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Unresectable hepatocellular carcinoma: transarterial chemoembolisation plus Huachansu – a single-center randomised controlled trial

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ABSTRACT

Objective Huachansu, a Chinese medicine derived from the dried skin glands of toad venom, has been used in China since the 1970s to treat liver cancer. Transarterial chemoembolisation (TACE) is the standard of care for patients with unresectable hepatocellular carcinoma (HCC). This study evaluated the efficacy and safety of the combination of TACE and Huachansu in unresectable HCC.

Methods From September 2012 to September 2016, 120 patients diagnosed with unresectable HCC were prospectively enrolled. Patients were randomised at a 1:1 ratio into the combined treatment group (Huachansu–TACE) and the TACE treatment group. The primary endpoint was progression-free survival (PFS) and secondary endpoints were overall survival (OS) and safety. The exploration outcome serum Na^+/K^+-ATPase (NKA) α3 at baseline and 3-month follow-ups were compared for a prognostic role. All patients were subjected to 36-month follow-up.

Results A total of 112 patients who completed the study were included in the analysis. PFS and OS were significantly better in the Huachansu–TACE group than in the TACE group (p=0.029 and p=0.025, respectively), with a median PFS of 6.8 and 5.3; and a median OS of 14.8 months and 10.7 months, respectively. Although no prognostic significance was found between the baseline NKA-low and NKA-high groups in the patients' OS (p=0.48), its changes after 3-month follow-up showed significant prognostic values, of which, were 8.5 months and 23.8 months, respectively (p<0.001). Treatment-related adverse events were comparable between groups.

Conclusions Huachansu–TACE is effective in prolonging the PFS and OS in patients with unresectable HCC.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Unresectable hepatocellular carcinoma (HCC) is a highly lethal disease with few treatment options. There is a lack of high-quality prospective randomised controlled trials evaluating the therapeutic efficacy of Huachansu in patients with unresectable diseases.

WHAT THIS STUDY ADDS

⇒ This study purposed a new treatment approach of Huachansu combined with transarterial chemoembolisation (TACE). The median progression-free survival of the Huachansu–TACE group was 6.8 months and the median overall survival was 14.8 months, similar to the clinical trials of targeted drugs (sorafenib or sunitinib) combined with TACE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Patients with lowered Na^+/K^+-ATPase (NKA) α3 from baseline at 3-month follow-up had significantly prolonged survival and changes in NKA expression could be used as a predictor of treatment efficacy in advanced HCC.

Trial registration number NCT01715532.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related death worldwide, and China is a country with a high incidence of HCC.[1] For patients without indications for curative treatment such as surgical resection, liver transplantation or local ablation, the 5-year survival rate remains abysmal.[2]

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/spcare-2022-003870).

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Received 11 August 2022
Accepted 8 March 2023

Check for updates

To cite: Gao H, He J, Cheng C, *et al*. *BMJ Supportive & Palliative Care* Epub ahead of print: [please include Day Month Year]. doi:10.1136/spcare-2022-003870

In patients with unresectable HCC and normal liver function (Barcelona Clinic Liver Cancer staging system stage B (BCLC stage B)), transarterial chemoembolisation (TACE) is the first-line therapy.[3 4] Although sorafenib is commonly used as systemic therapy, there is no standard treatment guideline for HCC patients with BCLC stage C liver function.[5]

TACE is commonly used in the palliative care of patients diagnosed with BCLC stage C HCC in China.[6] Previous studies have shown that TACE intervention significantly improves survival unresectable HCC patients compared with systemic chemotherapy or supportive care.[7] However, the recurrence rate remains high after TACE treatment, and the long-term survival rate is unsatisfactory.[8 9] Several studies have investigated TACE combination therapy, such as sorafenib, lenvatinib or internal radiotherapy, all of which have demonstrated an improved overall therapeutic effect of the combination therapy in patients with unresectable HCC.[10–12] More recent studies are increasingly focusing on the combination of TACE and immunotherapy.[13 14] However, the high treatment costs and resistance to therapy have limited the combination of targeted drugs and immunotherapy with TACE for the treatment of advanced HCC, especially in underdeveloped or developing countries.[15–17] For patients with unresectable HCC, improving the efficacy of TACE and finding effective combinations remain major clinical challenges.

Huachansu is a Chinese medicine derived from the dry skin glands of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider.[18] Clinical trials conducted in China since the 1970s have repeatedly demonstrated anticancer activity and can prolong the survival time of patients in various cancers.[19 20] As a potentially targeted drug of Na^+/K^+-ATPase (NKA), a versatile signal transducer of aberrant cell proliferation and adhesion, Huachansu has shown effects in apoptosis and autophagy inductions in HCC cells.[21] Our group previously conducted a pilot trial of Huachansu in 15 patients with advanced cancer using a phase I design. Of the included patients, 11 were diagnosed with HCC. The results demonstrated that six patients with HCC had stable disease with a median treatment duration of 6 months (range, 3.5–11.1 months).[22] However, there is currently a lack of high-quality, prospective randomised controlled trials (RCTs) investigating the effectiveness of Huachansu. A systematic review including 11 RCTs including 728 patients demonstrated that Huachansu may be of therapeutic benefit in patients with unresectable liver cancer[23]; however, the results should be interpreted with caution due to the low quality of the majority of the included trials.

The present study is a single-centre prospective RCT, aiming to investigate the efficacy and feasibility of Huachansu in combination with TACE for the treatment of unresectable HCC. The patients' progression-free survival (PFS) and overall survival (OS) and their correlation with the expression of NKA were studied. The findings were that the prognostic role of serum NKA expression could be used as a predictor of treatment efficacy in HCC. This study provides clinical evidence for the use of Huachansu combined with TACE in HCC patients with unresectable disease.

PATIENTS AND METHODS

Study design and sample size estimation

The present study is a prospective, single-centre, randomised, parallel, open-label, phase II study comparing PFS in patients with unresectable HCC treated with TACE with or without Huachansu. The study protocol was registered on https://www.clinicaltrials.gov (NCT01715532) on 17 August 2012.

Based on the relevant literature report, the median time-to-progression (TTP) time of patients with unresectable HCC was 5 months (range: 4–7 months)[24] and the treatment group was expected to prolong TTP by 50%. For superiority testing, we considered a two-sided α of 0.05, 80% power, SD of 20 and a follow-up loss of 20% in both arms. An estimated sample size of 120 cases was required to detect differences in PFS between the two arms.

Patients, study execution, randomisation and allocations

A total of 120 patients diagnosed with unresectable HCC according to the Diagnosing and Staging National Standards of China (2011) at the International Center of Integrative Oncology, Fudan University Cancer Hospital, from September 2012 to September 2016 were prospectively enrolled in this study. Treatment-naïve patients over 18 years of age and younger than 75 years with at least one imaging measurable lesion, Child-Pugh stage A or B, Eastern Cooperative Oncology Group performance status (ECOG PS)≤2, sufficient haematologic, hepatic and renal functions and signed informed consent were included. The detailed inclusion and exclusion criteria are shown in online supplemental table 1.

Following screening for eligibility and obtaining informed consent by one designated physician, eligible patients were enrolled by the research nurse. Registered patients were then assigned with a subject number using a computer-generated numbered randomisation list (http://www.randomization.com) for simple randomisation by another physician not participating in treatment and evaluation. Patients were allocated at a 1:1 ratio to the combined treatment group (Huachansu combined with TACE) and the TACE treatment group to receive the respective treatments by another designated physician. As an open-label study, no blinding was performed. The inclusion flow chart of patients is shown in figure 1.

TACE procedure

Both groups were treated with conventional TACE intervention according to the following protocol.

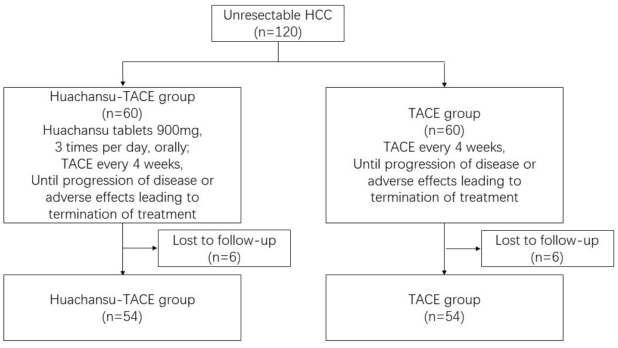

Figure 1 Inclusion flow chart of patients and treatment allocation. HCC, hepatocellular carcinoma; TACE, transarterial chemoembolisation.

Patients fasted for 4 hours prior to TACE. Before the procedure, an intravenous injection of dexamethasone (5 mg) was given. Under local anaesthesia, the right femoral artery was catheterised, followed by hepatic and superior mesenteric artery arteriography with digital subtraction angiography (DSA) to examine the size and location of tumour nodules. The tumour-feeding artery was super-selectively catheterised through the right or left hepatic artery, where appropriate. The 10 mL Lipiodol emulsions were mixed with 60 mg pirarubicin (THP, Shenzhen Main Luck Pharmaceuticals) and prepared by pumping two 10 mL syringes back-and-forth several times. Depending on the size of the tumour, up to 20 mL of pure Lipiodol may be injected to induce embolisation if necessary. All the emulsion and Lipiodol injection were performed and injected slowly at a steady rate under DSA supervision for any backflow and to ensure adequate delivery of the emulsion and Lipiodol to the tumour areas. For tumours in both hepatic lobes, emulsion and Lipiodol were injected through the distal proper hepatic artery or the gastroduodenal artery depending on the origin of the arterial vasculature of the individual tumour. TACE was repeated after 4 weeks. Treatment was withheld or terminated in the event of a vascular contraindication, compromised hepatic function, grade III or IV adverse events (AEs) or disease progression.

Huachansu treatment

Patients in the Huachansu–TACE treatment group received adjunctive treatment with open-label Huachansu, 1 day after TACE treatment. The dosage of Huachansu oral tablet was 900 mg, taken three times a day for 4 weeks in a treatment cycle. Dosage may be modified according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) V.3.0[25] in case of grade III or IV AEs. Continuous use of Huachansu tablets was recommended if the side effects self-relieve or improve after the intervention.

Assessments of outcome

The primary endpoint was PFS, and the second endpoint was OS. The exploration outcome is the prognostic role of serum NKA. The PFS was defined as the date of randomisation to the date of first documented date of disease progression or death. The OS was defined as the date of randomisation until the date of death. Patients were followed up monthly at the outpatient clinic until 1 September 2019. Serum biochemistry, serum alpha-fetoprotein (AFP) detection, serum NKA and CT or MRI scan were repeated monthly in the first trimester, then every 2 months. The serum NKA was measured using an ELISA according to the manufacturer's instructions (MBS2886186, MyBioSource, San Diego, California, USA) and a pre-established standard curve. All patient deaths were regarded as endpoints, regardless of the cause. Treatment-related death was defined as any death within 30 days of initial TACE therapy.

Statistical analysis

Statistical analyses were performed using the IBM SPSS V.22.0 statistical software and GraphPad Prism V.9.0 (GraphPad Software, USA). Continuous variables were described as the mean and SD for the baseline characteristics. Categorical variables were expressed as frequency and percentage. Student's t-test and the χ^2 test were used to compare the differences of the respective variables between groups, where appropriate. The Kaplan-Meier method was employed to determine the PFS and the OS using R (V.4.1.0) with the 'survival' package and the 'ggplot2' data visualisation package. Univariate and multivariate Cox regression analyses were also performed. Statistically significant variables ($p<0.05$) in the univariate analysis were further analysed by multivariate Cox regression to determine and identify risk factors of PFS. All statistical tests were two sided, and $p<0.05$ was considered to be statistically significant.

RESULTS

Study population

One hundred and twenty patients diagnosed with unresectable HCC at Fudan University Shanghai Cancer Center were prospectively included from September 2012 to September 2016. Twelve patients (six in each group) were excluded from further analysis due to lack of follow-up and no scheduled treatment after randomisation. Finally, 112 patients were included in the analysis; 54 patients received the combination therapy of Huachansu and TACE, while the remaining 54 patients received TACE. The baseline characteristics of the two groups were comparable without significant difference, as shown in table 1.

Treatment and follow-up

On the day of clinical data cut-off (30 September 2019), the median time of TACE procedures per

Table 1 Patient baseline characteristic

Characteristics	Huachansu–TACE (n=54)	TACE (n=54)	P value
Age, median±SD, years	53.65±10.1	52.94±10.69	0.725
Gender			0.053
Male	19	29	
Female	35	25	
HBV infection			0.678
Yes	52	54	
No	2	4	
Cirrhosis			0.540
Yes	47	49	
No	7	5	
Child-Pugh			0.839
A	35	36	
B	19	18	
AFP			0.661
<400 mg/mL	15	13	
≥400 mg/mL	39	41	
Na^+/K^+-ATPase α3, median±SD, U/mL	50.16±8.56	50.01±10.80	0.936
BCLC stage			0.494
B	11	14	
C	43	40	
ECOG PS			0.380
0–1	42	38	
2	12	16	
PVTT			0.661
Present	13	15	
Absent	41	39	
Ascites			0.407
Present	15	19	
Absent	39	35	

P<0.05 is considered statistically significant.

AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer staging system; ECOG PS, Eastern Cooperative Oncology Group performance status; HBV, hepatitis B virus; PVTT, portal vein tumour thrombus; TACE, transarterial chemoembolisation.

patient in the Huachansu–TACE and the TACE groups was 7.24 (range: 2–24) and 6.0 (range: 2–23), respectively. The median duration of Huachansu administration in the Huachansu–TACE group was 6.6 months (2–23 months). The median duration of follow-up was 10.5 months (10.9 months in the Huachansu–TACE group and 10.2 in the TACE group). Follow-up therapies included TACE, targeted therapy, radiotherapy, immunotherapy and best supportive care.

Primary outcome

Median PFS was 6.8 months (95% CI, 5.87.8 months) for the Huachansu–TACE group and 5.3 months (95% CI, 4.26.4 months) for the TACE group, as shown in figure 2. The PFS was significantly better in the Huachansu–TACE group than in the TACE group (p=0.029). The 3-month, 6-month and 1-year PFS rates were 94.4%, 64.8% and 14.8%, respectively, in the Huachansu–TACE group, and 88.8%, 38.8% and 7.4%, respectively in the TACE group.

Univariate analysis revealed that the treatment received Child-Pugh class, history of liver cirrhosis, ECOG PS and presence of ascites are independent

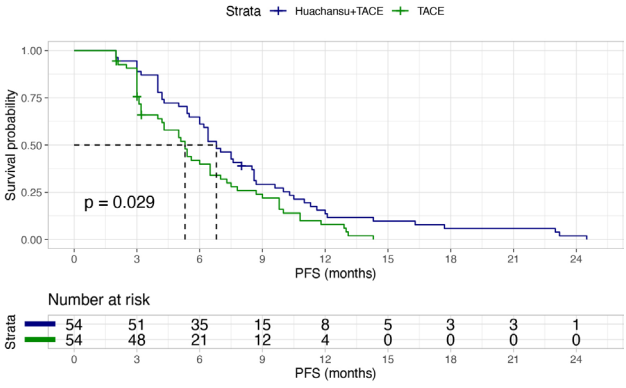

Figure 2 Kaplan-Meier analysis of progression-free survival (PFS) comparing the Huachansu–transarterial chemoembolisation (TACE) group with the TACE group.

Table 2 Univariate and multivariate analysis of the treatment for predicting PFS

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI for HR**	**P value**	**HR**	**95% CI for HR**	**P value**
Age (<60 vs ≥60)	1.039	0.667 to 1.614	0.864			
Gender (male vs female)	1.118	0.750 to 1.666	0.583			
HBV infection (yes vs no)	1.854	0.688 to 4.996	0.222			
Cirrhosis (yes vs no)	3.554	1.294 to 9.758	**0.014**	3.217	1.426 to 7.260	**<0.001**
Child-Pugh (A vs B)	2.041	1.246 to 3.342	**0.005**			
AFP (mg/mL) (<400 vs ≥400)	0.792	0.416 to 1.510	0.480			
BCLC stage (B vs C)	1.171	0.710 to 1.932	0.535			
ECOG PS (0–1 vs 2)	3.669	2.012 to 6.690	**<0.001**	2.799	1.622 to 4.831	**<0.001**
PVTT (present vs absent)	1.050	0.637 to 1.731	0.847			
Ascites (present vs absent)	3.055	1.762 to 5.294	**<0.001**	3.540	2.120 to 5.991	**<0.001**
Treatment (Huachansu+TACE vs TACE)	1.792	1.160 to 2.768	**0.009**	1.660	1.092 to 2.523	**0.018**

Bolded p<0.05 is considered statistically significant.

AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer staging system; ECOG PS, Eastern Cooperative Oncology Group performance status; HBV, hepatitis B virus; HR, Hazard ratio; PVTT, portal vein tumour thrombus; TACE, transarterial chemoembolisation.

prognostic factors of PFS. Multivariate Cox proportional hazard analysis indicated history of liver cirrhosis (HR 3.217; 95% CI: 1.426 to 7.260; p<0.001), ECOG PS (HR 2.799; 95% CI: 1.622 to 4.831; p<0.001), presence of ascites (HR 3.540; 95% CI: 2.120 to 5.991; p<0.001) and (HR 1.660; 95% CI: 1.092 to 2.523; p=0.018) was the independent prognostic factor for PFS, as shown in table 2.

Secondary outcome

Median OS was 14.8 months (95% CI: 9.5 to 20.1) in the Huachansu–TACE group and 10.7 months (95% CI: 4.7 to 16.7) in the TACE group. The OS in the Huachansu–TACE group was significantly better than in the TACE group (p=0.025), as shown in figure 3.

Treatment-related AEs for both treatment groups (Huachansu–TACE and TACE groups) are presented in table 3. The most common AEs in both groups were diarrhoea and liver dysfunction. A total of 31 grade 3 or higher AEs were observed or reported in 23 patients (21.3%) and 1 patient (0.1%) died within 2 weeks of the last TACE procedure due to pulmonary embolism. Grade 3 or 4 AEs occurred in 29.6% of 54 patients in the Huachansu–TACE group and in 22.2% of 54 patients in the TACE group.

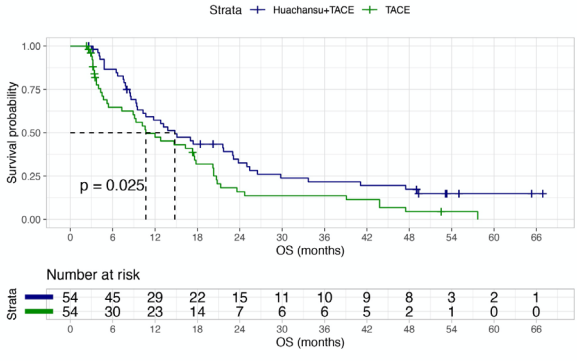

Figure 3 Kaplan-Meier analysis of overall survival (OS) comparing the Huachansu–transarterial chemoembolisation (TACE) group with the TACE group.

Exploration outcome

To evaluate the prognostic role of NKA and its association with clinical characteristics (online supplemental figure S1), we assessed the baseline median NKA levels and changes in NKA levels at 3-month follow-up, which is after two treatment cycles. At baseline, the median NKA of the patients studied was 50.13, ranging from 23.70 to 69.25 U/mL. There was no difference between the Huachansu–TACE group

Table 3 Treatment-related adverse events

	Huachansu–TACE group (n=54)		TACE group (n=54)	
Adverse event	**Any grade**	**Grade 3 or 4**	**Any grade**	**Grade 3 or 4**
Abdominal pain	8	1	10	2
Fever (>38.5°C)	15	1	16	1
Vomiting	10	1	13	1
Diarrhoea	23	2	5	1
Fatigue	8	0	6	0
Rash	2	1	1	0
New ascites	10	2	14	3
Pleural effusion	8	1	9	1
Liver dysfunction	22	3	25	4
Hepatorenal syndrome	1	1	2	1
Inguinal haematoma	5	1	6	0
Gastrointestinal bleeding	4	1	5	1
Pulmonary embolism	1	1	0	0
Spontaneous bacterial peritonitis	0	0	1	0

TACE, transarterial chemoembolisation.

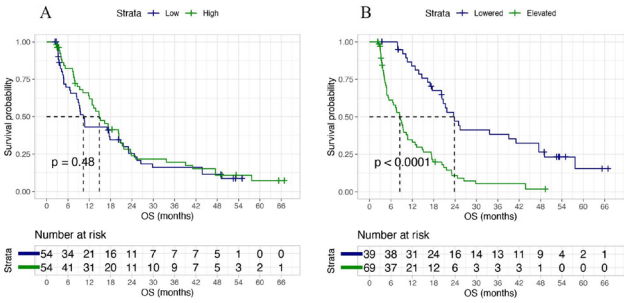

Figure 4 Kaplan-Meier analysis of overall survival (OS) comparing the (A) Na^+/K^+-ATPase α3-low and α3-high group. (B) Lowered Na^+/K^+-ATPase α3 from baseline and elevated Na^+/K^+-ATPase α3 from baseline group.

and TACE group with baseline NKA levels, which were 50.16±8.56 and 50.01±10.80, respectively (p=0.936). Based on the median baseline NKA level, we further divided patients into the baseline NKA-low (<50.13 U/mL) group and the baseline NKA-high (≥50.13 U/mL) group. No prognostic significance was found between the low and high baseline NKA groups in patients' OS (p=0.48), as shown in figure 4A.

The changes in NKA level 3-month follow-ups from baseline were elevated in 69 patients and lowered in 39 patients. To further investigate whether the change in NKA is associated with prognosis, a Kaplan-Meier analysis of PFS and OS comparing individuals with elevated or lowered NKA from baseline was performed. The results showed that the median PFS of patients in the elevated and the lowered NKA change groups was 4.2 months and 9.8 months, respectively (p<0.001). The median OS of patients in the elevated and the lowered NKA change groups was 8.5 months and 23.8 months (p<0.001, figure 4B). Depending on the type of treatment received, the OS in the Huachansu–TACE group was 9.4 months and 33.7 months, respectively, in the elevated (n=33) and the lowered (n=21) NKA change subgroups.

DISCUSSION

In this study, we evaluated the efficacy and safety of combination therapy of Huachansu, TACE and TACE alone in patients with unresectable HCC. The results showed that compared with TACE alone, we observed significant benefits in terms of PFS and OS when combining Huachansu with TACE in patients with advanced HCC. The median PFS of 6.8 months and the median OS of 14.8 months for the Huachansu–TACE group were similar to those of targeted drug clinical trials (sorafenib, sunitinib) in combination with TACE.[26–28] However, it should be noted that PFS or OS results may be relevant to the large number of patients with ECOG 0–1 and Child-Pugh A among our included patients, which is possible partly explain better survival because these factors are known positive predictors for PFS or OS.[29]

The exploration outcome did not suggest a prognostic role of baseline NKA; however, after two treatment cycles at 3-month follow-up, those with reduced NKA from baseline had significantly longer OS. A previous study analysed the expression and prognostic role of eight genes encoding the NKA subunits α1–α4 and β1–β4. The study reported significant overexpression of NKA subunits α1, β1 and β3 in the cancerous tissue compared with adjacent normal tissues of HCC patients. In additional, the overexpressions of NKA subunit α1, β1 and β3 were prognostic in patients with HCC in the Cancer Genome Atlas and Gene Expression Omnibus datasets.[30] In this study, baseline NKA levels, focusing on the levels of the α3 subunit of NKA, did not demonstrate a significant association with disease prognosis, which is consistent with the findings from the aforementioned database analysis.[30] Although an in vitro study suggested that NKA α3 could serve as a therapeutic target for bufalin in HCC,[31] contrary to many AFP studies, NKA α3 has not been confirmed to have prognostic significance in HCC.[32] On the other hand, the expression status of NKA can help predict the sensitivity of HCC cells to treatment with bufalin, which is the main active component of Huachansu.[31] It should be emphasised that in the present study, NKA was identified as an independent prognostic factor for PFS. Whether NKA alteration can be used as a predictor of Huachansu treatment efficacy in HCC patients deserves a further comprehensive clinical investigation.

Huachansu is an agent approved by the Food and Drug Administration of China (ISO9002, cFDA) for the treatment of various cancers, including HCC.[22] Its main active constituent, bufalin, is a cardiac glycoside with widely reported anticancer effects in vitro and in vivo. Notably, studies have suggested the promising potential of bufalin as a novel targeted suppressor of the NKA subunits α1 and α3 and is safe for long-term use without side effects.[21 31] The study also observed antiproliferation, antimigration and anti-invasion effects on the downregulation of NKA in vitro and suppression of tumorigenesis in vivo.[31 33] These results raise the possibility that administering Huachansu or its main active constituent bufalin in patients with high NKA expression might improve the therapeutic efficacy of bufalin and Huachansu.

The present study showed mild-to-moderate treatment-related AEs following treatment with TACE. While most AEs were manageable, one patient suffered from irreversible incidents of pulmonary embolism, and despite efforts and urgent treatment, death was inevitable. The AEs of the Huachansu and TACE combination treatment group with the TACE treatment group were similar. It should be noted that this study focused on advanced HCC in the Chinese population, where HBV infection is the primary cause of HCC and is often accompanied by liver cirrhosis. Impaired hepatic function increased the

risk of irreversible hepatotoxicity after TACE treatment, which may arise from chemotherapy drugs, Lipiodol and embolic agents. Although the incidence and severity of AEs in the present study were similar to those in the previous report,[34] the AEs related to treatment and the safety of patients should be carefully monitored.

The results of this study have some limitations. First, this is not a blinded study. Second, the data came from a single centre. Finally, the trial was conducted in a population of patients with preserved liver function (Child-Pugh class A or B). The safety of Huachansu and TACE combination therapy in the broader population deserves further investigation.

CONCLUSION

In patients with unresectable HCC, Huachansu in combination with TACE provided in better PFS and OS compared with TACE alone. Alterations in NKA expression can be used as a predictor of treatment efficacy in advanced HCC.

Acknowledgements The authors would like to thank research nurses for their support in this project.

Contributors HG participated in study design, blood analysis, data collection, data extraction, statistical analysis, drafted the manuscript and served as the guarantor of this study. JH participated blood analysis, data collection, data extraction, statistical analysis and drafted the manuscript. C-sC and LZ participated in blood collection, data extraction and drafted the manuscript. HC and ZM participated in study design, study coordination and substantially revised the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the National key clinical specialty programme of China (grant number 2DZK1102).

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants. The study was approved by the ethics committee of Fudan University Cancer Center (reference: 1207111-2). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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