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ORIGINAL RESEARCH

Impact of Cytokeratin 19 Expression on the Outcomes of Unresectable Hepatocellular Carcinoma Treated with Targeted Therapy and Immunotherapy: A Propensity Score Matched Analysis

Junjie Liu[1,2], Tong Yuan[1,2], Xing Lv[1,2], Guan Tan[1,2], Lin Xue[1,2], Zhiyong Huang[1,2]

[1]Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China; [2]Hubei Key Laboratory of Hepato-Pancreato-Biliary Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China

Correspondence: Zhiyong Huang, Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China, Email huangzy@tjh.tjmu.edu.cn

Background: Cytokeratin 19 (CK19) serves as a significant prognostic indicator for hepatocellular carcinoma (HCC). However, it is uncertain if CK19 expression influences the prognosis and effectiveness of treatment in patients undergoing targeted therapy and immunotherapy for unresectable HCC. This study aimed to evaluate the prognostic value of CK19 expression in this patient population.

Methods: Patients with unresectable HCC who received combined targeted therapy and immunotherapy between January 2021 and December 2023 were retrospectively analyzed in this study. CK19 expression in tumor biopsy samples from patients before treatments were identified using immunohistochemistry. Propensity score matching (PSM) was conducted in a 1:2 ratio to balance baseline features between the CK19-positive and CK19-negative groups. Survival outcomes were analyzed using the Kaplan-Meier method along with the Cox regression model. Treatment response was evaluated based on mRECIST criteria.

Results: A total of 247 patients were included, and 126 were selected after-PSM (43 CK19-positive, 83 CK19-negative), with balanced baseline characteristics. After PSM, the CK19-negative group had a markedly prolonged median OS compared to the CK19-positive group (42.2 months vs 15.6 months, $p < 0.001$), along with an extended median PFS (28.9 months vs 7.3 months, $p < 0.001$). The ORR was significantly higher in the CK19-negative group than in the CK19-positive group (59.0% vs 18.6%, $p < 0.001$), and the DCR was also superior (96.4% vs 79.1%, $p = 0.005$). Multivariate Cox analysis found CK19 expression as an independent factor predicting OS and PFS.

Conclusion: In patients with unresectable HCC undergoing targeted therapy and immunotherapy, CK19 expression correlated with poorer survival outcomes and diminished therapeutic response. CK19 may serve as a valuable biomarker for prognosis and treatment stratification in advanced HCC, and CK19 screening may be incorporated into clinical trial stratification and clinical decision-making.

Keywords: hepatocellular carcinoma, cytokeratin 19, immunotherapy, targeted therapy, prognosis, propensity score matching

Introduction

Hepatocellular carcinoma (HCC) ranks as the sixth most prevalent malignancy worldwide and is the third main cause of cancer-related mortality, representing a substantial threat to human health.[1–3] Owing to the absence of obvious clinical manifestations in this initial phase, the majority of patients are diagnosed with advanced or unresectable HCC, thereby forfeiting the opportunity for surgical intervention.[4] Recently, the continuous development of systemic treatment has led to targeted therapy combined with immunotherapy emerging as the primary treatment choice for advanced HCC.[5–7]

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Nonetheless, while combined therapy has enhanced the overall response rate and survival time, significant variations in efficacy among patients indicate an urgent necessity to identify meaningful biomarkers for efficacy prediction and population stratification.[8–10]

Cytokeratin 19 (CK19) is predominantly expressed in biliary epithelium and hepatic progenitor cells, with less expression in classical HCC.[11] Previous studies indicate that CK19-positive HCC exhibits more harmful features of biology, including enhanced cell proliferation, susceptibility to vascular invasion, elevated tumor recurrence rates, and significantly poor prognosis.[12–14] Research indicates that in clinical settings, roughly 4–28% of HCC exhibit CK19 expression.[15] Patients with HCC that show positive CK19 expression have different clinical, molecular, genetic, and environmental characteristics, which are linked to lower survival and higher rates of recurrence.[16]

While CK19 possesses a definitive prognostic significance in surgical patients, its function in unresectable HCC patients undergoing targeted therapy and immunotherapy remains inadequately explored. Furthermore, the association of CK19 with the resistance mechanism of combination therapy and its potential role as a poor prognostic indicator for immunotherapy require additional clarification.

In this study, we retrospectively analyzed patients with unresectable HCC who received combined targeted therapy and immunotherapy. CK19 expression was assessed by immunohistochemistry on pretreatment biopsy samples, and propensity score matching (PSM) was used to balance baseline characteristics. We evaluated the prognostic impact of CK19 expression on overall survival (OS), progression-free survival (PFS), objective response rate (ORR), and disease control rate (DCR), aiming to clarify its potential role as a biomarker in systemic treatment strategies.

Patients and Methods

Study Design and Patients

In this study, data from patients with unresectable HCC who received targeted therapy and immunotherapy at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, between January 2021 and December 2023, were retrospectively analyzed. The patients were categorized into CK19-positive and CK19-negative groups according to the immunohistochemistry staining results of biopsy samples prior to treatment. Patients lacking accessible biopsy specimens for CK19 evaluation, including those diagnosed exclusively through AFP or imaging, patients with limited clinical information, or those who had undergone past anticancer treatment, were excluded. The follow-up concluded on April 1, 2025 (Figure 1).

Immunohistochemical Staining and Interpretation of the Results

Liver biopsy specimens were fixed in 10% formalin, embedded in paraffin blocks, and cut into sections that were 4 μm thick. The expression of cytokeratin 19 (KRT19, A0247, ABclonal) in HCC tissue was detected using the PV-6000 two-step immunohistochemical staining technique. For quality assurance, CK19-positive sections served as positive controls, while phosphate-buffered saline (PBS) was used in lieu of the primary antibody for negative control purposes.

The staining results were viewed at 400× magnification using a light microscope. To ensure consistency and representative evaluation, five randomly selected non-overlapping high-power fields were analyzed on each slide. Tumors were classified as CK19-positive if any tumor cells exhibited distinct cytoplasmic and/or membranous staining, and as CK19-negative if no tumor cells showed staining. All assessments were independently conducted by two qualified pathologists blinded to clinical information. In case of disagreement, a third senior pathologist was consulted to reach consensus.

Treatment Procedure

Immunotherapy was administered intravenously every three weeks, and targeted therapy was given orally on a daily basis until disease progression or the occurrence of severe adverse events. Blood samples were obtained before each

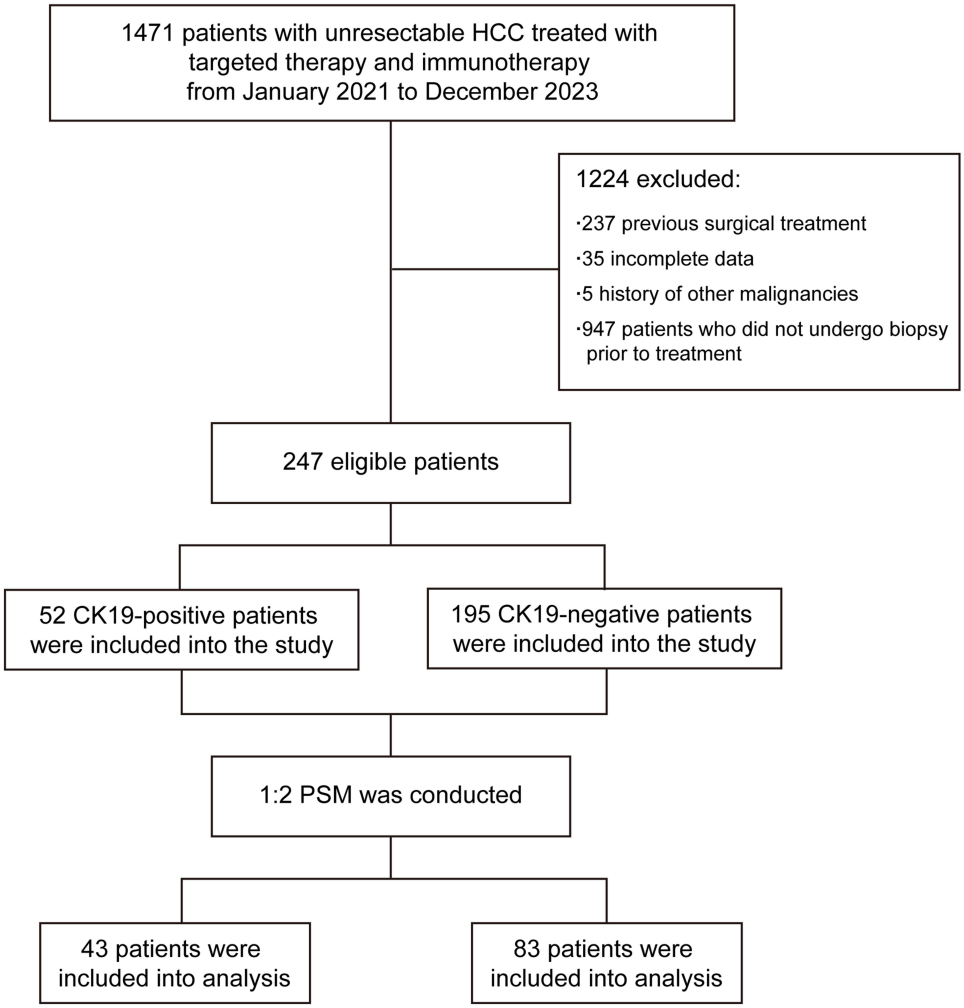

Figure 1 Flow chart of the study.

administration of immunotherapy during every treatment cycle. Tumor response was assessed every six weeks using contrast-enhanced CT or MRI according to the mRECIST criteria.

Endpoints of the Study

The study endpoints included OS and PFS. OS was measured from the initiation of targeted therapy and immunotherapy to the occurrence of death from any cause. PFS was defined as the duration from the initial administration of targeted therapy and immunotherapy to progression of the tumor based on the mRECIST criteria or death, whichever occurred first. The best overall response was defined as the best response recorded from the start of treatment until disease progression or recurrence, whichever occurred first.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data, and as median (interquartile range, IQR) for skewed data. Comparisons of continuous variables between groups were conducted using the Student's *t*-test or the Mann–Whitney *U*-test, if applicable. Differences in categorical variables were assessed using the χ^2-test or Fisher's exact test. Survival curves were constructed utilizing the Kaplan-Meier method, and survival differences were assessed employing the Log rank test. Cox proportional hazards regression models were employed to

ascertain characteristics correlated with patient survival. Variables with a p-value <0.05 in univariable Cox regression were subsequently included in the multivariable analysis.

PSM analysis was conducted to minimize selection bias and balance baseline features between groups. Table 1 describes the variables included in the PSM model for each patient. A stepwise logistic regression model was employed to identify variables. Nearest-neighbor matching was conducted at a 1:2 ratio utilizing a caliper width of 0.1 without replacement. Ultimately, 43 CK19-positive patients were successfully matched to 83 CK19-negative patients, as some negative cases could not be paired within the caliper.

All statistical analyses were conducted using R software version 4.4.2. P-values below 0.05 were regarded as significant.

Table 1 Baseline Characteristics of Patients Before and After Propensity Score Matching

Characteristics	Before PSM				After PSM			
	CK19 Negative (n=195)	CK19 Positive (n=52)	P	SMD	CK19 Negative (n=83)	CK19 Positive (n=43)	P	SMD
Age, years	56.0 (49.0–63.0)	53.0 (47.8–61.0)	0.215	0.190	55.0 (45.0–61.0)	52.0 (47.5–60.0)	0.863	0.032
Gender			0.120	0.224			1.000	0.011
Male	179 (91.7)	44 (84.6)			75 (90.4)	39 (90.7)		
Female	16 (8.2)	8 (15.4)			8 (9.6)	4 (9.3)		
BMI, kg/m^2	23.1 (21.0–25.3)	23.5 (20.8–25.3)	0.726	0.053	23.0 (21.2–25.0)	23.9 (21.6–25.2)	0.671	0.079
HBsAg			0.670	0.108			1.000	0.031
Negative	13 (6.7)	5 (9.6)			7 (8.4)	4 (9.3)		
Positive	182 (93.3)	47 (90.4)			76 (91.6)	39 (90.7)		
ECOG PS			0.919	0.016			0.622	0.092
0	155 (79.5)	41 (78.8)			65 (78.3)	32 (74.4)		
1	40 (20.5)	11 (21.2)			18 (21.7)	11 (25.6)		
Child-Pugh class			0.530	0.102			0.796	0.106
A	170 (87.2)	47 (90.4)			76 (91.6)	38 (88.4)		
B	25 (12.8)	5 (9.6)			7 (8.4)	5 (11.6)		
Liver cirrhosis			0.357	0.144			0.752	0.059
Absent	91 (46.7)	28 (53.8)			43 (51.8)	21 (48.8)		
Present	104 (53.3)	24 (46.2)			40 (48.2)	22 (51.2)		
BCLC stage			**0.004**	0.470			0.507	0.126
B	104 (53.3)	16 (30.8)			32 (38.6)	14 (32.6)		
C	91 (46.7)	36 (69.2)			51 (61.4)	29 (67.4)		
AFP, μg/L			0.066	0.291			0.591	0.101
<400	103 (52.8)	20 (38.5)			35 (42.2)	16 (37.2)		
≥400	92 (47.2)	32 (61.5)			48 (57.8)	27 (62.8)		
Largest tumor size			0.154	0.220			0.765	0.056
≤10.0 cm	126 (64.6)	28 (53.8)			44 (53.0)	24 (55.8)		
>10.0 cm	69 (35.4)	24 (46.2)			39 (47.0)	19 (44.2)		
Tumor number			0.792	0.041			0.514	0.121
≤3	146 (74.9)	38 (73.1)			66 (79.5)	32 (74.4)		
>3	49 (25.1)	14 (26.9)			17 (20.5)	11 (25.6)		
Albumin, g/L	38.0 (35.0–40.1)	38.3 (35.7–40.8)	0.935	0.015	39.0 (36.0–41.1)	38.2 (35.7–40.5)	0.426	0.171
ALT, U/L	28.0 (20.0–39.0)	31.0 (19.8–42.5)	0.393	0.017	31.0 (17.0–39.5)	29.0 (19.0–41.5)	0.767	0.047
Bilirubin, μmol/L	12.0 (9.0–16.5)	12.8 (10.4–19.6)	0.144	0.108	11.4 (8.6–16.0)	12.7 (10.2–17.7)	0.142	0.083
PVTT	77 (39.5)	25 (48.1)	0.264	0.174	38 (45.8)	22 (51.2)	0.566	0.108
Extrahepatic metastasis	17 (8.7)	13 (25.0)	**0.001**	0.446	14 (16.9)	8 (18.6)	0.808	0.045

Note: Bold values indicate statistical significance ($p < 0.05$).

Abbreviations: PSM, propensity score matching; CK19, Cytokeratin 19; SMD, standardized mean difference; BMI, body mass index; HBsAg, Hepatitis B surface antigen; ECOG PS, Eastern Cooperative Oncology Group performance status; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; PVTT, portal vein tumor thrombosis.

Results

Baseline Characteristics of the Patients

This retrospective analysis included 247 patients with unresectable HCC who received targeted therapy and immunotherapy. The cohort comprised 223 males and 24 females. Among them, 195 patients were confirmed to be CK19 negative by immunohistochemical staining of tumor biopsy tissue before treatment, and 52 patients were CK19 positive. In the CK19-negative group, 104 patients were diagnosed with Barcelona Clinical Liver Cancer (BCLC) stage B, while 91 patients were diagnosed with stage C, whereas in the CK19-positive group, 16 patients were stage B and 36 were stage C. Extrahepatic metastasis was observed in 17 patients (8.7%) in the CK19-negative group and 13 patients (25%) in the CK19-positive group. Before performing PSM, statistically significant differences existed in BCLC stage ($p = 0.004$) and the presence of extrahepatic metastasis ($p = 0.001$) between the two groups. After PSM, the CK19-negative group comprised 83 patients, whereas the CK19-positive group had 43 patients, with no statistically significant differences observed in the baseline characteristics between the two cohorts. Table 1 summarizes the baseline characteristics before and after matching. In addition, to provide an overview of treatment distribution, the utilization of different targeted therapies and immunotherapy agents in the entire cohort is summarized in Supplementary Tables 1 and 2.

Tumor Response Evaluation

Representative histological images are shown in Figure 2. Figure 2A displays hematoxylin-eosin (H&E) staining and CK19 immunohistochemical staining of tumor tissue from a CK19-negative patient, while Figure 2B shows the corresponding staining from a CK19-positive patient. Table 2 describes the optimal tumor response assessed in accordance with mRECIST criteria. The ORR was higher in the CK19-negative group than in the CK19-positive group (59.0% vs 18.6%; $p < 0.001$). The DCR was greater in the CK19-negative group than in the CK19-positive group (96.4% vs 79.1%, $p = 0.005$).

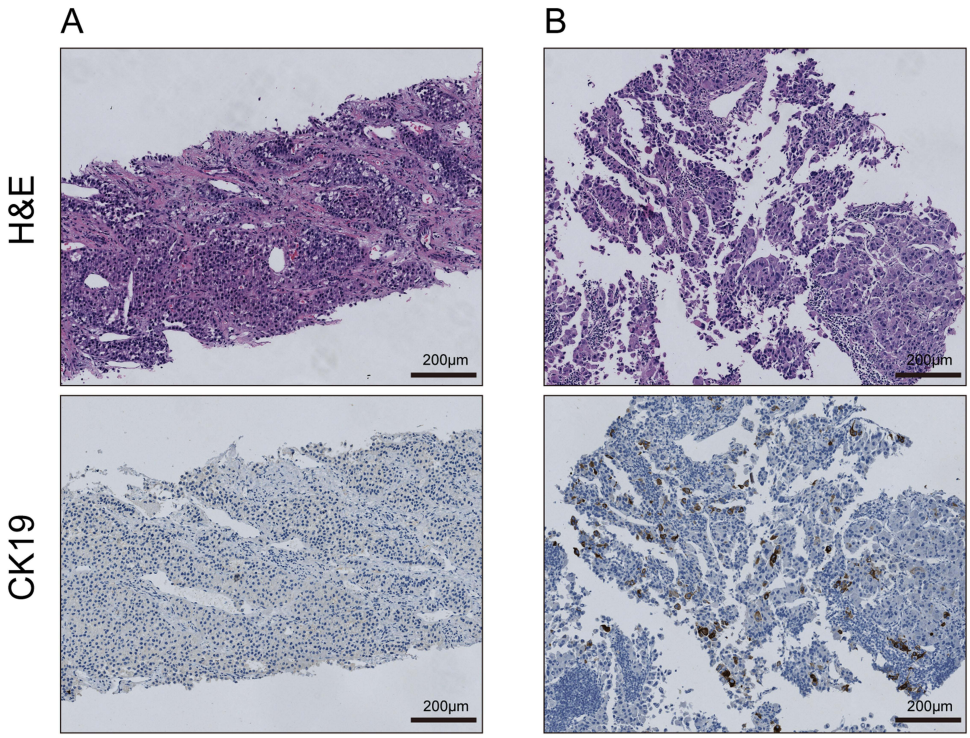

Figure 2 Representative histological images of CK19-negative and CK19-positive HCC. (**A** and **B**) Hematoxylin and eosin (HE) staining and CK19 immunohistochemical staining of biopsy specimens from CK19-negative and CK19-positive HCC patients. The black scale bar is 200 μm.

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Table 2 Tumor Response for the Patients in Matched Cohorts

Best Overall Response	CK19 Negative (n=83)	CK19 Positive (n=43)	P
CR, n (%)	4(4.8)	0(0.0)	
PR, n (%)	45(54.2)	8(18.6)	
SD, n (%)	31(37.3)	26(60.5)	
PD, n (%)	3(3.6)	9(20.9)	
ORR, %	59.0	18.6	**<0.001**
DCR, %	96.4	79.1	**0.005**

Note: Bold values indicate statistical significance ($p < 0.05$).
Abbreviations: CK19, Cytokeratin 19; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate.

Survival Outcomes

Before PSM, the median OS (mOS) for the CK19-negative group was 46.1 months (95% CI, 42.1-NA), whereas the mOS for the CK19-positive group was 14.8 months (95% CI, 9.9–22.8) ($p < 0.001$, Figure 3A). Additionally, the median PFS (mPFS) for the CK19-negative group was longer than that of the CK19-positive group (27.5 months (95% CI, 22.1–35.5) vs 6.8 months (95% CI, 5.7–12.2)) ($p < 0.001$, Figure 3B).

After PSM, the mOS of the CK19-negative group was 42.2 months (95% CI, 42.2-NA), and the mOS of the CK19-positive group was 15.6 months (95% CI, 11.2–25.0) ($p < 0.001$, Figure 4A). Similarly, the mPFS was prolonged in the CK19-negative group (28.9 months, 95% CI, 21.9–NA) compared with the CK19-positive group (7.3 months, 95% CI, 6.0–12.7; $p < 0.001$, Figure 4B).

Analysis of Prognostic Factors

In the matched cohort, prognostic factors were assessed using the Cox proportional hazards regression model. The analysis identified the number of tumors (>3 vs ≤3) (hazard ratio (HR) = 2.045, 95% CI, 1.175–3.562, p = 0.011), bilirubin (HR = 1.021, 95% CI, 1.010–1.033, $p < 0.001$), and CK19 expression (positive vs negative) (HR = 3.895, 95% CI, 2.276–6.664, $p < 0.001$) as independent predictors of OS (Table 3).

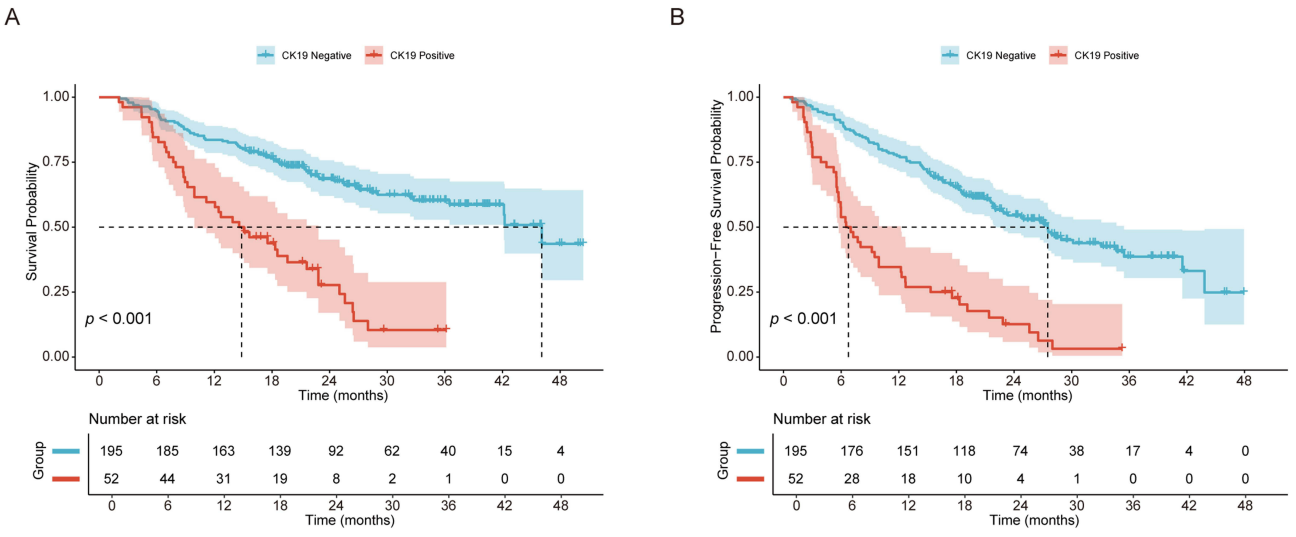

Figure 3 Kaplan–Meier survival curves comparing CK19-positive and CK19-negative patients before PSM. (**A**) Kaplan–Meier curves for OS. (**B**) Kaplan–Meier curves for PFS. Survival differences between the two groups were evaluated using the Log rank test. Before PSM, patients in the CK19-negative group exhibited significantly longer OS and PFS compared to those in the CK19-positive group (both $p < 0.001$).

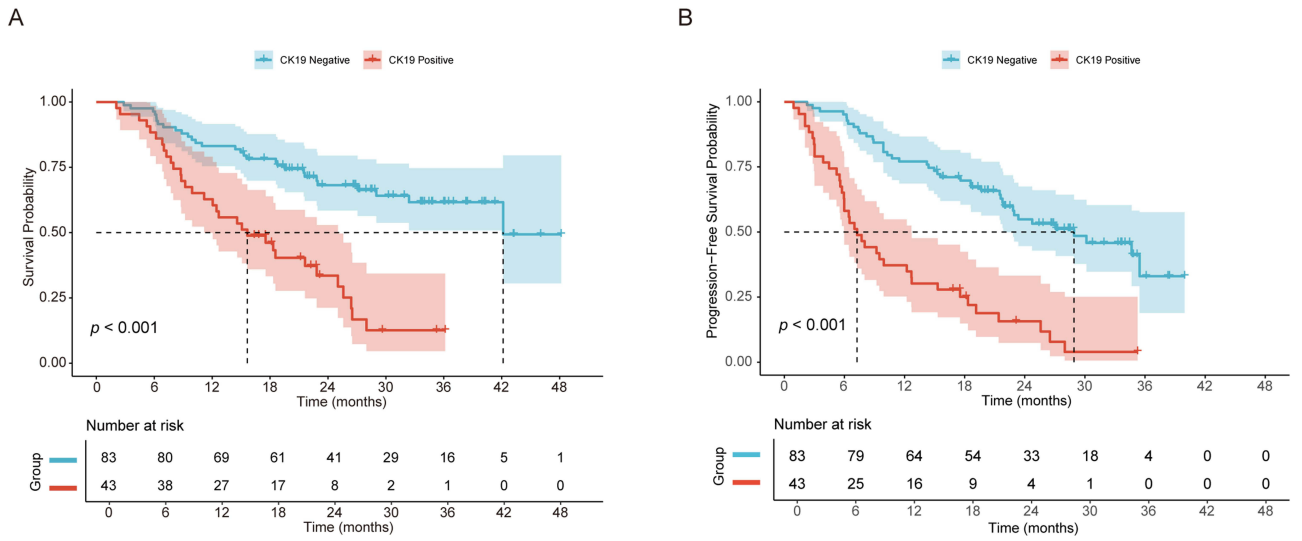

Figure 4 Kaplan–Meier survival curves comparing CK19-positive and CK19-negative patients after PSM. (**A**) Kaplan–Meier curves for OS. (**B**) Kaplan–Meier curves for PFS. Survival differences between the two groups were evaluated using the Log rank test. After PSM, patients in the CK19-negative group exhibited significantly longer OS and PFS compared to those in the CK19-positive group (both p < 0.001).

Furthermore, multivariate analysis for PFS indicated that BCLC stage (C vs B) (HR=1.734, 95% CI, 1.050–2.863, p = 0.032), tumor number (>3 vs ≤3) (HR = 1.868, 95% CI, 1.110–3.144, p = 0.019), and CK19 expression (positive vs negative) (HR=4.588, 95% CI, 2.855–7.372, p < 0.001) served as significant and independent prognostic factors (Table 4).

Table 3 Univariable and Multivariable Cox Regression Analysis for OS

Variables	Univariable Analysis HR (95% CI)	P	Multivariable Analysis HR (95% CI)	P
Age, years	0.998 (0.975–1.022)	0.891		
Gender (vs Female)	0.696 (0.316–1.532)	0.368		
BMI, kg/m^2	1.001 (0.924–1.085)	0.973		
HBsAg (vs Negative)	3.355 (0.819–13.739)	0.092		
ECOG PS (vs 0)	1.569 (0.905–2.723)	0.109		
Child-Pugh class (vs grade A)	1.900 (0.901–4.008)	0.092		
Liver cirrhosis (vs Absent)	1.155 (0.698–1.911)	0.576		
BCLC stage (vs B)	1.746 (0.985–3.095)	0.056		
AFP ≥400μg/L	0.953 (0.573–1.584)	0.851		
Largest tumor size (vs ≤10.0 cm)	1.222 (0.739–2.019)	0.434		
Tumor number (vs ≤3)	1.853 (1.075–3.193)	**0.026**	2.045 (1.175–3.562)	**0.011**
Albumin, g/L	0.982 (0.932–1.034)	0.491		
ALT, U/L	1.006 (0.994–1.020)	0.326		
Bilirubin, μmol/L	1.015 (1.005–1.026)	**0.005**	1.021 (1.010–1.033)	**<0.001**
PVTT	1.454 (0.878–2.408)	0.145		
Extrahepatic metastasis	1.480 (0.799–2.743)	0.212		
CK19 (vs Negative)	3.514 (2.090–5.909)	**<0.001**	3.895 (2.276–6.664)	**<0.001**

Note: Bold values indicate statistical significance (p < 0.05).
Abbreviations: OS, overall survival; HR, hazard ratio; BMI, body mass index; HBsAg, Hepatitis B surface antigen; ECOG PS, Eastern Cooperative Oncology Group performance status; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; PVTT, portal vein tumor thrombosis; CK19, Cytokeratin 19.

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Table 4 Univariable and Multivariable Cox Regression Analysis for PFS

Variables	Univariable Analysis HR (95% CI)	P	Multivariable Analysis HR(95% CI)	P
Age, years	1.001 (0.980–1.021)	0.955		
Gender (vs Female)	0.995 (0.457–2.166)	0.990		
BMI, kg/m^2	0.987 (0.918–1.062)	0.731		
HBsAg (vs Negative)	1.716 (0.693–4.249)	0.243		
ECOG PS (vs 0)	1.663 (1.015–2.725)	**0.043**	1.649 (0.993–2.737)	0.053
Child-Pugh class (vs grade A)	1.322 (0.634–2.755)	0.457		
Liver cirrhosis (vs Absent)	1.153 (0.741–1.796)	0.528		
BCLC stage (vs B)	1.860 (1.135–3.047)	**0.014**	1.734 (1.050–2.863)	**0.032**
AFP ≥400μg/L	0.930 (0.595–1.454)	0.751		
Largest tumor size (vs ≤10.0 cm)	1.007 (0.646–1.570)	0.975		
Tumor number (vs ≤3)	1.866 (1.137–3.063)	**0.014**	1.868 (1.110–3.144)	**0.019**
Albumin, g/L	0.985 (0.947–1.026)	0.472		
ALT, U/L	1.006 (0.994–1.019)	0.297		
Bilirubin, μmol/L	1.010 (0.999–1.021)	0.064		
PVTT	1.360 (0.873–2.118)	0.174		
Extrahepatic metastasis	1.634 (0.952–2.806)	0.075		
CK19 (vs Negative)	4.021 (2.541–6.363)	**<0.001**	4.588 (2.855–7.372)	**<0.001**

Note: Bold values indicate statistical significance ($p < 0.05$).
Abbreviations: PFS, progression-free survival; HR, hazard ratio; BMI, body mass index; HBsAg, Hepatitis B surface antigen; ECOG PS, Eastern Cooperative Oncology Group performance status; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; PVTT, portal vein tumor thrombosis; CK19, Cytokeratin 19.

Discussion

In this retrospective study, we found that positive expression of CK19 in HCC tumor biopsies was associated with significantly reduced PFS and OS. After controlling for baseline characteristics with PSM, CK19-positive continued to be an independent predictor of adverse prognosis in both the univariate and multivariate Cox regression models. To our knowledge, no previous investigations have assessed the prognostic significance of CK19 expression in patients with unresectable HCC receiving combination therapy with targeted therapy and immunotherapy. This study therefore provides the first clinical evidence supporting the role of CK19 as a potential predictor of treatment efficacy in the context of systemic combination therapy. Our results highlight its potential as a therapeutically significant biomarker in this setting.

The predictive significance of CK19 in hepatocellular carcinoma has been validated in numerous investigations.[17–19] CK19-positive HCC is characterized by increased aggressiveness, a greater rate of vascular invasion, early recurrence, and poor differentiation.[20] However, the majority of these investigations have concentrated on patients who underwent surgical resection.[16,21] Extending these findings to a previously underexplored population, our cohort of unresectable HCC undergoing combination targeted therapy and immunotherapy demonstrated that CK19 expression continued to correlate with diminished PFS and OS. This indicates that the stemness-associated biology of CK19-positive tumors is linked to a poor prognosis in both surgical and systemic treatment situations, thereby supporting its application for risk stratification within contemporary systemic regimens.

The poor prognosis of CK19-positive patients may be related to multiple biological mechanisms. CK19 is utilized as a marker for hepatic progenitor cells, and its presence in HCC correlates with tumor stemness, facilitating resistance to apoptosis and enhancing rapid proliferation.[22–24] CK19-positive HCC frequently exhibits epithelial-mesenchymal transition (EMT) and enhanced TGF-β signaling.[25] Research on various malignancies has demonstrated that the EMT and enhanced TGF-β signaling facilitate immune evasion by fostering an immunosuppressive tumor microenvironment and diminishing T-cell infiltration.[26–28] These immunosuppressive features may compromise the efficacy of immune checkpoint blockade and targeted therapies, which rely on functional anti-tumor immunity. Furthermore, a recent study has presented the concept of PANoptosis, a coordinated cell death mechanism that includes pyroptosis, apoptosis, and

necroptosis. PANoptosis has been associated with the growth of HCC and resistance to systemic treatments, whereas its activation may reprogram an immunosuppressive tumor microenvironment and augment innate immune responses.[29] Although direct evidence linking CK19 to PANoptosis is lacking, a plausible hypothesis is that CK19-high, progenitor-like/EMT tumors may attenuate PANoptotic signaling and thereby facilitate treatment resistance.

In addition, CK19-positive HCC has been closely associated with angiogenesis. Kim et al reported that CK19 expression correlates with increased vascular invasion and activation of VEGF signaling.[13] VEGF not only promotes tumor vascularization but also contributes to immune evasion by inhibiting dendritic cell maturation and impairing T-cell activation.[30] These mechanisms indicate that CK19 expression may simultaneously enhance angiogenesis and immunosuppression, potentially reducing the effectiveness of both anti-angiogenic targeted treatments and immunotherapies. This interaction highlights the rationale for assessing CK19 as a biomarker to enhance patient classification and inform the development of combination therapies. In particular, integration of molecular data such as gene expression profiles and immune cell infiltration patterns could help clarify the immunobiological features that underpin CK19-driven resistance.

Our findings indicate that CK19 expression may function as a significant biomarker for the risk classification of patients with unresectable HCC undergoing targeted therapy and immunotherapy. Exploring the combination therapy with additional medicines that target immunosuppressive pathways may be valuable in clinical trials. Furthermore, routine assessment of CK19 status in biopsy specimens may help guide treatment plans. Given the convenience of immunohistochemical detection, CK19 could be integrated into the diagnostic workflow to support personalized treatment strategies. In addition, ongoing efforts have explored CK19 not only as a biomarker but also as a potential therapeutic target. Future research should integrate CK19 biology with drug development to determine whether it can serve as both a stratification marker and a therapeutic target.

There are also limitations to this study. Firstly, this is a retrospective study, and the samples analyzed were collected from a single hospital, which may be subject to selection bias. In addition, only patients with available tumor biopsy specimens and complete clinical data were included, resulting in the exclusion of a large proportion of the initial cohort (1224/1471, 83%). This necessary restriction, while unavoidable because CK19 status could only be determined in patients with biopsy tissue, may have introduced additional selection bias and partly contributed to the relatively favorable survival outcomes compared with historical cohorts. Secondly, the sample size of CK19-positive HCC was relatively small and may not represent the characteristics of all CK19-positive HCC. Larger, multicenter prospective trials are required to validate our findings and investigate the predictive significance of CK19 for targeted therapy and immunotherapy. Thirdly, the lack of spatial or temporal evaluation of CK19 expression limits our ability to assess dynamic changes under treatment pressure. Although our cohort was treatment-naïve at baseline, we did not evaluate longitudinal changes in CK19 following therapy. In particular, we did not measure transcriptomic or spatial expression of TGF-β/EMT or PANoptosis-related genes within tumor tissue, which limits mechanistic inference. Prospective studies using single-cell sequencing, spatial transcriptomics, and multiplex immunohistochemistry in CK19-positive versus CK19-negative regions are warranted. Finally, an important limitation is that our study concentrated only on CK19. Growing evidence suggests that no single biomarker can comprehensively predict targeted therapy and immunotherapy efficacy, as treatment response is influenced by tumor-intrinsic modifications (such as PD-L1, TMB, and MSI),[31] characteristics of the immune microenvironment (such as TILs, TLS, and TAM polarization), host variables (such as HLA diversity and microbiome), and fluctuating circulating markers such as ctDNA.[32] AI-driven grading algorithms and comprehensive biomarker panels are emerging to tackle this complexity.[33–35] Our findings thus offer supplementary evidence for CK19 as a potential biomarker that may eventually be integrated into multimodal predictive models. Future work should also address whether CK19 expression differentially influences response to ICIs, anti-angiogenic therapies, or their combination. As all patients in our cohort received combined regimens, this distinction could not be clarified in the present study. Larger prospective cohorts and animal experiments are warranted to further validate and disentangle these effects.

Our clinical data indicate that the identification of CK19 expression in HCC may function as a biomarker for determining inadequate response and prognosis in patients with unresectable HCC undergoing targeted immunotherapy. Therefore, our results offer new insights into the heterogeneity of tumor development. Recognizing CK19 as a molecular

determinant of treatment heterogeneity may help reshape patient stratification and drive more precise therapeutic strategies in advanced HCC.

Conclusion

This study revealed that in patients with unresectable HCC receiving targeted therapy and immunotherapy, CK19 positivity was strongly associated with poorer treatment response and survival outcomes. After controlling for clinical confounding variables using propensity score matching, CK19-positive expression remained an independent adverse prognostic factor for PFS and OS. This outcome indicates that CK19 not only reflects the aggressive biological characteristics of the tumor but may also affect the effectiveness of systemic therapy for HCC.

Ethical Approval

This study was retrospectively conducted in accordance with the Declaration of Helsinki and received approval from the Ethics Committee of Tongji Hospital (TJ-IRB202504090). The informed consent of patients was waived due to the retrospective nature of the study. All patient data were anonymized and handled with strict confidentiality to ensure privacy protection.

Disclosure

None of the authors have a conflict of interest to disclose.

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