, PR, SD and progressive disease, respectively; thus, the ORR and DCR were 29.4 and 68.6%, respectively (Fig. 2A). Notably, blood MALT1 levels were significantly decreased in patients with ORR (vs. without ORR; P=0.043; Fig. 2B) and DCR (vs. without DCR; P=0.004) (Fig. 2C).

Association between blood MALT1 levels and PFS and OS in patients with advanced HCC who received ICI therapy. Accumulating PFS was shortened in patients with high blood MALT1 levels compared to those with low blood MALT1 levels (P=0.008). Specifically, the 6-, 12-18- and 24-month accumulating PFS rates in patients with high blood MALT1 levels were 50.0, 20.8, 8.3 and 0.0%, respectively, whereas they were 63.5, 44.8, 30.7 and 0.0% in patients with low blood MALT1 levels (Fig. 3A).

Moreover, accumulating OS was shortened in patients with high blood MALT1 levels in comparison with those with low blood MALT1 levels (P=0.040). Specifically, the 6-, 12-, 18-, 24- and 30-month cumulative OS rates were 84.4, 55.3, 28.7, 17.2 and 17.2% in patients with high blood MALT1 levels, respectively, whereas the rates at the aforementioned time points were 100.0, 86.1, 61.1, 29.1 and 29.1% in patients with low blood MALT1 levels, respectively (Fig. 3B).

Risk factors associated with a shorter PFS in patients with advanced HCC who received ICI therapy. High blood MALT1 levels (P=0.011), age ≥60 years (P=0.006), higher ECOG PS score (P=0.011), Child-Pugh stage B (vs. A) (P=0.038), largest tumor size >10 cm (P=0.021), portal vein invasion (P=0.024), extrahepatic disease (P=0.006), AFP ≥200 ng/ml (P=0.020), treatment line of 2 (vs. 1; P=0.029), sintilimab monotherapy (vs. camrelizumab + apatinib; P=0.035), and camrelizumab monotherapy (vs. camrelizumab + apatinib; P=0.013) were significantly associated with a shorter PFS; however, PD-L1 CPS ≥1 (P=0.004) was significantly associated with a longer PFS in patients with advanced HCC who received ICI therapy (Fig. 4A). After adjustment, high blood MALT1 levels [HR=2.419; P=0.009], higher ECOG PS score (HR=2.925; P=0.007) and treatment line of 2 (vs. 1; HR=2.213; P=0.036) were independent factors significantly associated with a shorter PFS in patients with advanced HCC who received ICI therapy (Fig. 4B).

Risk factors associated with a shorter OS in patients with advanced HCC who received ICI therapy. High blood MALT1 levels (P=0.046), higher ECOG PS score (P=0.031), largest tumor size >10 cm (P=0.004), extrahepatic disease (P=0.022), AFP ≥200 ng/ml (P=0.001), treatment line of 2 (vs. 1; P=0.002), sintilimab monotherapy (vs. camrelizumab

Table III. Relationship between mucosa-associated lymphoid tissue 1 in patients with advanced hepatocellular carcinoma and different tumor features.

Feature	MALT1, median (IQR)	P-value
ECOG PS score		0.175[a]
0	2.250 (1.595-5.055)	
1	3.665 (2.905-5.853)	
2	4.520 (2.050-NA)	
Child-Pugh stage		0.145[b]
A	3.420 (2.125-5.120)	
B	4.315 (3.073-6.555)	
Largest tumor size >10 cm		0.053[b]
No	3.100 (1.745-5.120)	
Yes	3.985 (3.350-6.413)	
Portal vein invasion		0.010[b]
No	1.810 (1.160-2.880)	
Yes	3.875 (2.420-6.145)	
Hepatic vein invasion		0.157[b]
No	3.405 (2.198-4.903)	
Yes	3.800 (2.285-6.820)	
Extrahepatic disease		0.026[b]
No	3.230 (1.810-3.800)	
Yes	4.740 (2.308-6.615)	
AFP ≥200 ng/ml		0.040[b]
No	2.980 (2.195-4.315)	
Yes	4.315 (3.133-6.278)	
PD-L1 CPS ≥1		0.095[b]
No	4.580 (3.193-7.045)	
Yes	3.420 (2.195-4.935)	

[a]Spearman's rank correlation coefficient test; [b]Wilcoxon rank-sum test. IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group Performance Status; NA, not available; AFP, α-fetoprotein; PD-L1 CPS, programmed cell death 1 ligand 1 combined positive score.

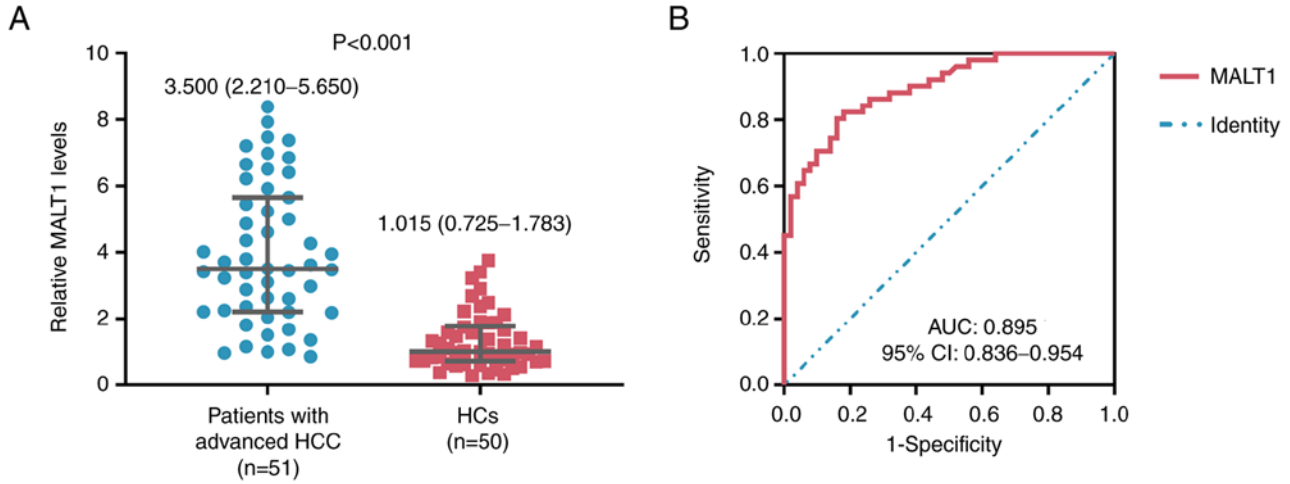

Figure 1. Blood MALT1 levels can be used to discern patients with advanced HCC from HCs. (A) Comparison of blood MALT1 levels between patients with advanced HCC and HCs, and (B) the associated receiver operating characteristics curve. The median (interquartile range) level of MALT1 in patients with advanced HCC and HCs was 3.500 (2.210-5.560) and 1.015 (0.725-1.783), respectively. MALT1, mucosa-associated lymphoid tissue 1; HCC, hepatocellular carcinoma; HC, healthy control; AUC, area under the curve; CI, confidence interval.

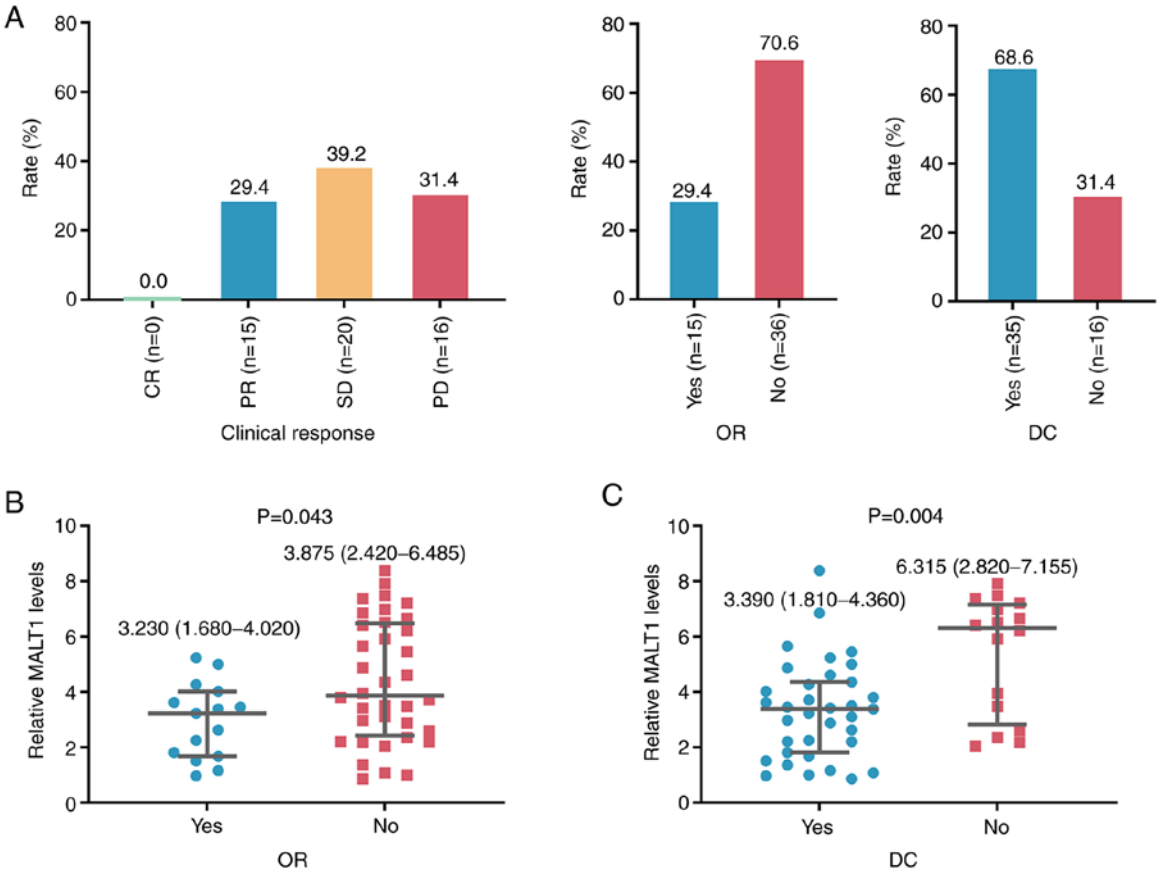

Figure 2. Blood MALT1 levels are negatively associated with ICI treatment response. (A) Proportions of patients with advanced HCC with different treatment responses after ICI therapy. Association between blood MALT1 levels and (B) ORR and (C) DCR in patients with advanced HCC who received ICI therapy. The median (interquartile range) level of MALT1 in patients with and without OR was 3.230 (1.680-4.020) and 3.875 (2.420-6.485), respectively; and it was 3.390 (1.810-4.360) and 6.315 (2.820-7.155) in patients with and without DC, accordingly. MALT1, mucosa-associated lymphoid tissue 1; HCC, hepatocellular carcinoma; OR, objective response; DC, disease control; ICI, immune-checkpoint inhibitor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

+ apatinib; P=0.002) and camrelizumab monotherapy (vs. camrelizumab + apatinib; P=0.014) were significantly associated with a shortened OS; however, PD-L1 CPS ≥1 (P=0.020) was significantly associated with a longer OS in patients with advanced HCC who received ICI therapy (Fig. 5A). Furthermore, high blood MALT1 levels (HR=2.706; P=0.018),

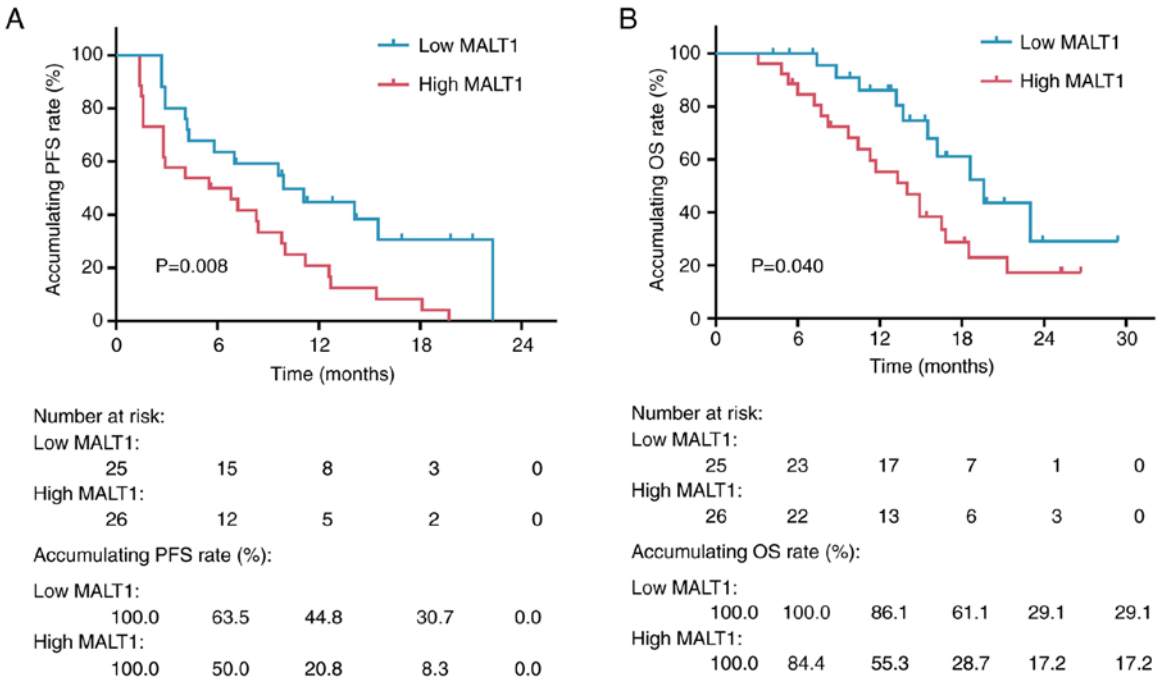

Figure 3. High blood MALT1 levels are associated with a worse prognosis in patients with advanced HCC after ICI therapy. Kaplan-Meier curves demonstrate the association between blood MALT1 levels and (A) PFS and (B) OS in patients with advanced HCC who received ICI therapy. MALT1, mucosa-associated lymphoid tissue 1; HCC, hepatocellular carcinoma; PFS, progression-free survival; OS, overall survival; ICI, immune-checkpoint inhibitor.

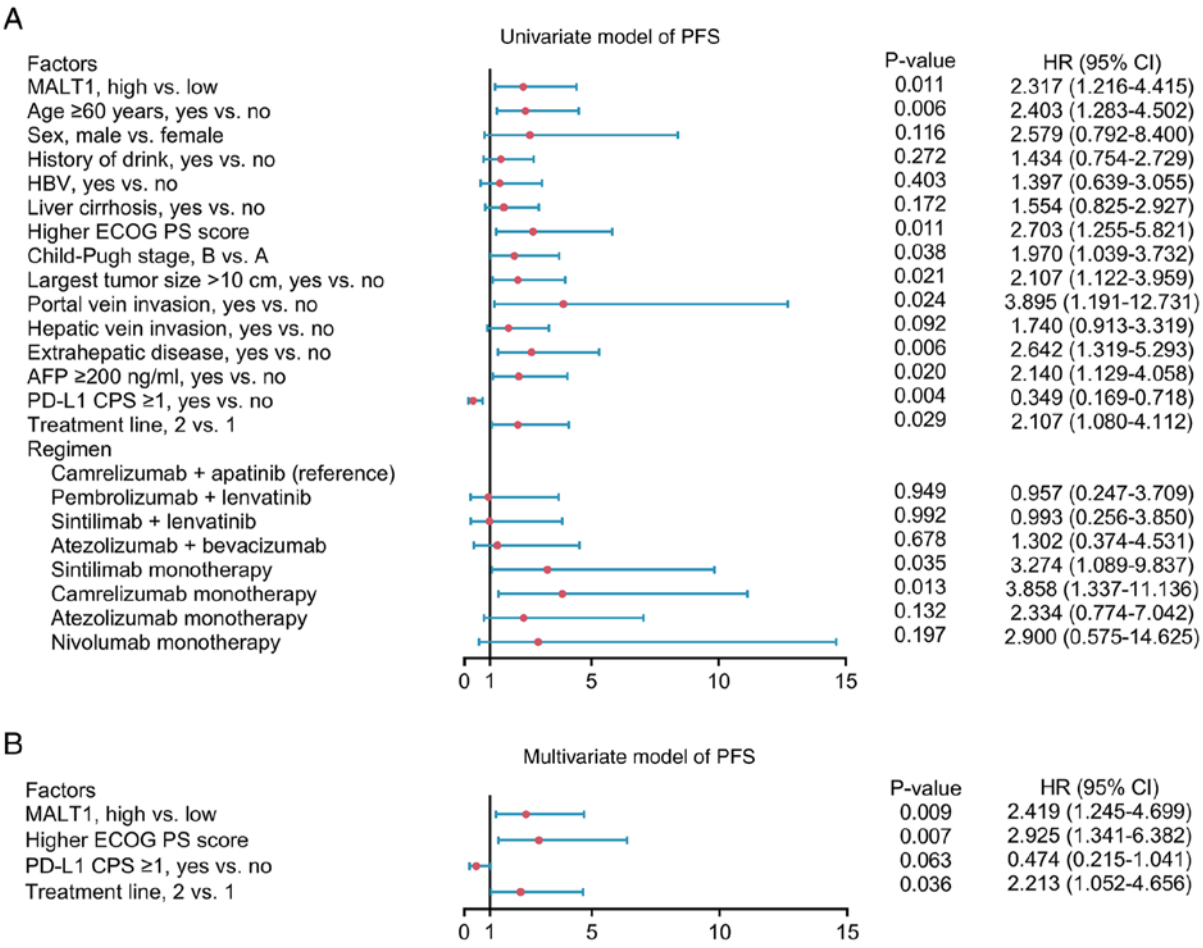

Figure 4. High blood MALT1 levels are independently associated with shorter PFS. (A) Univariate and (B) multivariate models for PFS in patients with advanced hepatocellular carcinoma who received immune-checkpoint inhibitor therapy. MALT1, mucosa-associated lymphoid tissue 1; PFS, progression-free survival; HBV, hepatitis B virus; ECOG PS, Eastern Cooperative Oncology Group Performance Status; AFP, α-fetoprotein; PD-L1 CPS, programmed cell death 1 ligand 1 combined positive score; HR, hazard ratio; CI confidence interval.

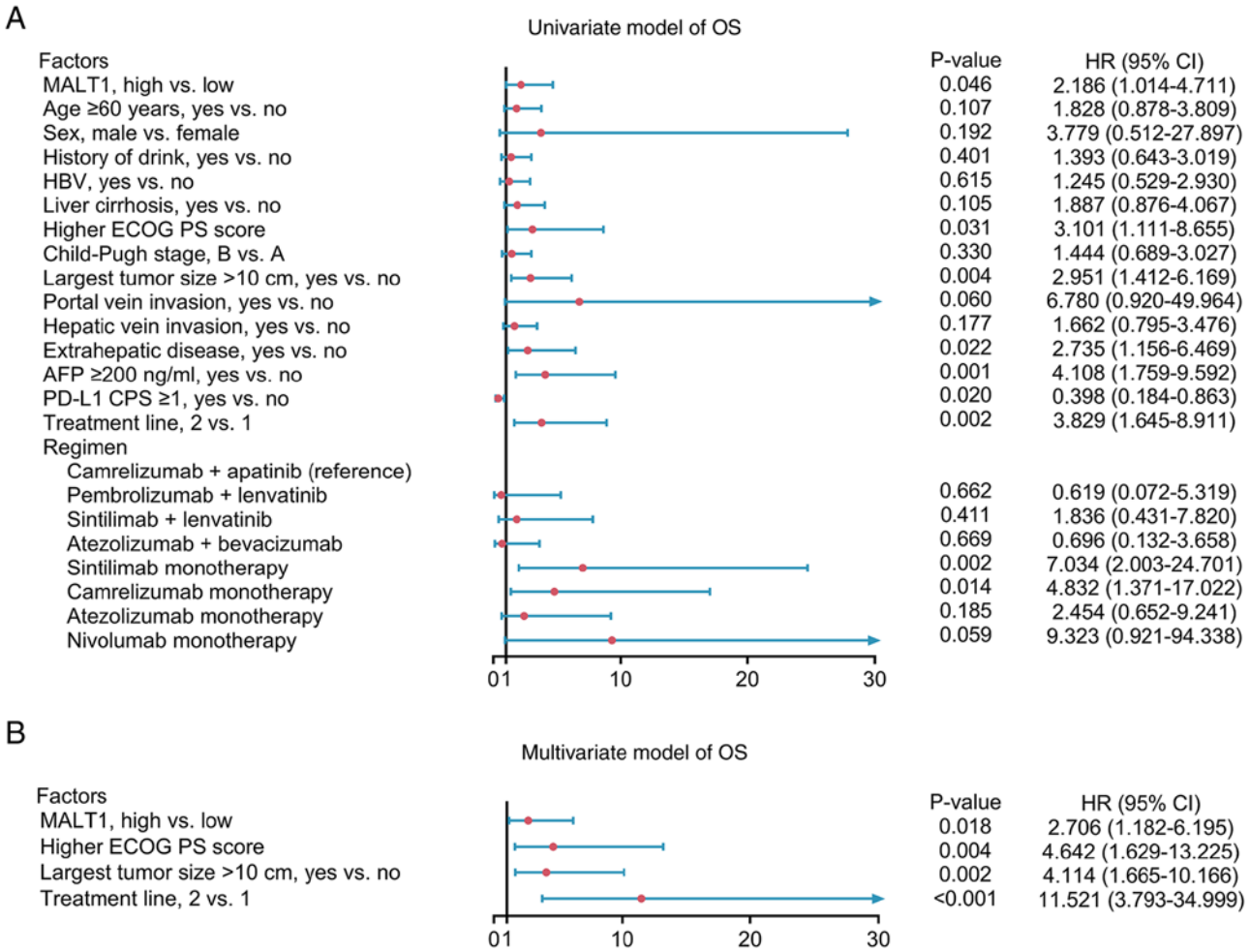

Figure 5. High blood MALT1 levels are independently associated with shorter OS. (A) Univariate and (B) multivariate models for OS in patients with advanced hepatocellular carcinoma who received immune-checkpoint inhibitor therapy. MALT1, mucosa-associated lymphoid tissue 1; OS, overall survival; HBV, hepatitis B virus; ECOG PS, Eastern Cooperative Oncology Group Performance Status; AFP, α-fetoprotein; PD-L1 CPS, programmed cell death 1 ligand 1 combined positive score; HR, hazard ratio; CI confidence interval.

higher ECOG PS score (HR=4.642; P=0.004), largest tumor size >10 cm (HR=4.114; P=0.002) and treatment line of 2 (vs. 1; HR=11.521; P<0.001) were independently significantly associated with a shorter OS in patients with advanced HCC who received ICI therapy (Fig. 5B).

Discussion

Although the oncogenic role of MALT1 is well-elucidated, only two previous studies have investigated MALT1 in HCC, to the best of our knowledge (34,35). For instance, one of the aforementioned studies reported that MALT1 inhibited HCC cell apoptosis and facilitated HCC progression through competitively binding to tumor necrosis factor receptor-associated factor (TRAF)6 with TRAF-interacting protein with Forkhead-associated domain (34). The other study reported that MALT1 was elevated and promoted migration, invasion and tumor-forming ability in human HCC cell lines (35). The aforementioned studies provide evidence of molecular implications of MALT1 in HCC, whereas the clinical role of blood MALT1 in patients with advanced HCC remains unclear.

The present study demonstrated that blood MALT1 levels were elevated in patients with advanced HCC compared with HCs, and increased blood MALT1 levels was associated with portal vein invasion, extrahepatic disease and AFP ≥200 ng/ml in patients with advanced HCC. A possible explanation could be as follows: i) MALT1 is a well-known oncogene, whose elevation promoted tumor development (6,7). Consequently, blood MALT1 levels were elevated in patients with advanced HCC compared with that in HCs; and ii) MALT1 has been reported to promote migration and invasion in an HCC cell line (35). As a result, blood MALT1 levels were positively associated with portal vein invasion and extrahepatic disease in patients with advanced HCC.

AFP, identified 60 years ago, is the most widely used serum biomarker to detect HCC and predict the prognosis (36). The results of the present study demonstrated that elevated blood MALT1 levels were associated with AFP ≥200 ng/ml in patients with advanced HCC, which may be explained as follows: MALT1 aggravated the progression of HCC which is typically reflected by elevated AFP (37). Consequently, elevated blood MALT1 levels was associated with AFP ≥200 ng/ml in patients with advanced HCC. As AFP is a well-known marker of HCC, this finding of the present study further provides evidence supporting the clinical utilization of MALT1 in patients with advanced HCC.

Furthermore, MALT1 has recently gained additional attention due to its role in regulating the immunological environment (9,11,38,39). For example, a previous study reported that MALT1 paracaspase activity mediated the T cell receptor-induced NF-κB activation in Tregs, which induced the conversion of resting Tregs into effector Tregs, thus facilitating the immune escape of tumor cells. Conversely, inhibiting MALT1 paracaspase activity could enhance antitumor immunity (11). Another study reported that MALT1 self-cleavage promoted interleukin-2 expression in conventional $CD4^+$ T cells to regulate Treg homeostasis. Moreover, inhibition of MALT1 self-cleavage can cause Treg deficit, which enhances the antitumor immune reactivity (40). Based on the aforementioned results, a bioinformatic analysis identified that MALT1 could eliminate the antitumor effect of ICI by impairing the activation of $CD8^+$ T cells (39). Notably, the density of liver-infiltrated Treg cells is increased in HCC and associated with the suppression of antitumor immunity, meanwhile, exhausted $CD8^+$ T cells are the landmark of the HCC tumor microenvironment (41-43). Therefore, the regulatory role of MALT1 on Treg cells and $CD8^+$ T cells suggests its involvement in antitumor immunotherapy of HCC. In the current study, it was demonstrated that blood MALT1 levels were negatively associated with ORR and DCR in patients with advanced HCC who received ICI therapy. The possible reasons are as follows: i) MALT1 attenuated the immune surveillance function of $CD8^+$ T cells and promoted Treg cell-mediated immune escape, which further restrained the treatment response of ICI therapy (12,44,45); and ii) MALT1 activated dendritic cells to regulate immunosuppressive factors, thus the immunotherapy resistance of HCC cells was facilitated (46). Blood MALT1 levels were therefore negatively associated with a reduced ORR and DCR after ICI therapy in patients with advanced HCC.

Apart from treatment response, the present study also demonstrated that high blood MALT1 levels were an independent risk factor for a shortened PFS and OS in patients with advanced HCC who received ICI therapy. The possible explanations are as follows: i) MALT1, together with B-cell lymphoma/leukemia 10 (BCL10) and caspase recruitment domain family member (CARD) to form the CARD-BCL10-MALT1 (CBM) complex, promoted tumor progression and resulted in a worse survival in patients with advanced HCC (47); and ii) MALT1 restrained the treatment response towards ICI; thus, the survival benefits of ICI therapy were impaired in patients with advanced HCC. Therefore, high blood MALT1 levels were independently associated with a shortened PFS and OS in patients with advanced HCC who received ICI therapy.

However, the present study had the following limitations: i) Considering that ICI treatment was only recently used in advanced HCC, the present study could only enroll 51 eligible patients, and the small sample size weakened the statistical power; ii) the mean age of the enrolled patients was 59.0±8.3 years, whilst the prognostic value of blood MALT1 levels in elderly patients with HCC (generally defined as age ≥65 years) remained unknown; and iii) MALT1 may have formed a CBM complex to exert a biological regulatory effect; however, the other two components of the CBM complex (BCL10 and CARD) were not detected in the present study, which warrants further investigations; and iv) the change of blood MALT1 during treatment was not evaluated and its association with treatment response and survival should be explored in the future.

In summary, high blood MALT1 levels reflect a worse ICI-treatment response and survival in patients with advanced HCC, and therefore, this may be a potential target to improve ICI treatment outcomes in patients with advanced HCC that warrants further exploration.

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Availability of data and materials

The datasets generated in the present study may be requested from the corresponding author.

Authors' contributions

WM and LT designed the study and analyzed the data. YY and BD collected the data and reviewed the relevant literature. WM, YY, BD and LT wrote the original draft. LW reviewed the relevant literature, analysed the data, prepared the tables and figures, and revised the manuscript. WM and LT confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of Handan Central Hospital (Handan, China) approved the present study, and all subjects provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J and Finn RS: Hepatocellular carcinoma. Nat Rev Dis Primers 7: 6, 2021.
2. Singal AG, Lampertico P and Nahon P: Epidemiology and surveillance for hepatocellular carcinoma: New trends. J Hepatol 72: 250-261, 2020.
3. Park JW, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS and Sherman M: Global patterns of hepatocellular carcinoma management from diagnosis to death: The BRIDGE Study. Liver Int 35: 2155-2166, 2015.
4. Giraud J, Chalopin D, Blanc JF and Saleh M: Hepatocellular carcinoma immune landscape and the potential of immunotherapies. Front Immunol 12: 655697, 2021.

5. Llovet JM, De Baere T, Kulik L, Haber PK, Greten TF, Meyer T and Lencioni R: Locoregional therapies in the era of molecular and immune treatments for hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 18: 293-313, 2021.
6. O'Neill TJ, Tofaute MJ and Krappmann D: Function and targeting of MALT1 paracaspase in cancer. Cancer Treat Rev 117: 102568, 2023.
7. Gomez Solsona B, Schmitt A, Schulze-Osthoff K and Hailfinger S: The Paracaspase MALT1 in Cancer. Biomedicines 10: 344, 2022.
8. Tsui KH, Chang KS, Sung HC, Hsu SY, Lin YH, Hou CP, Yang PS, Chen CL, Feng TH and Juang HH: Mucosa-Associated lymphoid tissue 1 is an oncogene inducing cell proliferation, invasion, and tumor growth via the upregulation of NF-κB activity in human prostate carcinoma cells. Biomedicines 9: 250, 2021.
9. Cheng L, Deng N, Yang N, Zhao X and Lin X: Malt1 protease is critical in maintaining function of regulatory T cells and may be a therapeutic target for antitumor immunity. J Immunol 202: 3008-3019, 2019.
10. Mazi FA, Cakiroglu E, Uysal M, Kalyoncu M, Demirci D, Sozeri PYG, Yilmaz GO, Ozhan SE and Senturk S: The paracaspase MALT1 is a downstream target of Smad3 and potentiates the crosstalk between TGF-β and NF-kB signaling pathways in cancer cells. Cell Signal 105: 110611, 2023.
11. Rosenbaum M, Gewies A, Pechloff K, Heuser C, Engleitner T, Gehring T, Hartjes L, Krebs S, Krappmann D, Kriegsmann M, *et al*: Bcl10-controlled Malt1 paracaspase activity is key for the immune suppressive function of regulatory T cells. Nat Commun 10: 2352, 2019.
12. Di Pilato M, Kim EY, Cadilha BL, Prussmann JN, Nasrallah MN, Seruggia D, Usmani SM, Misale S, Zappulli V, Carrizosa E, *et al*: Targeting the CBM complex causes T(reg) cells to prime tumours for immune checkpoint therapy. Nature 570: 112-116, 2019.
13. Liu Z, Liu X, Liang J, Liu Y, Hou X, Zhang M, Li Y and Jiang X: Immunotherapy for Hepatocellular Carcinoma: Current Status and Future Prospects. Front Immunol 12: 765101, 2021.
14. Yang C, Zhang H, Zhang L, Zhu AX, Bernards R, Qin W and Wang C: Evolving therapeutic landscape of advanced hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 20: 203-222, 2023.
15. Finn RS, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, *et al*: Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: A Randomized, double-blind, phase III trial. J Clin Oncol 38: 193-202, 2020.
16. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, *et al*: Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 382: 1894-1905, 2020.
17. Llovet JM, Pinyol R, Kelley RK, El-Khoueiry A, Reeves HL, Wang XW, Gores GJ and Villanueva A: Molecular pathogenesis and systemic therapies for hepatocellular carcinoma. Nat Cancer 3: 386-401, 2022.
18. Llovet JM, Brú C and Bruix J: Prognosis of hepatocellular carcinoma: The BCLC staging classification. Semin Liver Dis 19: 329-338, 1999.
19. Zhou J, Sun H, Wang Z, Cong W, Wang J, Zeng M, Zhou W, Bie P, Liu L, Wen T, *et al*: Guidelines for the diagnosis and treatment of hepatocellular carcinoma (2019 Edition). Liver Cancer 9: 682-720, 2020.
20. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET and Carbone PP: Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5: 649-655, 1982.
21. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC and Williams R: Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60: 646-649, 1973.
22. Schwartz LH, Litiere S, de Vries E, Ford R, Gwyther S, Mandrekar S, Shankar L, Bogaerts J, Chen A, Dancey J, *et al*: RECIST 1.1-Update and clarification: From the RECIST committee. Eur J Cancer 62: 132-137, 2016.
23. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
24. Chen X, Zhang X, Lan L, Xu G, Li Y and Huang S: MALT1 positively correlates with Th1 cells, Th17 cells, and their secreted cytokines and also relates to disease risk, severity, and prognosis of acute ischemic stroke. J Clin Lab Anal 35: e23903, 2021.
25. Xia Y, Tang W, Qian X, Li X, Cheng F, Wang K, Zhang F, Zhang C, Li D, Song J, *et al*: Efficacy and safety of camrelizumab plus apatinib during the perioperative period in resectable hepatocellular carcinoma: A single-arm, open label, phase II clinical trial. J Immunother Cancer 10: e004656, 2022.
26. Finn RS, Ikeda M, Zhu AX, Sung MW, Baron AD, Kudo M, Okusaka T, Kobayashi M, Kumada H, Kaneko S, *et al*: Phase Ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. J Clin Oncol 38: 2960-2970, 2020.
27. Ning S, Li X, Ma X, Liu J and Chang X: Efficacy of TACE combined with lenvatinib plus sintilimab for hepatocellular carcinoma with tumor thrombus in the inferior vena cava and/or right atrium. J Hepatocell Carcinoma 10: 1511-1525, 2023.
28. Yu Q, Wang Y, Ungchusri E, Patel M, Kumari D, Van Ha T, Pillai A, Liao CY and Ahmed O: Combination of transarterial radioembolization with atezolizumab and bevacizumab for intermediate and advanced staged hepatocellular carcinoma: A preliminary report of safety and feasibility. J Interv Med 6: 187-193, 2023.
29. Chen J, Hu X, Li Q, Dai W, Cheng X, Huang W, Yu W, Chen M, Guo Y and Yuan G: Effectiveness and safety of toripalimab, camrelizumab, and sintilimab in a real-world cohort of hepatitis B virus associated hepatocellular carcinoma patients. Ann Transl Med 8: 1187, 2020.
30. Ren Y, Liu Z, Makamure J, Kan X, Song S, Liu Y, Qian K, Zheng C and Liang B: Addition of camrelizumab to transarterial chemoembolization in hepatocellular carcinoma with untreatable progression. Technol Cancer Res Treat 21: 15330338221131385, 2022.
31. Zhang L, Gong JF, Pan HM, Bai YX, Liu TS, Cheng Y, Chen YC, Huang JY, Xu TT, Ge FJ, *et al*: Atezolizumab therapy in Chinese patients with locally advanced or metastatic solid tumors: An open-label, phase I study. Beijing Da Xue Xue Bao Yi Xue Ban 54: 971-980, 2022 (In Chinese).
32. El-Khoueiry AB, Trojan J, Meyer T, Yau T, Melero I, Kudo M, Hsu C, Kim TY, Choo SP, Kang YK, *et al*: Nivolumab in sorafenib-naive and sorafenib-experienced patients with advanced hepatocellular carcinoma: 5-year follow-up from CheckMate 040. Ann Oncol 35: 381-391, 2024.
33. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92: 205-216, 2000.
34. Shen W, Du R, Li J, Luo X, Zhao S, Chang A, Zhou W, Gao R, Luo D, Wang J, *et al*: TIFA suppresses hepatocellular carcinoma progression via MALT1-dependent and -independent signaling pathways. Signal Transduct Target Ther 1: 16013, 2016.
35. Kurden-Pekmezci A, Cakiroglu E, Eris S, Mazi FA, Coskun-Deniz OS, Dalgic E, Oz O and Senturk S: MALT1 paracaspase is overexpressed in hepatocellular carcinoma and promotes cancer cell survival and growth. Life Sci 323: 121690, 2023.
36. Hu X, Chen R, Wei Q and Xu X: The landscape of alpha fetoprotein in hepatocellular carcinoma: Where are we? Int J Biol Sci 18: 536-551, 2022.
37. Zheng Y, Zhu M and Li M: Effects of alpha-fetoprotein on the occurrence and progression of hepatocellular carcinoma. J Cancer Res Clin Oncol 146: 2439-2446, 2020.
38. Frizinsky S, Rechavi E, Barel O, Najeeb RH, Greenberger S, Lee YN, Simon AJ, Lev A, Ma CA, Sun G, *et al*: Novel MALT1 mutation linked to immunodeficiency, immune dysregulation, and an abnormal T cell receptor repertoire. J Clin Immunol 39: 401-413, 2019.
39. Yang N, Ji F, Cheng L, Lu J, Sun X, Lin X and Lan X: Knockout of immunotherapy prognostic marker genes eliminates the effect of the anti-PD-1 treatment. NPJ Precis Oncol 5: 37, 2021.
40. Baens M, Stirparo R, Lampi Y, Verbeke D, Vandepoel R, Cools J, Marynen P, de Bock CE and Bornschein S: Malt1 self-cleavage is critical for regulatory T cell homeostasis and anti-tumor immunity in mice. Eur J Immunol 48: 1728-1738, 2018.
41. Magen A, Hamon P, Fiaschi N, Soong BY, Park MD, Mattiuz R, Humblin E, Troncoso L, D'Souza D, Dawson T, *et al*: Intratumoral dendritic cell-CD4(+) T helper cell niches enable CD8(+) T cell differentiation following PD-1 blockade in hepatocellular carcinoma. Nat Med 29: 1389-1399, 2023.
42. Barsch M, Salie H, Schlaak AE, Zhang Z, Hess M, Mayer LS, Tauber C, Otto-Mora P, Ohtani T, Nilsson T, *et al*: T-cell exhaustion and residency dynamics inform clinical outcomes in hepatocellular carcinoma. J Hepatol 77: 397-409, 2022.

43. Zhang H, Jiang Z and Zhang L: Dual effect of T helper cell 17 (Th17) and regulatory T cell (Treg) in liver pathological process: From occurrence to end stage of disease. Int Immunopharmacol 69: 50-59, 2019.
44. Zheng X, Jin W, Wang S and Ding H: Progression on the roles and mechanisms of tumor-infiltrating T lymphocytes in patients with hepatocellular carcinoma. Front Immunol 12: 729705, 2021.
45. Lainé A, Labiad O, Hernandez-Vargas H, This S, Sanlaville A, Léon S, Dalle S, Sheppard D, Travis MA, Paidassi H and Marie JC: Regulatory T cells promote cancer immune-escape through integrin αvβ8-mediated TGF-β activation. Nat Commun 12: 6228, 2021.
46. Li Q, He J, Li S, Tian C, Yang J, Yuan H, Lu Y, Fagone P, Nicoletti F and Xiang M: The combination of gemcitabine and ginsenoside Rh2 enhances the immune function of dendritic cells against pancreatic cancer via the CARD9-BCL10-MALT1/NF-κB pathway. Clin Immunol 248: 109217, 2023.
47. Hayashi H, Chiba T, Mihara-Tomiyama N, Negishi T, Kodama Y, Sakashita H and Imai K: Domain structures of mucosa-associated lymphoid tissue lymphoma translocation 1 protein for nuclear localization in oral carcinoma cells and the proliferation inhibition. Biochem Biophys Res Commun 522: 799-804, 2020.