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Real-world study of hepatic artery infusion chemotherapy combined with anti-PD-1 immunotherapy for hepatocellular carcinoma patients with portal vein tumor thrombus

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Abstract

Background: Patients with hepatocellular carcinoma (HCC) with portal vein tumor thrombus (PVTT) present a poor prognosis. Current systemic therapies offer limited benefits. Hepatic artery infusion chemotherapy (HAIC) is a local regional treatment for advanced HCC, particularly in selected patients such as patients with PVTT or high intrahepatic tumor burden.

Objectives: The purpose of this study is to retrospectively evaluate the efficacy and safety of HAIC combined with anti-PD-1 immunotherapy for HCC patients with PVTT, and explore factors related to survival prognosis, providing clues for treatment decisions for HCC patients.

Design: This is a single-center retrospective study conducted over 2 years on consecutive PVTT patients receiving HAIC combined anti-PD-1 antibodies.

Methods: The primary endpoint was overall survival (OS). Univariate and multivariate analyses were performed to identify prognostic factors affecting OS. Treatment-associated adverse events were evaluated as well.

Results: A total of 119 patients were analyzed. The median OS and PFS were 14.9 months and 6.9 months. A total of 31.1% of grade 3–4 adverse events were reported, with elevated transaminase and total bilirubin being the most common. The independent variables correlated with survival include treatment-related alpha-fetoprotein (AFP) response, the presence of extrahepatic organ metastasis, absolute value of platelet (PLT), neutrophil-to-lymphocyte ratio, and combined usage of tyrosine kinase inhibitors (TKIs).

Conclusion: In HCC patients with PVTT, combination therapy with HAIC and anti-PD-1 antibodies might be a promising therapy. The efficacy and safety of this combination protocol on patients with HCC complicated by PVTT warrants further investigation prospectively, especially in combination with TKIs.

Keywords: anti-PD-1 immunotherapy, combination therapy, hepatic artery infusion chemotherapy, hepatocellular carcinoma, portal vein tumor thrombus

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Introduction

Hepatocellular carcinoma (HCC), a global health problem, is the fifth leading cause of cancer-related death worldwide.[1] Recently, diagnosis of early HCC is feasible in 30–60% of cases, due to improvements in surveillance procedures, diagnostic tools, and therapeutic options.[2] However, a substantial part of patients still present portal vein tumor thrombus (PVTT) either at the onset of the disease or as a result of HCC recurrence or

progression, leading to an advanced stage of disease with an expected survival of around 3 months without treatment.[3]

Recently, atezolizumab plus bevacizumab has been recommended as the preferred first-line treatment for unresectable advanced HCC.[4] Other clinical trials, such as the KEYNOTE-524 trial, RESCUE, and the newest reported CARES-310 study,[5] have also demonstrated promising antitumor activity for advanced HCC for systemic treatments combining tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs). Building upon this foundation, other systemic treatment approaches, both as monotherapies and combinations, have also demonstrated encouraging clinical research outcomes. Notable examples include the RATIONALE-301 and HIMALAYA phase III trials.[6,7] However, despite these advances, the therapeutic effects of current approved systemic therapies for HCC patients complicated with PVTT are unsatisfactory because the baseline of such patients' survival is extremely poor. It is also a concern that the outcome data about these high tumor burden patients were limited because they had been usually excluded from previous clinical trials. Besides, the response time of immunotherapy may be long which may in part negate the effects of combined systemic therapies. Moreover, once first-line therapy is ineffective or discontinued, the formation of PVTT may markedly accelerate, decreasing the portal blood flow and leading to rapid liver function deterioration, eventually complicating the administration of the following treatment. Thus, there remains a need for further progress in the management of HCC complicated with PVTT.

Combination with local regional treatment may be a favorable option, especially for patients with intrahepatic tumor progression. Hepatic artery infusion chemotherapy (HAIC) has been reported as an effective treatment for HCC with PVTT, especially in Asia.[8] A large-scale retrospective study reported HAIC had a significantly better prognosis compared with sorafenib in advanced HCC patients with macrovascular invasion.[9] By infusing chemotherapeutic agents directly into HCC feeding arteries and avoiding first-pass effects, HAIC is basically a systemic therapy with more prominent locoregional efficacy. Several features of HAIC make it a suitable candidate that could be compatible with other systemic strategies. First, it is associated with fewer hepatocellular injuries and systemic adverse events than intravenous chemotherapy. Besides, the antitumor mechanisms between HAIC, TKIs, and ICIs are entirely distinct and may even have synergistic effects in some cases. For example, HAIC of the FOLFOX regimen plus sorafenib improved OS and had acceptable toxic effects in HCC patients with PVTT compared with sorafenib alone.[10] In another phase III trial, although the addition of HAIC to sorafenib did not significantly improve OS in advanced HCC, subgroup analysis revealed a significant additive effect in patients with HCC invasion into the portal trunk.[11] Also, it has been reported that triple combination therapy of HAIC, programmed cell death protein-1 (PD-1) inhibitor, and TKIs yielded promising clinical efficacies in advanced HCC.[12–14] However, most of these published reports were based on small sample sizes, and few pure data explored the efficacy and safety of this combination for HCC particularly with PVTT.

The current study aims to explore the efficacy and safety of HAIC combined with anti-PD-1 immunotherapy for HCC patients with PVTT in a prospectively collected series of patients. Factors independently associated with OS are also analyzed. Furthermore, based on the results of the multivariate analysis using Cox regression, an easy-to-use prognostic stratification that may help to select patients who would benefit from the combination therapy was explored.

Materials and methods

Study population

This retrospective analysis is based on the prospective database of patients diagnosed with HCC at the Department of Hepatic Oncology, Liver Cancer Institute, Zhongshan Hospital, Fudan University. Patients were diagnosed by biopsy examination or the American Association for the Study of Liver Diseases imaging criteria. The informed consent was waived for the retrospective nature of the study. It conformed to the ethical principles for medical research of the Declaration of Helsinki. The reporting of this study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology statement (Supplemental Material).

Between 1 January 2020 and 31 December 2021, all consecutive HCC patients who received HAIC treatment were included. The inclusion criteria consisted of the following: (1) radiologic evidence of PVTT on contrast-enhanced computed tomography (CT) or magnetic resonance (MRI) images; (2) HAIC of FOLFOX or modified FOLFOX regimen; (3) patients had at least one cycle of HAIC combined anti-PD-1 immunotherapy, regardless TKIs use; and (4) patients with preserved liver function (Child-Pugh score $\leqslant 7$) and performance status (score $\leqslant 2$). Excluded criteria included the following: (1) patients with other malignancies; (2) patients with an absence of baseline imaging information or a significant amount of missing data; (3) patients who had received prior HAIC; (4) patients with allergies to related drugs; and (5) patients with severe cardiovascular and renal diseases (Supplemental Figure 1). Through comprehensive data collection, standardized outcome measures, transparent reporting, and rigorous statistical approaches, our study has made concerted efforts to minimize potential sources of bias.

HAIC procedure and anti-PD-1 immunotherapy

HAIC was performed as the standard modality of the institution.[15] Briefly, tumor-feeding arteries were clarified by angiographic surveys. Then, 4Fr or 5Fr RH, MPA catheter, or 2.7Fr microcatheter was advanced into the hepatic artery at the level of selective segmental, lobar, or whole liver, based on tumor size, location, and arterial supply. Chemotherapy drugs were delivered by an external infusion pump connected to the catheter or microcatheter. The following regimen of modified FOLFOX was administered: oxaliplatin, 60–85 mg/m^2 for 2 h; leucovorin, 300–400 mg/m^2; and 5-fluorouracil, 300–400 mg/m^2 bolus for 2 h, 2400 mg/m^2 for 46 h (48-h protocol) or 1200 mg/m^2 for 22 h (24-h protocol) at the discretion of the treating physician. HAIC was administered every 3–4 weeks and repeated until tumor progression, unacceptable toxicities, or deterioration of hepatic function or clinical conditions.

Anti-PD-1 antibodies were administrated intravenously within 3–5 days after or before HAIC and repeated every 3 weeks with or without HAIC. The dose of these agents was administrated according to the guidelines. Decisions on the dose adjustment, disruption, or discontinuation were made at the discretion of the investigator based on the patient's clinical status.

Follow-up, tumor response assessments, and safety analyses

Tumor response was evaluated every 1–3 cycles by dynamic contrast-enhanced CT or MRI according to mRECIST by two investigators. The follow-up period ended on 31 May 2023. The median follow-up time was 13.8 months.

Adverse events were recorded based on CommonTerminology Criteria for Adverse Events (CTCAE) version 5.0 (US Department of Health and Human Services). Given that almost all patients included in the current study had a background of liver cirrhosis, which typically results in a low baseline white blood cell and platelet count, the incidence of grade 1–2 leukopenia and thrombocytopenia was not taken into account in our study.

Statistical analyses

Quantitative data were presented as the median with interquartile range, and categorical data were presented as counts with percentages unless otherwise specified.

Overall survival (OS) was the primary endpoint in this analysis, defined from the date of the initial HAIC to the date of death from any cause. Patients who were alive at the last follow-up (31 May 2023) or lost to follow-up were considered as censored data. PFS was defined as the time from initial HAIC to disease progression or death for any reason. Disease progression included intrahepatic tumor or extrahepatic tumor progression. If patients were not tolerant to treatment or their liver function was categorized as Child–Pugh grade C, PFS was recorded. Kaplan–Meier survival curves compared by log-rank test were used to estimate the median OS and PFS and the survival rate.

Continuous baseline data were converted into categorical data. The optimal cutoff point for continuous data was determined based on normal reference values, relevant previously reported cutoffs, and the results of maximally selected rank statistics from the R package 'maxstat'. Missing data were addressed by treating them as null values in the analysis. Variables showed as significant ($p < 0.05$) on univariate analysis were entered into multivariate Cox proportional hazard regression analysis. A stepwise forward selection model was employed to identify independent prognostic factors. The proportional hazard assumption was

verified using the Schodenfeld residuals test and plot of the final model (Supplemental Figure 2). The Bootstrap method was employed for internal validation. By performing 1000 rounds of random sampling with replacement, resampled datasets of the same size as the original datasets were generated. The differences in survival rates (mean and 95% confidence interval) were then calculated using Kaplan–Meier estimation.

All statistical analyses were conducted using SPSS version 25 and *R* statistical software (version 3.6.1, R Foundation for Statistical Computing). A two-tailed $p < 0.05$ was considered statistically significant.

Results

Patients baseline characteristics

A total of 119 patients were included. Baseline characteristics are summarized in Table 1. Most patients were male, had compensated liver function, heavy tumor burden (large lesions and multiple tumor numbers), and Hepatitis B Virus (HBV) was the commonest etiological cause. All participants had portal vein invasion, with 45.4% of patients presenting with Vp1/2 PVTT and 54.6% with Vp3/4 PVTT. In all, 64 (53.8%) patients had extrahepatic spread.

Out of the 119 patients, the majority had previously undergone local therapy, with 68 patients receiving conventional TACE and 14 patients receiving RFA/MVI/PEI. Several patients had previously received systemic therapy, 29 patients with prior target therapy exposure, 17 with anti-PD-1 antibodies, 2 with anti-PD-L1 antibodies, and 1 with chemotherapy.

The regimens of the current combination therapy are summarized in Table 2. Briefly, the median cycle of HAIC procedure and anti-PD-1 antibody usage were 2 and 4, respectively. Most patients (89.9%) received combined TKI treatment. As listed, the regimens of anti-PD-1 immunotherapies and TKIs were diverse, with the most common regimen being Sintilimab plus Lenvatinib (a total of 43 patients).

Subsequent therapy

Most patients continued to receive follow-up treatment (Supplemental Table 1). In patients with intrahepatic tumor regression, two patients received conversion surgery, and two patients underwent local ablation treatment. Otherwise,

Table 1. Patients' baseline demographic and clinical characteristics of all 119 patients.

Characteristics	Number (%)/median (IQR)
Gender	
Male	105 (88.2)
Female	14 (11.8)
Age (years)	56 (47–64)
Etiology	
HBV	116 (97.5)
Others	3 (2.5)
Largest tumor diameter, cm	9 (6.2–13.3)
<10	63 (52.9)
⩾10	56 (47.1)
Tumor number	
1	13 (10.9)
⩾2	106 (89.1)
PVTT	
Vp1/2	54 (45.4)
Vp3/4	65 (54.6)
Extrahepatic spread	
Lymph node	47 (39.5)
Organ	32 (26.9)
Both	15 (12.6)
AFP, ng/ml	2671.5 (69.5–28219.3)
<400	41 (34.6)
⩾400	75 (63)
PIVKA	13834 (1435–58641.5)
Child-Pugh score	
A	110 (92.4)
B	9 (7.6)
ALBI grade	
1	42 (35.3)
2	77 (64.7)
3	0
ALT, U/L	42 (29–62.5)
ALB, g/L	38 (35–41)

(Continued)

Table 1. (Continued)

Characteristics	Number (%)/median (IQR)
TB, umol/L	17.1 (12.9–23.6)
ALP, U/L	174.5 (124.3–234.3)
GGT, U/L	190 (122.5–317)
PT, s	13.1 (12.2–13.8)
PLT, 10^9/L	132 (93–196.5)
WBC, 10^9/L	5.2 (3.2–6.7)
NEUT, 10^9/L	3.3 (2.3–4.2)
LYMPH, 10^9/L	1.0 (0.7–1.4)
MONO, 10^9/L	0.5 (0.34–0.71)
With previous treatment	78 (65.5)
TACE	68
Target therapy	29
Immunotherapy	19
RFA/MW/PEI	14
Surgery	6
Radiotherapy	0
Chemotherapy	1

Medians with interquartile range are shown for quantitative variables, whereas counts with proportions are shown for categorical variables.
AFP, alpha-fetoprotein; ALB, albumin; ALBI, albumin–bilirubin grade; ALP, alkaline phosphatase; ALT, alanine transaminase; GGT, gamma-glutamyl transferase; LYMPH, lymphocyte; MONO, monocyte; MW, microwave ablation; NEUT: neutrophil; PEI, percutaneous ethanol injection; PIVKA, protein induced by vitamin K absence or antagonist; PLT, platelet; PT, prothrombin time; PVTT, portal vein thrombus; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TB, total bilirubin; TKI, tyrosine kinase inhibitor; WBC, white blood cell.

Table 2. Anti-PD-1 agents and targeted drugs used in the current study ($n = 119$).

Treatment	Number
Median HAIC cycle	2
Median anti-PD-1 cycle	4
Camrelizumab	23
Sintilimab	75
Toripalimab	11
Pembrolizumad	3
Tislelizumad	7
Combined TKIs	107 (89.9)
Sorafenib	22
Lenvatinib	70
Donafenib	1
Regorafenib	10
Apatinib	4

HAIC, Hepatic artery infusion chemotherapy; PD-1, programmed cell death protein-1; TKIs, tyrosine kinase inhibitors.

for patients with tumor progression or treatment intolerance, systemic treatment was still the very first choice for these populations, in which the majority of patients continued to immunotherapy and target treatment.

Treatment efficacy

The median OS was 14.9 months (range: 1.0–30.8 months, Figure 1(a)). The corresponding OS rates at 6 and 12 months were 70% and 54%, respectively. Besides, the median PFS was 6.9 months [range: 1.0–15.9 months, Figure 1(b)]. The best tumor response was summarized in Table 3, with an overall response rate (ORR) of 21.2% and a disease control rate (DCR) of 83.7%.

Safety and tolerability

No treatment-related death was recorded. As listed in Table 4, there were a total of 37 grade 3–4 AEs (31.1%). Elevated transaminase and total bilirubin were the most common adverse effects both in grade 3–4 and 1–2 AEs. The most common adverse reaction related to anti-PD-1 therapy was hypothyroidism, and the most common adverse reaction related to TKI therapy was hypertension. No severe vascular complications were related to the HAIC procedure. Varying degrees of abdominal pain was the most common adverse reaction related to the HAIC procedure, whereas only two patients discontinued drug infusion for intolerance abdominal pain.

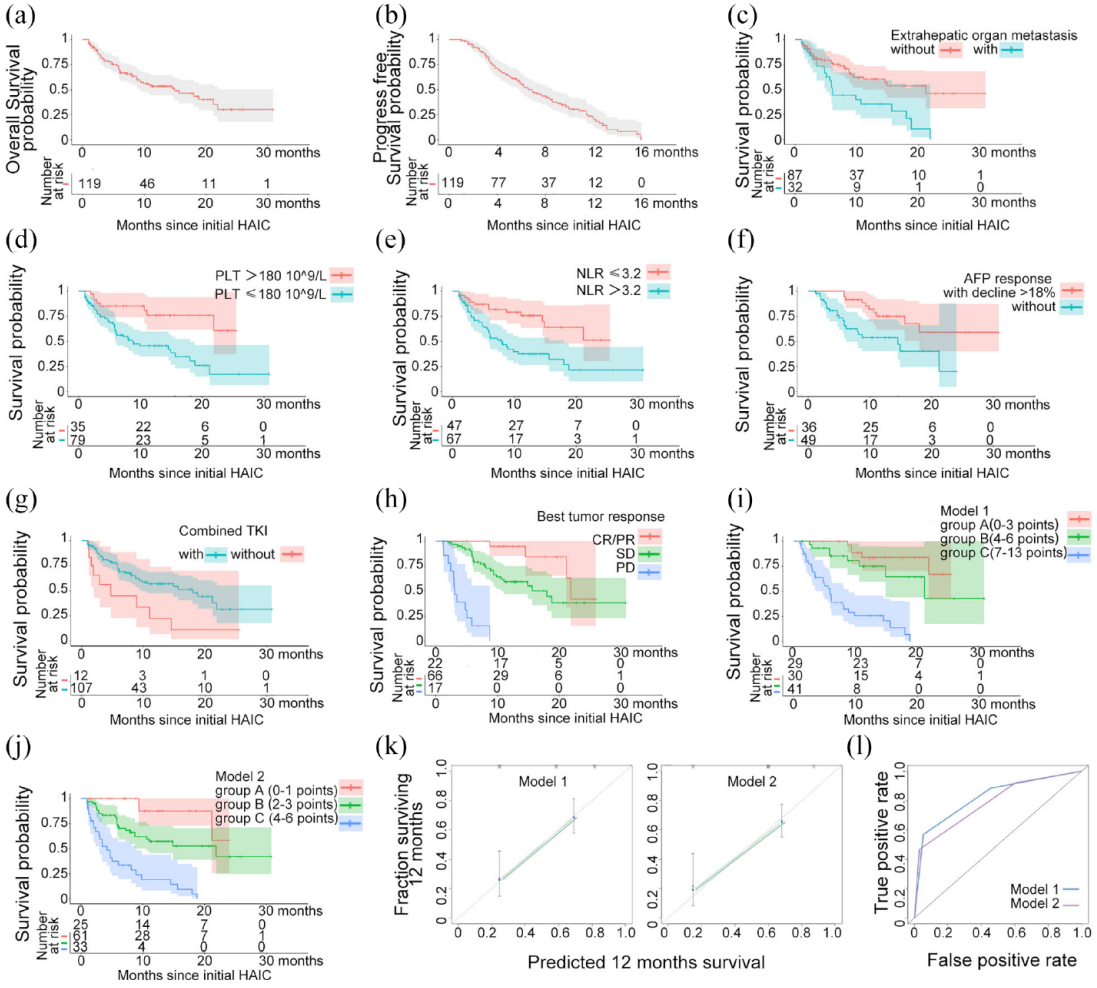

Figure 1. Kaplan–Meier estimated survival curves of the 119 HCC patients with PVTT treated with HAIC combined anti-PD-1 treatment. Kaplan–Meier curves for OS (a) and progression-free survival (b). Kaplan–Meier curves of OS between without and with extrahepatic organ metastasis group (c), PLT high and PLT low group (d, cutoff value, 180×10^9/L), NLR high and low group (e, cutoff value, 3.2), with and without AFP response group (f, cutoff value, AFP declined >18% compared to baseline), with and without combined TKIs treatment (g), best tumor response CR/PR, SD, and PD group (h). Kaplan–Meier estimated curves of overall survival of the studied patients stratified by the current Model 1 staging system (i). Kaplan–Meier estimated survival curves by Model 2 (j). Calibration curves of the Model 1 and Model 2 (k). The *y*-axis represents the actual survival rate. The *x*-axis represents the predicted possibility. The diagonal dashed line indicates the ideal prediction by a perfect model. Model 1 and Model 2 related operating characteristics curve curves for survival at 12 months (l).

AFP, alpha-fetoprotein; CR, complete response; HCC, hepatocellular carcinoma; PLT, platelet; PR, partial response; NLR, neutrophil-to-lymphocyte ratio; PD progressive disease; SD, stable disease; TKI, tyrosine kinase inhibitors.

Univariate and multivariate analyses

The results of the univariate analysis for both OS and PFS based on patients' characteristics are shown in Table 5 and Figure 1(c)–(h). As listed, variables identified as significant in univariate analysis were entered into further Cox regression analyses. After conducting a stepwise variable selection analysis to identify significant predictors, the following variables were found to remain strongly associated with poorer OS: less AFP response, the presence of extrahepatic organ metastasis, higher NLR, and decreased platelet count. While combined TKI usage remained significantly related to better OS, variables, such as higher PVTT stage and ALBI grade, less PIVKA response, lower lymphocyte-to-monocyte ratio (LMR) level, and lower body mass index (BMI) were found to have trended toward worse survival outcome but was not statistically significant in multivariate analysis.

Table 3. Best tumor response in the current study ($n = 119$).

Overall response	Number	Valid percent
CR	2	1.9
PR	21	19.2
SD	65	62.5
PD	17	16.3
Not assessable	15	
ORR	22	21.2
DCR	87	83.7

CR, complete response; DCR, disease control rate; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

Table 4. Frequency of treatment-related adverse events ($n = 119$).

Adverse event	Grades 1–2	Grade 3–4
Elevated ALT/AST	46	12
Hyperbilirubinemia	28	4
Leukopenia	–	2
Thrombocytopenia	–	2
Hypothyroidism	38	0
Proteinuria	15	2
Weight loss	19	0
Hemorrhage	–	2
Pain	12	2
Diarrhea	4	5
Fever	9	1
Nausea/vomiting	3	0
Rash	16	4
Hypertension	31	0
Thromboembolic event	1	0
Hand–foot syndrome	17	1

ALT, alanine transaminase; AST, Aspartate Aminotransferase.

Likewise, less AFP response, higher NLR, and decreased platelet count were independently associated with poorer PFS. Besides, jaundice, elevated ALP, as well as decreased lymphocytes were independent predictors of poorer PFS. Factors that represented heavy tumor burden and impaired live function, such as tumor number, PVTT stage, ALBI grade, Child-Pugh classification, and BMI exhibited trends toward worse survival outcomes but were not statistically significant.

The best tumor response based on mRECIST was related to both OS and PFS significantly. However, to find indicators that could help to predict prognosis and prevent potential confounding effects, the best tumor response was not included in the further multivariate analysis.

In addition, in the subgroup analysis, it was found that the history of prior treatments, including immunotherapy, target treatment, and TACE, as well as the specific drug selection for immunotherapy, and the specific drug selection of targeted therapy did not have a significant impact on the OS of patients. Also, it showed no significant difference in OS prognosis among patients who received full dosage of FOLFOX regimens or modified dosage in this study.

Development of a prognostic model

Based on the results of the Cox regression analysis, prognostic models were developed. To simplify the calculation of model scores, the estimated regression coefficients were multiplied by a factor of 2 and rounded to the nearest unit. Consequently, in this scoring system, patients were assigned 3 points for the presence of extrahepatic organ metastasis, less AFP response, and the absence of combined TKIs, 2 points for decreased platelet count, and higher NLR. The range of scores for this Model 1 system was from 0 to 13. Then patients were categorized into three groups, depending on their scores at the 15th and 85th percentiles (2 and 7 points). Survival curves and the calibration plots for the survival probability at 6 and 12 months are shown in Figure 1(i) and (k).

Considering that the factor AFP response in Model 1 can only be obtained 4–8 weeks after treatment, we excluded this factor and reanalyzed the data using Cox regression. This approach allows us to build a pretreatment prognostic model (Model 2) before the commencement of HAIC treatment. The six following variables were found to remain strongly associated with poor

Table 5. Univariate and multivariate analyses of potential prognostic factors of OS and PFS for HCC patients with PVTT treated with HAIC combined anti-PD-1 immunotherapy.

Characteristics	OS			PFS		
	Univariate *p*	Multivariate *p*	HR (95% CI)	Univariate *p*	Multivariate *p*	HR (95% CI)
Gender (female/male)	0.938			0.5		
Age > 65 (no/yes)	0.845			0.307		
BMI < 23.5 (no/yes)	0.048*			0.235		
Largest tumor diameter (<10/≥10)	0.792			0.989		
Tumor number (single/multiple)	0.347			0.079		
Lymph node metastasis (without/with)	0.288			0.954		
Extrahepatic organ metastasis (without/with)	0.001*	0.001*	3.900 (1.799–8.505)	0.337		
Extrahepatic spread (without/with)	0.107			0.821		
PVTT (Vp1/2/Vp3/4)	0.049*			0.077		
AFP ≥ 400 ng/mL (no/yes)	0.852			0.956		
AFP response (with decline > 18%/without)	0.006*	0.001*	4.651(1.913–11.309)	0.01*	0.002*	3.216 (1.727–5.987)
PIVKA ≥6000 mAu/mL (no/yes)	0.272			0.875		
PIVKA response (with decline > 50%/without)	0.041*			0.093		
Child-Pugh score (A/B)	0.562			0.047*		
ALBI grade (1/2)	0.021*			0.029*		
ALT > 60 U/L (no/yes)	0.265			0.36		
ALB ≤ 3.6 g/L (no/yes)	0.844			0.067		
TB > 17 umol/L (no/yes)	0.017*			0.001*	0.006*	2.283 (1.226–4.118)
GGT > 390 U/L (no/yes)	0.393			0.395		
ALP > 190 U/L (no/yes)	0.006*			0.002*	0.003*	2.515 (1.383–4.575)
PT > 14s (no/yes)	0.553			0.371		
PLT ≤ 180×10^9/L (no/yes)	0.010*	0.043*	2.819 (1.033–7.693)	0.027*	0.022*	2.116 (1.114–4.019)
WBC < 6.1×10^9/L (no/yes)	0.028*			0.096		
NEUT < 4×10^9/L (no/yes)	0.042*			0.074		
LYMPH < 1.1×10^9/L (no/yes)	0.008*			0.032*	0.004*	6.239 (1.847–21.559)
MONO < 0.41×10^9/L (no/yes)	0.074			0.059		
CRP > 12.8 mg/L (no/yes)	0.859			0.949		

(Continued)

Table 5. (Continued)

Characteristics	OS			PFS		
	Univariate *p*	Multivariate *p*	HR (95% CI)	Univariate *p*	Multivariate *p*	HR (95% CI)
LMR ⩽ 2.5 (no/yes)	0.018*			0.059		
PLR > 175 (no/yes)	0.078			0.49		
NLR > 3.2 (no/yes)	0.001*	0.017*	2.852 (1.207–6.742)	0.003*	0.001*	8.564 (2.442–30.034)
With previous treatment(no/yes)	0.565			0.521		
Pre-target therapy	0.946			0.42		
Pre-immunotherapy	0.852			0.704		
Pre-TACE	0.435			0.823		
Pre-RFA/MW/PEI/surgery	0.066			0.329		
HAIC regiment (FOLFOX/modified FOLFOX)	0.572			0.253		
Combined TKI (with/without)	0.001*	0.009*	3.967(1.411–11.153)	0.487		
Best tumor response (CR PR/SD/PD)	0.001*			0.001*		

AFP, alpha-fetoprotein; ALB, albumin; ALBI, albumin-bilirubin grade; ALP, alkaline phosphatase; ALT, alanine transaminase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; LMR, lymphocyte-to-monocyte ratio; LYMPH, lymphocyte; MONO, monocyte; MW, microwave ablation; NEUT: neutrophil; NLR, neutrophil-to-lymphocyte ratio; OS, Overall Survival; PEI, percutaneous ethanol injection; PIVKA, protein induced by vitamin K absence or antagonist; PLR, platelet-to-lymphocyte ratio; PLT, platelet; PT, prothrombin time; PVTT, portal vein thrombus; RFA, radiofrequency ablation; TB, total bilirubin; WBC, white blood cell; TACE, transarterial chemoembolization; TKI, tyrosine kinase inhibitor.
*$p<0.05$.

OS: the presence of extrahepatic organ metastasis, higher NLR, decreased platelet count, higher ALBI grade, elevated ALP, and without combined TKIs. Similarly, following the rule of multiplying the estimated regression coefficients by a factor of 2 and rounding to the nearest whole number, the model was assigned values accordingly. In this Model 2 scoring system, patients were assigned 1 point for each of the six factors mentioned (the presence of extrahepatic organ metastasis, higher NLR, decreased platelet count, higher ALBI grade, elevated ALP, and the absence of combined TKIs). The range of scores for the Model 2 system was from 0 to 6. Then patients were categorized into three groups (Groups A, B, and C), depending on their scores at the 15th and 85th percentiles (1 and 4 points). As depicted in Figure 1(j) and (k), this category was effective in distinguishing the patients based on their prognosis, and the calibration plots for the survival probability at 6 and 12 months exhibit optimal agreement between the expected and observed outcomes.

Akaike information criterion (AIC), C-index, area under the curve (AUC), and likelihood ratio (LR) test of the candidate two models are listed in Table 6 and Figure 1(l). Considering the need for preoperative stratification of patients for treatment selection, as well as the relative simplicity of calculation, Model 2 with three categories was recommended. This model has a lower AIC value, higher C-index, and reasonable AUC, indicating its potential usefulness in predicting patient outcomes.

The current Model 2 score system was internally validated using the Bootstrap method. The Bootstrap method was employed to calculate the 0.5-year and 1-year survival rates, along with their corresponding 95% confidence intervals (95% CI), for each group (Groups A, B, and C) within Model 2. Internal validation was conducted by comparing the survival rates pairwise among these three groups. The results of the pairwise comparisons revealed that the lower confidence limit for the difference between each pair of

Table 6. Comparison of the performance and discrimination ability of current models.

Model	AIC	C-index	AUC	LRT loglik
Model 1 three categories	423.81	0.676	0.819	−97.629
Model 2 three categories	416.61	0.716	0.794	−102.504
COX model 1	199.66	0.82	0.932	−94.828
COX model 2	376.93	0.78	0.886	−98.198

AIC, Akaike information criterion; AUC, area under the curve; LRT, likelihood ratio test.

Groups A, B, and C was greater than zero (Supplemental Table 2). This indicates that all differences between the groups were statistically significant.

Discussion

We have undertaken a retrospective investigation into the effects of HAIC in combination with anti-PD-1 immunotherapy for HCC patients with PVTT. In our cohort of 119 patients, the median OS was 14.9 months (±3.618, 95% CI 7.775–21.958) and the median PFS was 6.9 months (±0.752, 95% CI 5.426–8.374). Treatment-related AFP response, the presence of extrahepatic organ metastases, platelet counts (PLT), neutrophil-to-lymphocyte ratio (NLR), and utilization of combined TKIs were identified as significant prognostic factors for OS.

In an era where systemic therapy options are limited, transarterial chemoembolization (TACE) was a treatment option for HCC with PVTT. As we previously reported, the median OS for HCC patients with main PVTT treated with conventional TACE as monotherapy was 6 months.[16] Then, sorafenib was the only approved agent for treating advanced HCC, whereas the survival benefits for PVTT patients were limited, with a median OS of about 8 months in the SHARP study.[17] Thereafter, lenvatinib demonstrated comparable therapeutic effects to sorafenib as a first-line treatment, and multiple VEGF TKIs showed survival benefits in later-line settings.[18,19] On the other hand, although immunotherapy showed promising results in several phase II/III trials, it was not until recently that the combination of atezolizumab and bevacizumab (IMbrave150 trial) showed, for the first time, an OS benefit compared with sorafenib in advanced treatment-naive patients with HCC. The sub-analysis revealed the median OS of advanced HCC patients treated with atezolizumab plus bevacizumab was 19.2 months.[20] However, when comes to HCC patients with Vp4 PVTT, the updated IMbrave150 showed a limited benefit with a median OS of 7.6 months.[21]

The present study showed a relatively satisfactory OS. In previous studies, it was shown that the combination of loco-regional therapies with systemic therapies might improve the survival of patients with high-risk advanced HCC.[12,22,23] However, due to the limitations of follow-up time, many prior studies failed to analyze the OS data. Till recently, a phase II trial involving 36 participants with 86% of high-risk HCC, evaluating the efficacy and safety of lenvatinib, toripalimab, and FOLFOX-HAIC combination therapy, showed a median OS of 17.9 months after the follow-up extension.[24] In addition, the ORR and PFS in our study of patients receiving current combined treatment seem comparable with those of patients receiving first-line systemic treatment for advanced HCC, in which ORR ranged from 2% to 27.3% and PFS ranged from 3.7 to 7.3 months.[18,19] But patients enrolled in the current study may be considered to have a poor prognosis because all the patients had PVTT and most of them had heavy tumor burden (larger tumor site and extrahepatic organ metastases). One of the main contributors to the current beneficial effects on patient survival may be the effectiveness of the perfused FOLFOX regimen in inducing immunogenic cell death (ICD),[25] enhancing tumor immunogenicity, and promoting the infiltration of CD8+ T cells into the tumor. The combined immune therapy can further enhance this antitumor immune response, enabling immune cells to better attack and eliminate cancer cells. In addition, previous studies have demonstrated that these chemotherapy drugs may upregulate the expression of checkpoint receptors in tumor cells and lymphocytes,[26] providing additional support for the rationale behind combined immunotherapy.

In terms of safety, the overall incidence of adverse reactions associated with the current combination therapy was tolerable, with a total of 37 patients experiencing serious adverse events and no reported treatment-related fatalities. The most common severe adverse events observed were liver dysfunction, diarrhea, and skin rash. Liver dysfunction, hypothyroidism, and hypertension were identified as frequent adverse events. These

findings are consistent with previous studies investigating the safety of HAIC combined with ICIs or ICIs in combination with TKIs for advanced HCC. No unexpected safety signals were reported, and there was no apparent potential for synergistic toxicity.[13,27,28] In most cases, impaired liver function was related to HAIC processors. Whereas most of these patients could recover without affecting the next cycle of treatment. Apart from liver dysfunction, myelosuppression is another common adverse event associated with HAIC, characterized by decreased neutrophils and platelets.[28] However, because patients enrolled in this real-world study generally had a cirrhosis background and low basal white blood cell (WBC) and platelet values, the incidence of grade 1–2 leukopenia and thrombocytopenia were not counted in our study. Grade 3–4 myelosuppression was also relatively low, which may be attributed to the use of the modified Folfox regimen and timely medication for leukopenia and thrombocytopenia. Regarding irAE, hypothyroidism, the most prevalent immune-related adverse event, occurred in 31.9% of the patients, consistent with previous research. In addition, five individuals discontinued PD-1 inhibitor therapy due to grade 3–4 irAE, including one case of immune therapy-associated colitis and diarrhea, one occurrence of bullous pemphigoid, one instance of severe rash, and two instances of reactive cutaneous capillary endothelial proliferation (RCCEP).

Identifying prognostic variables for survival in advanced HCC patients with PVTT treated with current combined therapy was also a crucial aspect of our study.

First, we demonstrated that in HCC patients with PVTT receiving current combined treatment, the presence of extrahepatic organ metastasis, platelet counts, and NLR were easily accessible preoperative independent prognostic factors. Factors, such as PVTT extent, ALBI grade, and LMR and PLR level, that had been demonstrated to affect post-treatment survival of HCC patients, were found to have trends with survival outcome but were not statistically significant in the current multivariate analysis. It is probably because, in current practice, these treated PVTT patients generally had heavy tumor burdens but relatively well-preserved liver function and performance status. Therefore, extrahepatic organ metastases played a major role in Cox regression analysis predicting survival, instead of factors such as the size and number of tumors, PVTT extension, ALBI grade, lymph node metastasis, BMI classification, and baseline AFP value. From another aspect, these results may reflect the effectiveness of HAIC in controlling intrahepatic local lesions. In addition, from the univariate analysis, it was observed that the classification of WBCs is closely related to prognosis as previously reported.[29] While in this study cohort, the classification effect of PLT and NLR was found to be even more significant.

Second, we showed that AFP response and best tumor response are good postoperative prognostic indicators. AFP decrease has been reported as a prognostic indicator in previous studies, whereas the cutoff point varied from 10% to 50%.[30] In our study, an 18% decrease in AFP value was selected, which was determined using the cutoff algorithm in *R* statistical software. Regarding the PIVKA response, according to R statistical software, a 50% decrease was used as the grouping threshold. Although this cutoff value was able to distinguish the prognosis of the study population, it did not reach statistical significance in the Cox analysis.

Third, we explored the impact of different treatment regimens on the prognosis. We showed in current PVTT patients, who received HAIC plus anti-PD1 immunotherapy, combined TKI treatment was an independent factor associated with better OS and PFS. Previous treatments, regardless of target therapy, immunotherapy, or TACE, did not impact the survival benefit. One major reason could be that the enrolled cases in this study had a heavy tumor burden, with most of them exceeding the UP-TO-SEVEN criteria, more than half had a 3/4 PVTT (PTTV) and extrahepatic lesions, while only a small portion of the patients belonged to the ALBI grade 1 category. Monotherapy, TACE or systemic therapy, has limited survival benefits for this group of patients. These findings align with recent trials, such as KEYNOTE-240 and CheckMate 459, where single-agent immunotherapy failed to meet predefined OS endpoints. Therefore, for this patient population, combination therapy plays a vital role in achieving synergistic antitumor effects, rather than relying solely on the individual antitumor mechanisms of each drug. The synergistic antitumor effects of HAIC plus TKIs and anti-PD-1 treatment may be attributed to several mechanisms:[31–33] (1) HAIC has a controlling effect on intrahepatic lesions, as well as induces

tumor antigen exposure and improves the tumor immune microenvironment to reduce off-targets, thereby enhancing the efficacy of systemic therapy; (2) chemotherapy drugs activate adaptive immunity and restore immune surveillance; (3) the combination of anti-PD-1 inhibitors and TKIs drugs promotes normalization of blood vessels, converting 'cold' tumors to 'hot' tumors; and (4) the anti-angiogenic effects of TKIs and anti-PD-1 inhibitors help eliminate tumor angiogenesis and recurrence.

Moreover, we try to build an easy-to-use prognostic stratification that may allow us to select patients who would benefit from the combination of HAIC and anti-PD-1 immunotherapy. Based on the results of the multivariate analysis using Cox regression, we have developed a prognostic scoring model. Although the AIC, C-index, and AUC parameters show satisfactory results, there is a significant overlap in the 95% confidence intervals for survival when dividing patients into three groups. In addition, due to the inclusion of the AFP response, it is not feasible to construct a pretreatment prognostic scoring model. Therefore, we excluded the AFP response and performed a subsequent Cox analysis, which led to the development of an alternative prognostic scoring model. In this scoring system, patients were assigned 1 point for each of the six factors that demonstrated significance: the presence of extrahepatic organ metastasis, higher NLR, decreased platelet count, higher ALBI grade, elevated ALP, and the absence of combined TKIs. According to the evaluation parameters of the model and the KM survival curves of the model's groups, using the 15th and 85th percentiles as cutoff points to divide patients into three groups is considered a favorable stratification approach. This finding may have clinical implications to help identify candidates who may benefit from the combined strategies while avoiding unnecessary treatments associated with poor OS. However, further validation using external data is still necessary.

This study should be interpreted in light of several limitations. First, being a retrospective study, there is a possibility of selection bias, which cannot be completely ruled out. Second, the sample size was relatively small, and the follow-up period was short, which may limit the generalizability of the study results. Compared to previous research, our study is a relatively large-scale study. Third, the absence of a control group makes it difficult to determine the true efficacy of the combination therapy. Then, the heterogeneous regimens of anti-PD-1 immunotherapies and TKIs used in this study may have had an impact on the treatment efficacy and safety profile, thereby introducing a potential confounding bias. Finally, this study lacks external validation. We hope to improve this part of the work in the follow-up research.

Conclusion

These studies suggest that the combination therapy of anti-PD-1 antibodies with HAIC may be an effective and safe treatment option for HCC patients with PVTT. The identification of prognostic factors may be useful in guiding treatment decisions and predicting survival outcomes. However, further research is needed to confirm the efficacy and safety of these approaches and determine the optimal patient selection criteria and treatment regimens.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Zhongshan Hospital, Fudan University (Approval Number: B2022-621).

Consent for publication

The informed consent was waived for the retrospective nature of the study.

Author contributions

Jinghuan Li: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft.

Bing Quan: Data curation; Formal analysis; Investigation.

Wenfeng Liu: Data curation; Formal analysis; Investigation.

Menglong Zhao: Formal analysis.

Fan Yao: Validation.

Rongxin Chen: Visualization.

Zhenggang Ren: Visualization.

Xin Yin: Conceptualization; Methodology; Project administration; Visualization; Writing – review & editing.

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Competing interests
The authors declare that there is no conflict of interest.

Availability of data and materials
The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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