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ORIGINAL RESEARCH

Efficacy and Safety of Hepatic Arterial Infusion Therapy with Cinobufacini in Advanced Hepatocellular Carcinoma with Macrovascular Invasion: A Retrospective Cohort Study

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Background: The presence of macrovascular invasion (MVI) is associated with poor prognosis in advanced hepatocellular carcinoma (HCC). This study aims to evaluate the efficacy and safety of Cinobufacini therapy via hepatic arterial infusion (HAI) in advanced HCC patients with MVI.

Methods: The clinical records of 130 consecutive patients with unresectable advanced HCC and MVI who had received Cinobufacini or cisplatin plus 5-fluorouracil (CF) treatment via HAI were retrospectively analyzed. The therapeutic efficacy, overall survival (OS), progression-free survival (PFS), and adverse events were compared between the two treatment groups.

Results: The Cinobufacini group demonstrated significant curative effects on treatment via HAI compared with the CF group, including the objective response rate (44.9% vs 27.9%, *P*=0.048), the median OS (14.8 months vs 11.1 months, *P*=0.010), and the median PFS (10.3 months vs 6.0 months, *P*=0.006). Result in subgroup analysis of portal vein invasion grade supported the efficacy in Cinobufacini treatment, especially in the median OS of Vp1-2 (18.3 months vs 14.3 months, *P*=0.043) and Vp3 (15.0 months vs 11.4 months, *P*=0.046), as well as the median PFS of Vp1-2 (14.8 months vs 10.2 months, *P*=0.028) and Vp3 (10.8 months vs 6.6 months, *P*=0.033) compared with CF treatment. Cox proportional hazards model and forest plot analysis of factors confirmed the survival benefit from HAI with Cinobufacini over CF (hazard ratio [HR], 0.61; 95% CI: 0.40–0.91; *P*=0.010). Multivariable analysis identified portal vein invasion grade (Vp4; HR, 1.78; 95% CI: 1.03–2.16; *P*=0.032) and AFP (>1000; HR, 1.61; 95% CI: 1.08–1.91; *P*=0.039) as the independent factors for prognosis. Moreover, the total incidence of adverse events in the Cinobufacini group was significantly lower than in the CF group (60.9% vs 82.0%, *P*=0.009).

Conclusion: Cinobufacini therapy via HAI is a viable strategy for curing advanced HCC with MVI, due to prolonged survival and a superior safety profile.

Keywords: hepatocellular carcinoma, macrovascular invasion, cinobufacini, hepatic arterial infusion, efficacy

Introduction

Hepatocellular carcinoma (HCC), a common malignancy, is the fifth incidence of malignant tumors and the third leading cause of tumor-related mortality globally with an increase of more than one million annually diagnosed cases.[1] It is highlighted that approximately half of the new patients with HCC worldwide are Chinese.[2] Further study on the current

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status of HCC in China demonstrates that the majority of HCC patients with the initial diagnosis are already at intermediate or advanced stages of the disease and no longer suitable for surgical resection.[3] Tumoral macrovascular invasion (MVI) is common during the natural process of advanced HCC, which mainly originates from a nest of malignant cells in vessels, develops to invade the portal vein system and forms portal vein tumor thrombosis.[4] HCC with MVI is usually featured by poor liver function, vulnerability to metastasis, more occurrence of complications, and refractory disease for treatment. MVI becomes an independent factor of poor HCC prognosis.[5] Although massive efforts, such as local and systemic therapy, have been exerted to cure the disease, the clinical efficacy is still limited. Therefore, studies on improving therapy for HCC and MVI are urgently needed.

Therapy via hepatic arterial infusion (HAI) is a traditional treatment approach for HCC at advanced stage, which could deliver drugs directly into the blood supply vessels of the tumor, and reduce systemic toxicities through a significant first-pass metabolism in the liver.[6] Evidence of clinical application is gradually accumulating which indicates the curative benefit, and HAI is considered as an effective locoregional treatment for advanced HCC. Recently, HAI has been used in advanced HCC as clinical guidelines by the Chinese Society of Clinical Oncology, and Japan Society of Hepatology.[7,8] Moreover, HAI is recommended in the current Pan-Asian Adapted ESMO Guidelines, as the first-line option for advanced and non-metastatic HCC with MVI.[9] The HAI regimen of platinum-based anticancer drugs provides encouraging therapeutic efficacy in advanced HCC, which results in favorable survival time, high response rate, and enhanced quality of life according to several clinical trials in population with high hepatic tumor burdens.[10–12] Therefore, the strategy of HAI with platinum-based regimens is considered as an alternative therapeutic option for advanced HCC in Asia by the Medical Society Guidelines. However, side effects are still the significant concerns, especially in platinum-related toxicity.

Cinobufacini, an active water-soluble extract from the dried skins of the Bufo bufo gargarizans Cantor, is a well-known traditional Chinese medicine utilized extensively for anticancer treatment in China, Japan, Korea, and other Asian countries.[13,14] It has been reported that Cinobufacini could increase therapeutic efficacy in liver, pancreatic, colon, and lung cancers, improve immunomodulation of patients, and diminish adverse events.[15] Experimental studies have indicated that Cinobufacini has a significant inhibited effect on numerous cancer cells through apoptosis.[16,17] Our previous study revealed that Cinobufacini could enhance the pharmacological effect on transarterial chemoembolization-treated HCC patients with an increase in prealbumin, as well as a reduction in VEGF levels.[18] However, the therapeutic efficacy of Cinobufacini in advanced HCC has not been investigated as yet.

In the present study, Cinobufacini therapy via HAI was applied to cure advanced HCC with MVI in clinic. Moreover, therapeutic adverse events were strictly monitored to evaluate whether HAI with Cinobufacini could improve prognosis and be a viable strategy of therapy for advanced HCC with MVI.

Methods

Study Design and Patients

This study was a multicenter retrospective review of the medical records of patients with unresectable advanced HCC treated with HAI regimens. Advanced HCC was diagnosed by radiographic image evaluation according to European Association for the Study of Liver Disease (EASL) Clinical Practice Guideline, and partly confirmed by pathological analyses in non-cirrhotic patients. Moreover, MVI without extrahepatic spread was determined by contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI). MVI was defined as portal vein tumor thrombosis, including Vp1-2 (distal to or in the second-order branches of the portal vein invasion), Vp3 (primary branch portal vein invasion), and Vp4 (main portal vein invasion), at the beginning of baseline radiographic assessments. Patients were excluded if they had hepatic decompensation, Eastern Cooperative Oncology Group (ECOG) performance status >2, extrahepatic metastasis, or other invasive malignant diseases.

All procedures of the studies performed were based on the ethical principles of the Declaration of Helsinki. The Institutional Review Board approved the protocol (Application for Approval of Research Protocol No. 2019-15). Written informed consent for the participants in this retrospective study was not required due to the feature of the retrospective data collection and the analysis. Finally, 130 patients diagnosed with unresectable advanced HCC and MVI fulfilled the entry criteria in the study between January 2014 and August 2019 from three institutions, including First People's Hospital Affiliated to Huzhou

University, Huzhou Traditional Chinese Medicine Hospital Affiliated to Zhejiang Chinese Medical University and Mingzhou Hospital.

HAI Treatment and Follow-Up

HAI was performed by guiding an implanted catheter through the femoral artery into the common hepatic artery, or the proper hepatic artery, and consecutive injection of drug by Seldinger method, using an implantable port assisted system for HAI. Antiemetic regimen of ondansetron plus dexamethasone was used according to researcher's discretion. Protection against cisplatin-induced kidney injury was administration of adequate hydration or diuretics. The cisplatin dose was reduced in appearance of grade 4 adverse events or events that had developed seriously during the initial treatment cycle. Patients were followed every two cycles of HAI treatment. Each follow-up performance included a detailed record and physical examination, laboratory tests of hematologic and biochemical analyses, and abdominal contrast-enhanced CT or MRI scan.

Therapeutic Drug Regimen

The patients were administered Cinobufacini injection (1 mg/mL, manufactured by Anhui Jinchan Biochemistry Company Ltd) with a dose of 40 mL/m^2 for 3 h on 5 days in the Cinobufacini group cohort. Cisplatin with a dose of 60 mg/m^2 for 2 h on 1 day and 5-fluorouracil (5-FU) with a dose of 500 mg/m^2 for 5 h on following three consecutive days were used in the cisplatin plus 5-FU (CF) group cohort. If Child-Pugh status maintained in grade A or B, patients underwent sustained regimen as a treatment cycle every 3 weeks until the appearance of tumor progression, severe adverse events, or patient refusal to continue.

Tumor Response Assessment

Baseline tumor evaluation was obtained within 1 week before treatment using contrast-enhanced CT or MRI as measured with the longest diameters of the tumor. Radiographic assessment was conducted 4–6 weeks after the beginning of treatment cycle and every 2–3 months during the follow-up. Evaluation of tumor response and post-hoc analysis were performed based on the modified Response Evaluation Criteria in Solid Tumors (mRECIST), including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The longest diameter of tumor on axial images was obtained for mRECIST. Overall survival (OS) was defined as the time from the beginning of the HAI treatment to the death from any cause. Progression-free survival (PFS) was defined as the time from the beginning of the HAI treatment to the diagnosis of PD or death. Objective response rate (ORR) was defined as the percentage of CR plus PR, and disease control rate (DCR) was defined as the percentage of ORR plus SD in all patients.

Evaluation of Adverse Events

Patients were evaluated for adverse events every 1 week during the study and at 2-month intervals during follow-up. Adverse events were evaluated based on subjective and objective symptoms and graded according to the standards of the Common Terminology Criteria for Adverse Events (CTCAE) in version 4.0.

Statistical Analysis

Continuous data was expressed as mean±SD or as median with range and analyzed using the Mann–Whitney *u*-test or Student's *t*-test. Categorical data were recorded as numerical values and percentages and analyzed using the rank sum test. Survival curves were estimated using the Kaplan–Meier method and were compared using the Log rank test. The Cox proportional hazards model was used to analyze hazard ratios (HRs) and 95% CIs for survival or efficacy. Statistical analyses were conducted by SPSS 21.0 software. A value of P <0.05 was accepted as statistically significant.

Results

Patient Characteristics

A total of 130 patients were involved in the retrospective cohort study (Figure 1), with 69 patients in the Cinobufacini group and 61 patients in the CF group. As shown in Table 1, the Cinobufacini group included 61 (88.7%) men and 8 (11.3%) women, with

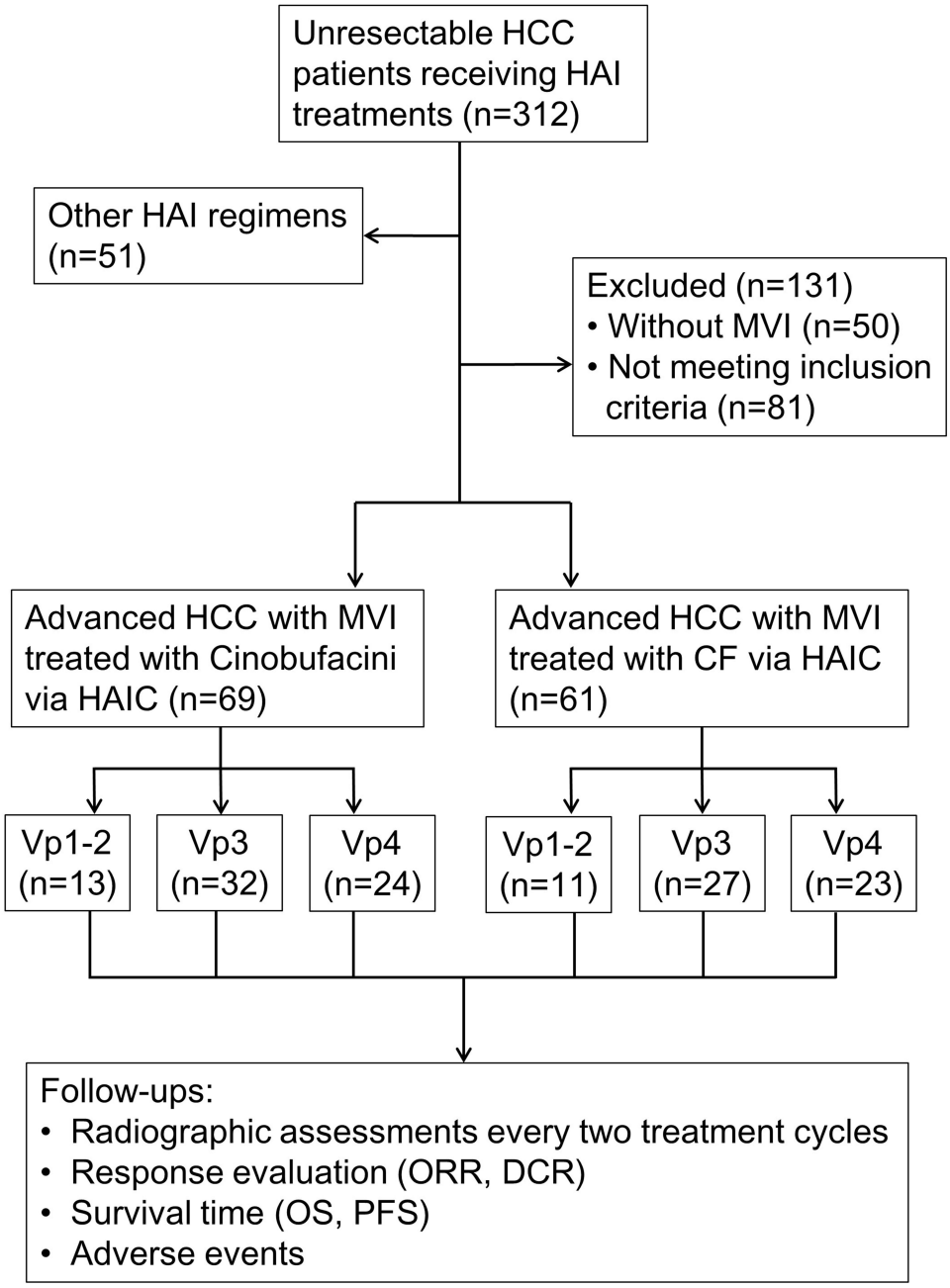

Figure 1 Flow diagram of the filtering process and study design. From the 312 reviewed patients with unresectable HCC receiving HAI treatment, we excluded the patients who were not employed for Cinobufacini or CF treatment regimen, without MVI and not meeting inclusion criteria in other conditions. 69 patients of Vp1-Vp4 invasion with Cinobufacini treatment, and 61 patients of Vp1-Vp4 invasion with CF treatment were selected for further analyses of follow-ups.
Abbreviations: HCC, hepatocellular carcinoma; MVI, macrovascular invasion; HAI, hepatic arterial infusion; CF, Cisplatin plus 5-FU; ORR, objective response rate; DCR, disease control rate; OS, overall survival; PFS, progression-free survival.

a mean age of 53.2±13.1 years old. Of the 69 patients, 49 (71.0%) belonged to Child-Pugh Grade A and 20 (29.0%) belonged to Child-Pugh Grade B. ECOG scores of 0, 1, and 2 were noted in 29 (42.0%), 31 (45.0%), and 9 (13.0%) patients, respectively. Chronic HBV infection was the main underlying etiology of HCC (n=60, 87.0%), followed by chronic HCV infection (n=5, 7.2%). The mean liver tumor size was 9.1±4.1 cm. There were 34 (49.3%) patients with fewer than 3 intrahepatic tumors and 35 (50.7%) patients with more than 3. The number of patients with Vp1-2 was 13 (18.8%), Vp3 was 32 (46.4%), and those with Vp4 was 24 (34.8%). The median value of lower AFP level (≤1000 ng/mL) was 719.5 ng/mL (range from 110.5 to 990.1 ng/mL), the median value of higher AFP level (>1000 ng/mL) was 5663.5 ng/mL (range from 1056.6 to 10,235.8 ng/mL).

Table 1 Baseline Characteristics of Patients in Entire Cohort Study

Features	Cinobufacini Group (n=69)	CF Group (n=61)	*P* value
Age	53.2±13.1	56.8±13.9	0.531
Gender			0.431
Male	61 (88.7%)	51 (83.3%)	
Female	8 (11.3%)	10 (16.7%)	
Child-Pugh classification			0.574
Grade A	49 (71.0%)	46 (75.4%)	
Grade B	20 (29.0%)	15 (24.6%)	
ECOG score			0.682
0	29 (42.0%)	23 (37.7%)	
1	31 (45.0%)	30 (49.2%)	
2	9 (13.0%)	8 (13.1%)	
Etiology			0.846
HBV infection	60 (87.0%)	55 (87.5%)	
HCV infection	5 (7.2%)	4 (6.5%)	
Tumor size (cm)	9.1±4.1	9.3±3.9	0.863
Number of intrahepatic tumors			0.449
≤ 3	34 (49.3%)	26 (42.6%)	
>3	35 (50.7%)	35 (57.4%)	
Portal vein invasion			0.758
Vp1-2	13 (18.8%)	11 (18.0%)	
Vp3	32 (46.4%)	27 (44.3%)	
Vp4	24 (34.8%)	23 (37.7%)	
AFP (ng/mL)			0.636
≤1000	719.5 (110.5–990.1)	743.6 (98.3–998.6)	
>1000	5663.5 (1056.6–10,235.8)	7332.4 (1243.3–14,387.9)	

Abbreviations: CF, Cisplatin plus 5-FU; ECOG, Eastern Cooperative Oncology Group; HBV, Hepatitis B virus; HCV, Hepatitis C virus; AFP, alpha-Fetoprotein.

Meanwhile, the CF group included 51 (83.3%) men and 10 (16.7%) women, with a mean age of 56.8±13.9 years old. Of the 61 patients, 46 (75.4%) belonged to Child-Pugh Grade A and 15 (24.6%) belonged to Child-Pugh Grade B. ECOG score of 0, 1, and 2 were noted in 23 (37.7%), 30 (49.2%), and 8 (13.1%) patients, respectively. Chronic HBV infection was also the main underlying etiology of HCC (n=55; 87.5%), followed by a chronic HCV infection (n=4; 6.5%). The mean liver tumor size was 9.3±3.9 cm. There were 26 (42.6%) patients with fewer than 3 intrahepatic tumors and 35 (57.4%) patients with more than 3. The number of patients with Vp1-2 was 11 (18.0%), Vp3 was 27 (44.3%), and those with Vp4 was 23 (37.7%). The median value of lower AFP level (≤1000 ng/mL) was 743.6 ng/mL (range from 98.3 to 998.6 ng/mL), the median value of higher AFP level (>1000 ng/mL) was 7332.4 ng/mL (range from 1243.3 to 14,387.9 ng/mL). Overall, the clinical features were not significantly different between the two groups.

Efficacy in Response Rate

The results of response rate with radiologic assessment are shown in Table 2 based on mRECIST criteria by post-hoc analysis. Of the 69 patients in Cinobufacini group, CR, PR, SD, ORR, and DCR were 1 (1.4%), 30 (43.5%), 18 (26.1%), 31 (44.9%), and 49 (71.0%), respectively. Meanwhile, of the 61 patients in the CF group, CR, PR, SD ORR, and DCR were 1 (1.6%), 16 (26.2%), 20 (32.8%), 17 (27.9%), and 37 (60.6%), respectively. Noticeably, PR and ORR were significantly higher in the Cinobufacini group than those in the CF group ($P<0.05$).

Efficacy in Overall Survival

As shown in Figure 2A, the median OS of patients in the Cinobufacini and CF groups was 14.8 months (95% CI: 10.1–21.7) and 11.1 months (95% CI: 7.6–16.3), respectively ($P=0.010$). Noticeably, in the subgroup of portal vein

Table 2 Therapeutic Effects in All Patients

Therapeutic Effects	Cinobufacini Group (n=69)	CF Group (n=61)	*P* value
CR	1 (1.4%)	1 (1.6%)	0.930
PR	30 (43.5%)	16 (26.2%)	0.041
SD	18 (26.1%)	20 (32.8%)	0.404
PD	17 (24.6%)	23 (37.7%)	0.109
ORR	31 (44.9%)	17 (27.9%)	0.045
DCR	49 (71.0%)	37 (60.6%)	0.215

Notes: ORR is equal to percentage of CR plus PR, and DCR is equal to percentage of SD plus CR and PR. CR is number of patients with complete response, PR is number of patients with partial response, SD is number of patients with stable disease, and PD is number of patients with progressive disease.
Abbreviations: CF, Cisplatin plus 5-FU; CR, Complete response; PR, Partial response; SD, Stable disease; PD, Progressive disease; ORR, Objective response rate; DCR, Disease control rate.

invasion grade, classified patients with Vp1-2 in the Cinobufacini group benefited in a longer median OS of 18.3 months (95% CI: 6.6–42.9) than those of 14.3 months (95% CI: 5.2–33.8; *P*=0.043) in the CF group (Figure 2B). Similarly, in patients with Vp3, a longer median OS of 15.0 months (95% CI: 8.9–26.9) was also observed in the

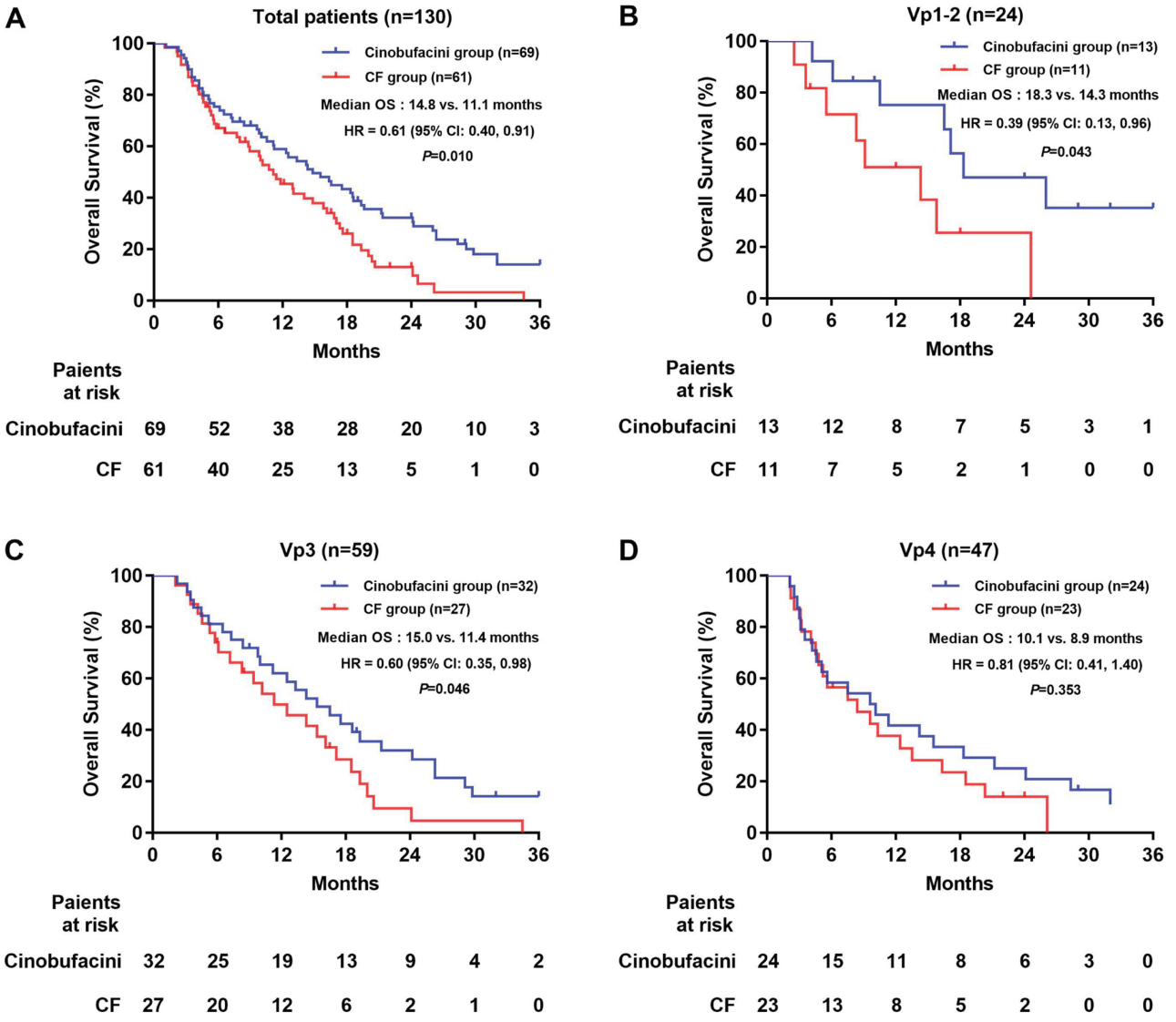

Figure 2 Kaplan-Meier analysis of overall survival in patients treated with Cinobufacini or CF via HAI. (**A**) Total patients; (**B**) Patients with Vp1-2; (**C**) Patients with Vp3; (**D**) Patients with Vp4.
Abbreviations: HR, hazard ratio; CI, confidence interval; CF, Cisplatin plus 5-FU.

Cinobufacini group compared with those of 11.4 months (95% CI: 6.4–19.3; *P*=0.046) in the CF group (Figure 2C). However, Cinobufacini treatment did not significantly prolong the median OS of patients with Vp4 compared with CF treatment, which were 10.1 months (95% CI: 5.7–19.3) and 8.9 months (95% CI: 4.7–15.9), respectively (*P*=0.353) (Figure 2D).

Efficacy in Progression-Free Survival

The patients in the Cinobufacini group achieved a longer median PFS of 10.3 months (95% CI: 7.1–14.6) than those of 6.0 months (95% CI: 4.3–8.9; *P*=0.006) in the CF group (Figure 3A). Patients with Vp1-2 in the Cinobufacini group also benefited in a longer median PFS of 14.8 months (95% CI: 6.4–36.8) compared with those of 10.2 months (95% CI: 4.1–23.7; *P*=0.028) in the CF group (Figure 3B). Similarly, the median PFS of patients with Vp3 patients in the Cinobufacini and CF groups was 10.8 months (95% CI: 5.9–17.6) and 6.6 months (95% CI: 4.0–12.1), respectively (*P*=0.033) (Figure 3C). PFS results of patients with Vp4 were relatively similar between the two groups. The median PFS of

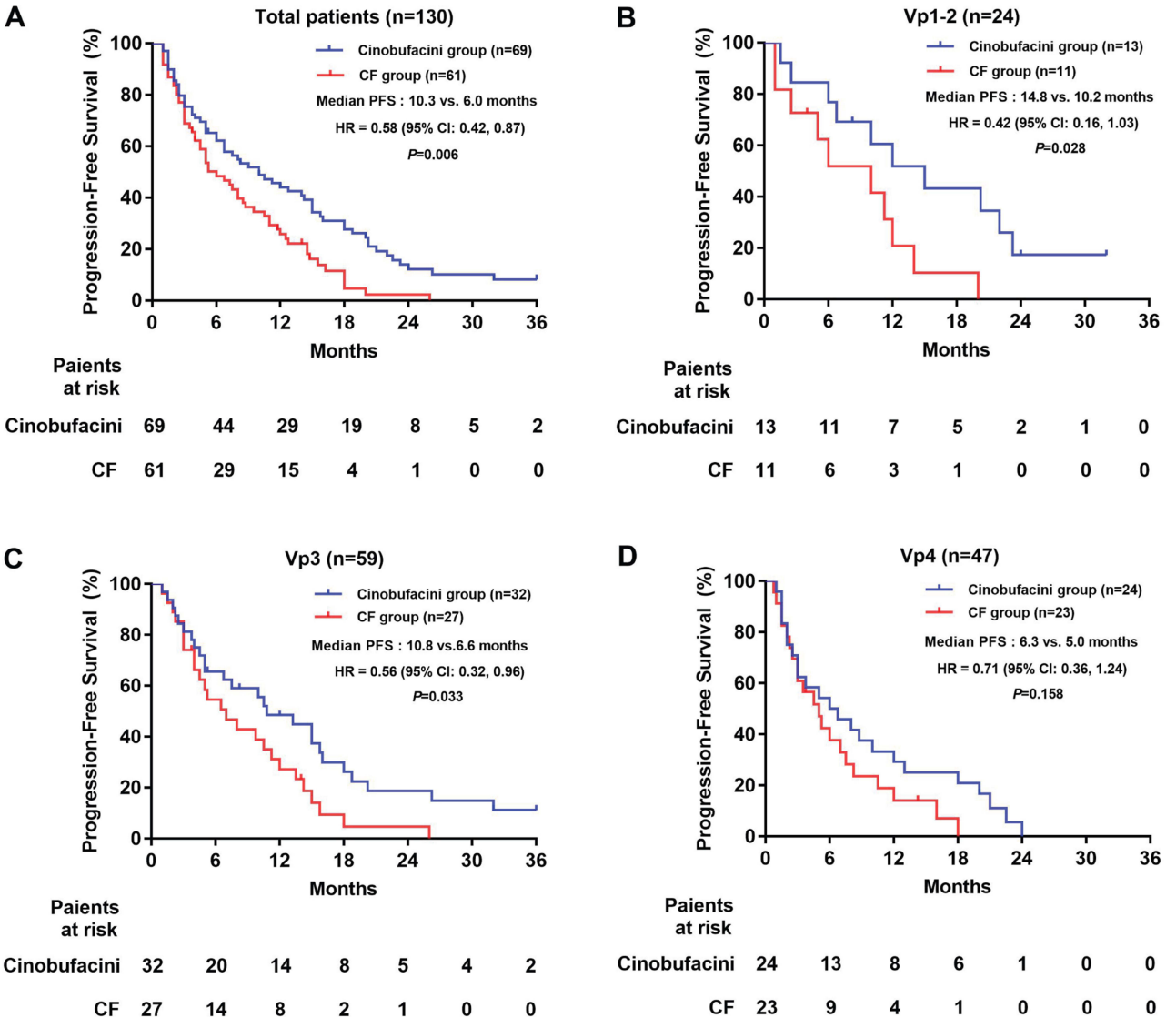

Figure 3 Kaplan-Meier analysis of progression-free survival in patients treated with Cinobufacini or CF via HAI. (**A**) Total patients; (**B**) Patients with Vp1-2; (**C**) Patients with Vp3; (**D**) Patients with Vp4.

Abbreviations: HR, hazard ratio; CI, confidence interval; CF, Cisplatin plus 5-FU.

Vp4 patients in the Cinobufacini group was 6.3 months (95% CI: 3.5–11.5), while in the CF group was 5.0 months (95% CI: 2.7–8.9), without statistical significance (P=0.158) (Figure 3D).

Cox Proportional Hazards Model Analysis

The longer OS in the Cinobufacini group (HR, 0.61; 95% CI: 0.40–0.91; P=0.010) was confirmed by the Cox proportional hazards model analysis, compared with the CF group. Moreover, factors associated with OS in forest plot analysis revealed that HAI with Cinobufacini indicated a benefit over CF in majority of subgroup, except in classified patients with more than three intrahepatic tumors, which was in favor of the CF treatment (Figure 4). Four baseline characteristics of patients were identified as prognostic indicators for OS by univariate analysis of survival, including tumor size, portal vein invasion grade, AFP, and regimen. Multivariate analysis confirmed portal vein invasion grade (Vp4; HR, 1.78; 95% CI: 1.03–2.16; P=0.032) and AFP (>1000; HR, 1.61; 95% CI: 1.08–1.91; P=0.039) as the independent factors for prognosis (Table 3).

Adverse Events

Cinobufacini and CF treatment-related adverse events are indicated in Table 4. The total incidence of adverse events was 60.9% and 82.0% in Cinobufacini and CF groups, respectively. In addition, the following occurrence rate of grade 3 or 4 adverse events was much lower in the Cinobufacini group (10.1%, n=7) than in the CF group (39.3%, n=24), especially in fatigue (0% vs 9.8%), thrombocytopenia (1.4% vs 16.4%), leukopenia (1.4% vs 14.8%), diarrhea (1.4% vs 14.8%), and abnormal liver function (4.3% vs 26.2%). Researchers tried to support and improve each patient's condition, when adverse events occurred. No treatment-related mortality was observed in either group.

Discussion

Currently, the standard treatment for advanced HCC is the administration of molecular targeting inhibitors, such as sorafenib and lenvatinib, which have achieved general evidence-based consensus.[19] However, the survival time of patients with highly progressed tumor conditions treated with molecular targeting inhibitors is still limited, and the cost is relatively expensive. Therefore, more effective and feasible therapies are needed to explore for advanced HCC.

MVI is present in advanced HCC approximately 10–60% of patients at diagnosis.[20] Portal vein tumor thrombosis is the most common form of MVI, which is divided into 4 types from Vp1 to Vp4, according to the severity of tumor thrombosis and anatomic structures of the portal vein.[21] The growth pattern of MVI is predominantly characterized by invasion from intrahepatic nodules that progresses toward the central trunk, and its occurrence indicates tumor spread in the liver, resulting in a reduction in patient survival.[10,22] In addition to standard treatment of molecular targeting inhibitors for advanced HCC with MVI, alternative treatment strategies such as radiotherapy (RT), transarterial chemoembolization (TACE), and radiofrequency ablation (RFA) have also been used to improve the therapeutic effects on selected patients. Noticeably, HAI is another such treatment that can deliver high concentrations of anticancer drugs directly through hepatic artery to the liver, representing a specific and beneficial treatment approach for advanced HCC patients with MVI.[23] Various chemotherapy regimens are applied for HAI, and the combined treatment of cisplatin with 5-FU is one of the common regimens.[24,25] Sustained HAI using cisplatin and 5-FU has been proven to be effective in the therapy of advanced HCC and portal vein tumor thrombosis, resulting in the ORR of 24.5–71%, the median OS of 6.0–15.9 months, and the median PFS of 4.1–7.0 months.[26] Our results were consistent with the abovementioned study, treatment with cisplatin combined with 5-FU demonstrated effects on the ORR of 27.9%, the median OS of 11.1 months, and the median PFS of 6.0 months.

Cinobufacini is a traditional animal-derived medicine utilized in China for a long history. Compared with chemical medicine, there is increasing evidence suggesting advantages of natural product-related drugs applied in traditional Chinese medicine and active constituents extracted from medicinal animals and plants. Moreover, Cinobufacini injection has been approved by the Chinese State Food and Drug Administration (SFDA) since 2005 and extensively accepted to cure cancer patients in oncology departments. An increasing number of studies have indicated its efficacy both in vitro and in vivo, and it is reported that Cinobufacini inhibited metastasis and invasion of HepG2 cells by suppressing epithelial–mesenchymal transition through downregulation of the expression of phosphorylated-c-Met.[27] Another

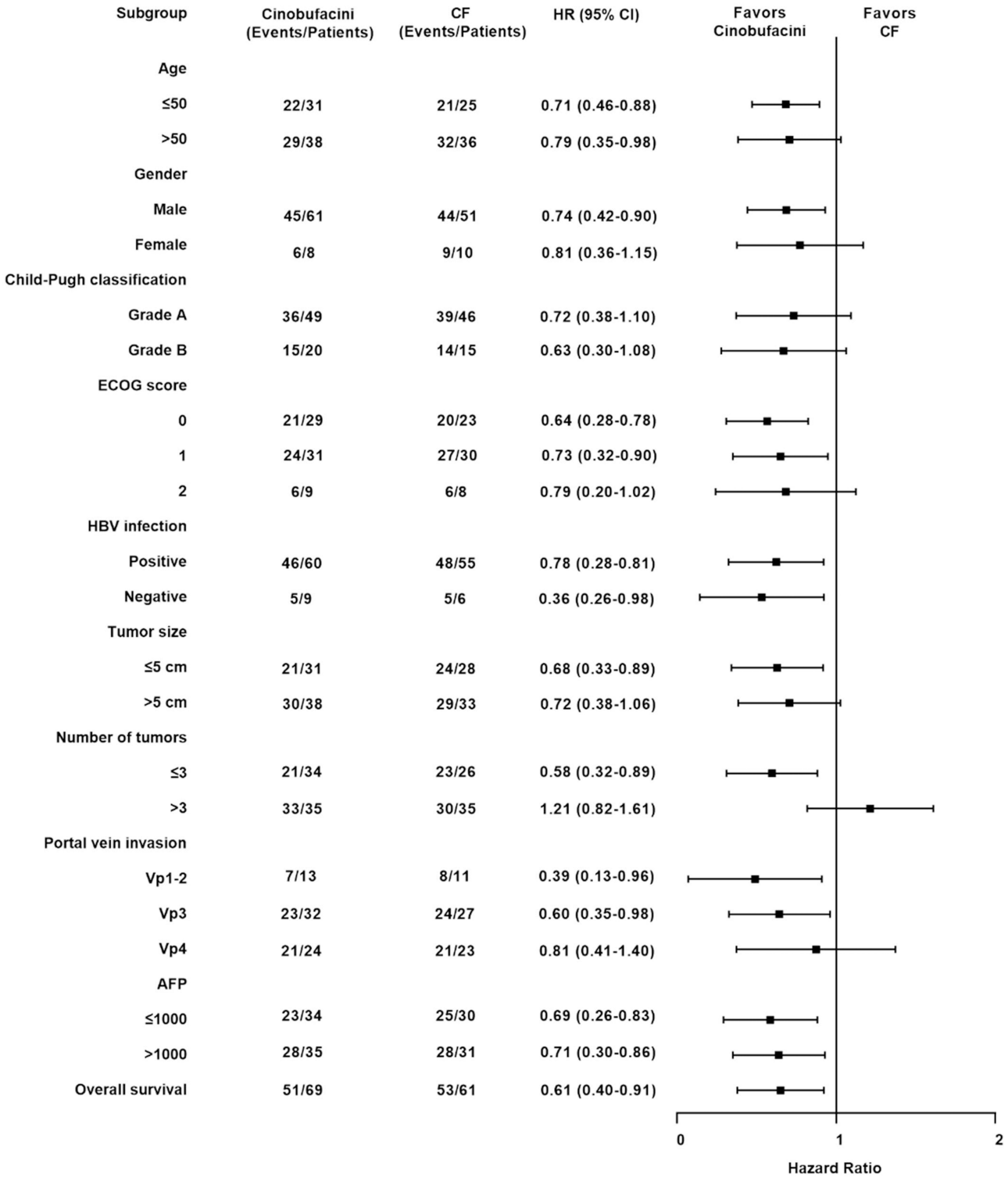

Figure 4 Forest plot analysis of factors associated with overall survival in patients who received Cinobufacini or CF treatment via HAI.
Abbreviations: HR, hazard ratio; CI, confidence interval; CF, Cisplatin plus 5-FU; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; AFP, alpha-fetoprotein.

research studied the underlying mechanism of inhibition induced by Cinobufacini in Bel-7402 cells, and the results demonstrated that Cinobufacini could induce apoptosis of HCC cells through caspase-related pathway by mediation of mitochondria and Fas with increasing treatment time.[28] A trial study has further shown the anticancer activity of Cinobufacini in enormous HCC in clinic.[29] The combination of Cinobufacini with conventional chemotherapy regimens

Table 3 Univariate and Multivariate Analyses of Overall Survival

Variable	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age (> 55/≤ 55 years)	1.15	0.63–1.67	0.385			
Gender (Male/Female)	1.12	0.46–1.59	0.434			
Child-Pugh (A/B)	0.78	0.51–1.45	0.687			
ECOG (1–2/0)	1.08	0.58–1.83	0.725			
HBV (±)	1.31	0.98–1.90	0.068			
Tumor size (> 5/≤ 5 cm)	1.73	1.05–2.12	0.036	1.35	1.21, 1.81	0.189
Number of intrahepatic tumors (> 3/≤ 3)	1.24	0.75–1.72	0.325			
Portal vein invasion						
Vp3/Vp1-2	1.81	1.21–2.56	0.010	1.43	1.02, 1.93	0.063
Vp4/Vp1-2	2.01	1.18–2.79	0.005	1.78	1.03, 2.16	0.032
AFP (> 1000/ ≤ 1000 ng/mL)	1.89	1.25–2.68	0.008	1.61	1.08, 1.91	0.039
Regimen (Cinobufacini/CF)	0.68	0.38–0.93	0.010	0.71	0.42, 1.06	0.089

Notes: The univariate and multivariate analyses were performed using Cox proportional hazards model.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; AFP, alpha-fetoprotein; CF, cisplatin plus 5-FU.

Table 4 Treatment-Related Adverse Events

Adverse Events	Cinobufacini Group (n=69)		CF Group (n=61)		*P* value	
	Any Grade (%)	Grade 3–4 (%)	Any Grade (%)	Grade 3–4 (%)	Any Grade	Grade 3–4
Total incidence	42 (60.9%)	7 (10.1%)	50 (82.0%)	24 (39.3%)	0.009	<0.001
Constitutional symptoms						
Fatigue	23 (33.3%)	0 (0)	27 (44.3%)	6 (9.8%)	0.203	0.008
Fever	4 (5.8%)	1 (1.4%)	9 (14.8%)	4 (6.6%)	0.091	0.132
Weight loss	16 (23.2%)	0 (0)	20 (32.8%)	1 (1.6%)	0.224	0.288
Hematologic system						
Thrombocytopenia	13 (18.8%)	1 (1.4%)	38 (62.3%)	10 (16.4%)	<0.001	0.002
Neutropenia	7 (10.1%)	1 (1.4%)	20 (32.8%)	4 (6.6%)	0.002	0.132
Leukopenia	12 (17.4%)	1 (1.4%)	29 (47.5%)	9 (14.8%)	<0.001	0.005
Anemia	7 (10.1%)	0 (0)	13 (21.3%)	4 (6.6%)	0.079	0.031
Gastrointestinal events						
Diarrhea	7 (10.1%)	1 (1.4%)	19 (31.1%)	9 (14.8%)	0.003	0.005
Abdominal pain	10 (14.5%)	1 (1.4%)	25 (41.0%)	4 (6.6%)	0.001	0.132
Nausea/Vomiting	4 (5.8%)	0 (0)	23 (37.7%)	4 (6.6%)	<0.001	0.031
Neurotoxicity	3 (4.3%)	0 (0)	20 (32.8%)	1 (1.6%)	<0.001	0.288
Nephrotoxicity	4 (5.8%)	0 (0)	32 (52.5%)	1 (1.6%)	<0.001	0.288
Abnormal liver function	16 (23.2%)	3 (4.3%)	37 (60.7%)	16 (26.2%)	<0.001	<0.001

Abbreviation: CF, Cisplatin plus 5-FU.

could improve patient immunity and reduce toxic effects.[30] In addition, Cinobufacini has been proven to prevent recurrence after resection of small HCC.[31] The anticancer activity of Cinobufacini observed in this study may be related to these aforementioned experimental and clinical results, as well as the water-soluble characteristic of implantable port-assisted systems for sustained infusion.

In the present study, the utilization of Cinobufacini showed good prospects in the therapy of advanced HCC with MVI since it had advantages of significant pharmacological effects and fewer side effects. Compared with cisplatin plus 5-FU treatment, Cinobufacini treatment demonstrated more effective therapeutic results in 17% increase of the ORR, 39% lower hazards of mortality, and 42% lower hazards of disease progression. In the subgroup analysis of portal vein

invasion grade, Cinobufacini treatment indicated remarkable therapeutic effects on Vp1-2 and Vp3, including the median OS and the median PFS. However, in contrast, the results of survival time had no significant differences in the patients with Vp4 between the two groups. This might be due to a higher refractory status and later stage of the tumor in Vp4 grade. Meanwhile, the presence of patients with multiple intrahepatic tumors indicated early metastases in HCC, platinum-based anticancer drugs exerted advantages in treating the aggressiveness of liver cancer.[32] Consequently, it was the possible cause of Cinobufacini treatment did not achieve a benefit over CF treatment in subgroup of patients with more than three intrahepatic tumors. Safety is an important concern in the treatment, because the main condition is that patients with advanced HCC are always accompanied by hypohepatia. As the sword has two edges, drugs of chemotherapy regimens used via HAI may further induce liver injury and cause adverse events. Here in the study, the categories and incidence of adverse events in the HAI regimen of the cisplatin plus 5-FU group were similar to those reported in previous studies,[11,33] including constitutional symptoms, hematologic system, gastrointestinal events, and liver function. The total incidence of grade 3–4 adverse events in the HAI regimen of the Cinobufacini group was much lower than that in the cisplatin plus 5-FU group, further suggesting the advantage in safety and efficacy of HAI with Cinobufacini as a treatment approach for advanced HCC with MVI. In addition, no treatment-related mortality was detected in the study. Taken together, application of Cinobufacini via HAI is an effective locoregional treatment with prominent effects on anticancer and liver function protection, resulting in increased therapeutic efficacy and reduced adverse effects.

Conclusions

The study provided strong evidence that Cinobufacini utilized as traditional Chinese medicine exhibited considerable therapeutic effects on advanced HCC with MVI. HAI with Cinobufacini improved the ORR, and prolonged OS and PFS compared with cisplatin plus 5-FU, especially in patients with Vp1-2 and Vp3. This therapy is effective, safe, and has fewer adverse events than platinum-based chemotherapy conventionally used in clinic. Consequently, application of Cinobufacini is a promising and viable strategy for the treatment of advanced HCC with MVI, and our investigation sheds light on a better understanding of the disease. The potential mechanism of the therapeutic effect of Cinobufacini requires further study.

Data Sharing Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request for further studies.

Ethics Approval

This study protocol was approved by the Institutional Review Board of the First People's Hospital Affiliated to Huzhou University (Application for Approval of Research Protocol No. 2019-15) and conducted in accordance with the Declaration of Helsinki in 2013.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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