

Combination therapy with PD-1 blockade and radiofrequency ablation for recurrent hepatocellular carcinoma—A retrospective study

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Research Article

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Abstract

Background: The aim of this study was to evaluate whether combined therapy with PD-1 blockade (anti-PD-1) and radiofrequency ablation (RFA) was superior to RFA monotherapy for recurrent hepatocellular carcinoma (HCC).

METHODS: A total of 127 patients who underwent anti-PD-1 plus RFA treatment

(n = 41) or RFA alone treatment (n = 86) for recurrent HCC were enrolled in this retrospective study. Clinical data including post-RFA HCC recurrence (the primary end point), overall survival (OS) (the secondary end point), adverse events and toxic effects were retrospectively analyzed.

RESULTS: The 1-year recurrence-free survival rates for the anti-PD-1 plus RFA and RFA groups were 36.6% and 16.3%, respectively. The corresponding overall survival rates for the two groups were 95.1% and 74.4%, respectively. There were statistically significant differences between the two groups in recurrence-free survival rate ($P = 0.002$) or overall survival rate ($P = 0.008$). Tumor number, TNM stage and anti-PD-1 treatment were demonstrated to be important factors associated with 1-year recurrence-free survival probability by univariate and multivariate analyses. Univariate and multivariate analyses demonstrated that tumor number, TNM stage and anti-PD-1 treatment were significant prognostic factors for OS. RFA treatment-related adverse events were pleural effusion requiring drainage and mild or moderate increase in body temperature. Grade 3 or higher events related to anti-PD-1 treatment occurred in 12.8% (6) patients and were infrequent.

CONCLUSIONS: Combination therapy of anti-PD-1 plus RFA was superior to RFA alone in improving survival for recurrent HCC.

Introduction

Hepatocellular carcinoma (HCC) is the most prevalent malignancy and the fourth most common cause of cancer-related death worldwide (1). Despite continuous advances in tumor detection, only about 10–20% of patients diagnose with early HCC and qualify for potentially surgical intervention (2). Even after curative treatment, recurrence rates were approximate 70% (3). In addition, due to multifocal intrahepatic or extrahepatic recurrence, impaired liver function and tumors in unresectable locations, repeated liver resection is feasible in a minority of patients (4). Radiofrequency ablation (RFA), a novel thermal ablation technique, has become an alternative curative therapeutics for preventing liver failure and preserving the hepatic parenchyma after surgical resection (5). In addition, RFA exhibits a lower complication and a less invasive in the treatment of solitary and small HCC when compared to surgical resection (6). However, the recurrent rate for HCC patients after RFA treatment is high (7), and such recurrent HCC appears to behave higher aggressive potential than before RFA (8, 9). Thus, monotherapy with RFA is still not satisfactory for HCC patients.

Recently, the combination therapy strategy is used to cure the disadvantage of RFA treatment in the high incidence of recurrence and take full advantage of in HCC management. For example, recent efforts have focused on the combination of RFA with other anti-cancer approaches, including transarterial chemoembolization (TACE) and molecular targeted therapy (10). Transarterial chemoembolization (TACE) plus RFA exhibits better overall survival and recurrence-free survival for patients with HCC less than 7 cm when compared to RFA alone (11, 12). However, there were no statistically significant differences in overall survival rate between TACE plus RFA and RFA alone in treating small HCC nodules (< 3 cm) (13). Sorafenib, a targeted molecular agent, is approved by the United States Food and Drug Administration (FDA) for the treatment of patients with advanced HCC. Recently, some authors found that the 1-, 2-, and 3-year recurrence rates were lower for patients with HCC at different stages of Barcelona Clinic Liver Cancer (BCLC) (0-B1) after sorafenib-RFA combination therapy, compared with RFA alone (14). Thus, the combined use of RFA with sorafenib is available for the treatment of HCC, but the reported response rates of sorafenib remains unsatisfactory, ranging from 2.3%~9.2% (15). Thus, further efforts are needed to optimize a better RFA-combined therapy strategy for the treatment of HCC.

In recent years, tumor immunotherapy has made a significant progress in inhibiting tumor progression (16). Among them, immune checkpoint inhibitors offer great promise for the approval of nivolumab by the FDA on September 23, 2017, based only on a phase I/II clinical trial for the treatment of patients with HCC (17). Recently, one study showed that nivolumab treatment improved overall survival and progression free survival, and seems safe in patients with post-operative recurrence of malignant pleural mesothelioma (18). Thus, it is a strong hint that immune checkpoint inhibitors may achieve a good clinical efficacy in patients with recurrent HCC. Meanwhile, several studies showed that RFA can induce massive necrotic cell death, thereby releasing large amounts of tumor antigens which might activate immunity and the presentation of cryptic antigens to induce tumor-specific T cell response (19–21). Moreover, the combined therapy of RFA and anti-PD-1 antibodies exhibited a stronger antitumor immunity and prolonged survival by enhancing T cell immune responses in mouse tumor models (22). These results suggest that immune checkpoint inhibitors plus RFA may provide promising results in patients with recurrent HCC. However, so far, it has not been reported. Therefore, we evaluated the combined safety and efficacy of RFA and PD-1 blockade (anti-PD-1) in patients with recurrent HCC in a retrospective cohort study for the first time.

Materials And Methods

Patients

This retrospective study was approved by the Ethical Committee of Southwest Hospital, Army Medical University (Ethical approval number: KY2021046). All patients provided written informed consent to participate in the study. The inclusion criteria for this study were as follows: (a) patients were diagnosed with recurrent intrahepatic HCC lesions after hepatic resection or RFA; (b) patients had solitary intrahepatic tumor or multiple tumors with three or two nodules each ≤ 3 cm in size; (c) patients were aged 18–75 years; (d) patients had BCLC stage 0-A; (e) patients were unwilling to undergo hepatectomy

or liver transplantation; (f) patients had no extrahepatic metastases; (g) patients had no invasion of the portal vein, the hepatic vein trunk or secondary branches, or the bile duct (h) No other anti-tumor therapy received before treatment, including radiotherapy, TACE and targeted drugs, etc. (i) patients had no residual lesions detected by contrast-enhanced ultrasound after RFA at 24 h, 48 h and confirmed by Contrast-enhanced computed tomography (CT) or gadoxetic acid-enhanced magnetic resonance imaging (EOB-MRI) one month after RFA. (j) patients received PD-1 blockade therapy for at least one month after RFA in this study. Patients were excluded from our study if they (a) patients had severe portal hypertension, a history of esophageal variceal hemorrhage, severe hypersplenism syndrome, or refractory ascites currently or in the history; (b) had serious heart, kidney and other organ dysfunction; (c) had autoimmune disease currently or in the history; (d) had other malignant tumors currently or in the history; (e) had serious adverse events after PD-1 blockade therapy; (f) received other treatments during study period.

We reviewed the electronic medical records of 180 consecutive patients with recurrent intrahepatic HCC lesions after RFA or hepatectomy who underwent RFA and received PD-1 blockade therapy or who underwent RFA from November 2013 to December 2019 at Southwest Hospital, Army Medical University. Collection of follow-up data was terminated on April 30, 2017. Collection of follow-up data was terminated on December 31, 2020.

RFA Procedure

All the patients enrolled in this study were treated percutaneously using an LDRF-120S radiofrequency ablation device (Lead Electron Corporation, Mianyang, China) according to the Guidelines of Chinese Expert Consensus Statement issued by the Chinese Society of Liver Cancer and Chinese Society of Clinical Oncology (22, 23). RFA procedures were performed by physicians with at least 5 years of experience in ultrasound-guided hepatic RFA. Before RFA, each lesion was confirmed by contrast-enhanced ultrasound. After local or general anesthesia, the radiofrequency electrode was placed to tumor lesions under ultrasound guidance. In order to eliminate residual tumor cells in the treatment area, the open cool-tip electrode was at least 2 cm larger than the maximum diameter of the tumor, and the open cluster electrode was rotated clockwise in situ by 15°C after closed and reopened for another treatment. Each tumor lesion was treated with one electrode. For patients with multiple tumors, all lesions were ablated according to the same procedure. After RFA for 15 min, 24 h and 48 h, contrast-enhanced ultrasound was used to detect whether each lesion was completely destroyed. Contrast-enhanced computed tomography (CT) or gadoxetic acid-enhanced magnetic resonance imaging (EOB-MRI) was performed to confirm whether residual tumor was detected one month after RFA. If residual tumor was still detected, the lesions were retreated according to the same procedure.

PD-1 blockade management

Patients in RFA plus PD-1 blockade treatment group (RFA + anti-PD-1) received 200 mg of Camrelizumab intravenously every 2 weeks, or 200 mg of Sintilimab intravenously every 2 weeks and the initial administration was within 72h after RFA. Patients received continual PD-1 blockade treatment until

unacceptable toxic effects occurred or there was loss of clinical benefit. It is possible to observe atypical reactions (e.g. temporary enlargement of the tumor or appearance of new small lesions in the first few months, followed by tumor shrinkage). If the patient's clinical symptoms are stable or continue to reduce, even if there is preliminary evidence of disease progression on imaging, based on the judgment of overall clinical benefit, the drug can be considered to continue to be used until the disease progression is confirmed.

Determination of complete ablation, tumor recurrence, survival and complications

Complete ablation was defined as the absence of any enhancing lesion at the ablation site by CT or MRI at 1 month after RFA. HCC recurrence was defined as the appearance of local and distant tumor progression. Local tumor recurrence was defined as the appearance of enhancing lesions at edge of the ablation site (< 2.0 cm from the edge of the ablation site) during follow-up imaging (24). Distant tumor progression was defined as the appearance of enhancing lesions > 2.0 cm from the edge of the ablation site or new HCC foci during follow-up imaging by CT or MRI. All imaging evaluations were decided by two independent diagnostic radiologists with at least 5 years of experience. Recurrence-free survival (RFS) time was defined as the time from complete RFA to HCC recurrence. Overall survival (OS) time was defined as the time from complete RFA to death or last follow-up. Patients who remained alive at last follow-up were considered a censored event in the statistical analysis. RFA-related complications were evaluated according to Dindo–Clavien classification (25).

Follow-up

All patients in this study were followed up 1 month after initial RFA, including CT (or MRI), physical examination, routine blood tests, liver function tests and complications. If complete ablation was attained, follow-up was conducted every 8 weeks. This study was censored on December 31, 2020.

Statistical analysis

Statistical analyses in this study were performed using SPSS software (version 19.0; SPSS, Chicago, Ill). Baseline characteristics between RFA + anti-PD-1 and RFA alone were compared by Fisher's exact test, Mann-Whitney U-tests, and the χ^2 test for categorical data. Survival curves were analyzed by the Kaplan-Meier method. The equivalences of the survival curves were evaluated by the log-rank statistics. The risk factors with statistical significance in the log-rank statistics analysis were subjected to a multivariate survival analysis. All statistical tests were two tailed, and statistical significance was considered when $P < 0.05$.

Results

Baseline characteristics of patients

A total of 180 patients with recurrent HCC underwent RFA plus anti-PD-1 (n = 62) or RFA alone (n = 118) in this study. As shown in Fig. 1, a total of 53 patients were excluded from this study because they met the exclusion criteria. As a result, 41 patients were treated with RFA plus anti-PD-1, while 86 patients were received RFA alone (Fig. 1).

Detailed baseline patient characteristics are presented in Table 1. There were no significant differences in age, gender, chronic hepatitis B, liver cirrhosis, tumor number, tumor size, tumor-node metastasis (TNM), RFA and Targeted drugs treatment between the two treatment groups (Table 1). A total of 67 patients had underwent hepatic resection, with significant difference between the two treatment groups (36.6% of the RFA + anti-PD-1 group and 60.5% of the RFA alone group, $P = 0.012$) (Table 1). However, the patients with recurrent HCC after monotherapy with hepatic resection in the RFA + anti-PD-1 group was not fewer than in RFA alone group (24.4% vs. 30.2%, $p = 0.495$) (**Supplementary Table 1**). The patients with recurrent HCC after monotherapy with RFA in the RFA + anti-PD-1 group was more common than in RFA alone group (63.4% vs. 39.5%, $P = 0.012$) (Supplementary Table 1). A total of 31 (24.4%) patients underwent hepatic resection and RFA treatments: 6 (12.2%) patients in the RFA + anti-PD-1 group, and 26 (30.2%) patients in the RFA alone group. There were significant differences between the two groups ($P = 0.027$) (**Supplementary Table 1**).

Table 1
Baseline patient characteristics

Characteristic	RFA + anti-PD-1 (n = 41)	RFA (n = 86)	T, Z or χ^2 value	P
<i>Age</i>	56.24 ± 10.22	54.44 ± 10.89	0.889	0.376
<i>Gender</i>			0.099	0.753
Male	37 (90.2%)	76 (88.4%)		
Female	4 (9.8%)	10 (11.6%)		
<i>Underlying liver disease</i>				
<i>Chronic hepatitis B</i>			3.080	0.079
Yes	34 (82.9%)	80 (93.0%)		
No	7 (17.1%)	6 (7.0%)		
<i>Liver cirrhosis</i>			0.970	0.325
Yes	25 (61.0%)	60 (69.8%)		
No	16 (39.0%)	26 (30.2%)		
<i>Tumor characteristics at initial diagnosis</i>				
<i>Tumor number</i>			0.576	0.750
1	33 (80.5%)	65 (75.6%)		
2	5 (12.2%)	15 (17.4%)		
3	3 (7.3%)	6 (7.0%)		
<i>Maximal tumor size (cm)</i>			2.325	0.127
≤ 1.8	25 (61.0%)	40 (46.5%)		
> 1.8 and < 3.0	16 (39.0%)	46 (53.5%)		
<i>TNM</i>			0.379	0.538
I	33 (77.3%)	65 (75.6%)		
II	8 (22.7%)	21 (24.4%)		
<i>Treatment history before this study</i>				

RFA, radiofrequency ablation; TNM, tumor-node metastasis. * $P < 0.05$.

Characteristic	RFA + anti-PD-1 (n = 41)	RFA (n = 86)	T, Z or χ^2 value	P
<i>Antiviral therapy</i>			0.848	0.357
Yes	31 (70.7%)	71 (82.6%)		
No	10 (29.3%)	15 (17.4%)		
<i>Hepatic resection</i>			6.352	0.012*
Yes	15 (36.6%)	52 (60.5%)		
No	26 (63.4%)	34 (39.5%)		
<i>RFA</i>			0.467	0.495
Yes	31 (75.6%)	60 (69.8%)		
No	10 (24.4%)	26 (30.2%)		
RFA, radiofrequency ablation; TNM, tumor-node metastasis. * $P < 0.05$.				

Complications or Adverse Events

As shown in Table 2, there was no RFA treatment-related hospital mortality in either group in this study. Adverse events in the RFA + anti-PD-1 group were as follows: pleural effusion requiring drainage (1) and mild or moderate increase in body temperature (3). Adverse events in the RFA alone group were as follows: pleural effusion requiring drainage (2) and mild or moderate increase in body temperature (5). The overall complication rate was 9.8% and 8.1% in the RFA + anti-PD-1 group and in the RFA alone group, respectively. There was no grade 3 or higher events in our study, and the overall complication rate was similar between the two groups ($P = 0.762$).

Table 2
Adverse events related to RFA treatment

Characteristic	RFA + anti-PD-1 (n = 41)	RFA (n = 86)	P
<i>In-hospital mortality</i>	0	0	-
<i>Overall morbidity</i>	4 (9.8%)	7 (8.1%)	0.762
Pleural effusion requiring drainage	1 (2.4%)	2 (2.3%)	0.969
Mild or moderate increase in body temperature	3 (7.3%)	5 (5.8%)	0.744
<i>Complications (Dindo–Clavien classification)</i>	4(9.8%)	7(8.1%)	0.762
Grade 1	3 (7.3%)	5(5.8%)	0.744
Grade 2	1(2.4%)	2 (2.3%)	0.969
RFA, radiofrequency ablation.			

The anti-PD-1-related adverse events were shown in Table 3. The frequency of any grade treatment-related adverse events (TRAEs) was 70.2% (33). The frequency of grade 3 or higher events was 12.8% (6). The most common TRAEs of any grade were fatigue and pruritus. Laboratory TRAEs were no frequent in our study. <10% of patients experienced TRAEs of any grade and < 3% of patients experienced grade 3 or higher TRAEs. In addition, the most common select TRAEs of any grade were skin, gastrointestinal and hepatic events, and grade 3 or higher sTRAEs occurred in < 3%. A total of 6 patients occurred grade 3 or higher TRAEs were excluded from this study because they met the exclusion criteria.

Table 3
Adverse events related to anti-PD-1 administration*

Adverse events	RFA + anti-PD-1 (n = 47)	
	All events	Grade 3 or higher events
<i>TRAEs</i>		
Any TRAE, n (%)	33 (70.2)	6 (12.8)
<i>TRAEs (≥ 10%), n (%)</i>		
Fatigue	8 (17.0)	1 (2.1)
Pruritus	7 (14.9)	0
Rash	6 (12.8)	0
Diarrhoea	6 (12.8)	1 (2.1)
Decreased appetite	5 (10.6)	1 (2.1)
<i>Laboratory TRAEs (≥ 5%), n (%)</i>		
Blood bilirubin increased	4 (8.5)	0
Platelet count decreased	4 (8.5)	1 (2.1)
Lipase increased	4 (8.5)	1 (2.1)
ALT increase	3 (6.4)	0
AST increased	3 (6.4)	0
Amylase increased	3 (6.4)	0
<i>sTRAEs</i>		
<i>sTRAEs, n (%)</i>		
Skin**	12 (25.5)	1 (2.1)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; sTRAE, select TRAE; TRAE, treatment-related adverse event.

*Includes events reported between first dose and 30 days after last dose of study therapy. Event terms were reported by investigators and were not predefined.

**Includes rash, pruritus, pruritus generalized, rash maculopapular, pemphigoid, skin exfoliation, rash pruritic, erythema, eczema, rash popular and palmar-plantar erythrodysesthesia syndrome.

§Includes colitis, enteritis, diarrhea and frequent bowel movements.

¶Includes increases in AST, ALT, blood alkaline phosphatase, aminotransferases, blood bilirubin, hepatitis, hyperbilirubinemia and liver disorder.

Adverse events	RFA + anti-PD-1 (n = 47)	
	All events	Grade 3 or higher events
gastrointestinal tract [§]	7 (14.9)	1 (2.1)
Hypothyroidism	2 (4.2)	0
Hepatic [¶]	5 (10.6)	1 (2.1)
Adrenal insufficiency	0	0
Hyperthyroidism	0	0
Thyroiditis	0	0
Pneumonitis	0	0
Renal	0	0
Hypersensitivity/infusion-related reaction	1 (2.1)	0
ALT, alanine aminotransferase; AST, aspartate aminotransferase; sTRAE, select TRAE; TRAE, treatment-related adverse event.		
*Includes events reported between first dose and 30 days after last dose of study therapy. Event terms were reported by investigators and were not predefined.		
**Includes rash, pruritus, pruritus generalized, rash maculopapular, pemphigoid, skin exfoliation, rash pruritic, erythema, eczema, rash popular and palmar-plantar erythrodysesthesia syndrome.		
§Includes colitis, enteritis, diarrhea and frequent bowel movements.		
¶Includes increases in AST, ALT, blood alkaline phosphatase, aminotransferases, blood bilirubin, hepatitis, hyperbilirubinemia and liver disorder.		

Survival

98 of 127 patients (77.2%) experienced HCC recurrence during the follow-up period; 26 (63.4%) were in the RFA + anti-PD-1 group, and 72 (83.7%) were in the RFA alone group. One patient was censored in the RFA alone group, and one patient was also censored in the RFA + anti-PD-1 group. Among these 98 patients, 78 underwent repeated RFA procedure and 20 underwent conservative management. In the RFA + anti-PD-1 group, anti-PD-1 treatment was continually administered. The median recurrence-free survival (mRFS) was 39.1 weeks (95% CI: 23.5–54.8) in the RFA + anti-PD-1 group and 19.3 weeks (95% CI: 15.1–23.5) in the RFA alone group. The RFS was significantly longer in the RFA + anti-PD-1 group than that in the RFA alone group ($P=0.002$) (Fig. 2).

24 of 127 patients (18.9%) died during the follow-up period, and 22 (25.6%) were in the RFA alone group, 2 (4.9%) were in the RFA + anti-PD-1 group. One patient was censored in the RFA alone group, and one patient was also censored in the RFA + anti-PD-1 group. 22 patients died due to tumor recurrence and extrahepatic metastases, and 2 patients died due to unknown reason. The OS was 51.0 weeks (95% CI:

49.0-52.9) in the RFA + anti-PD-1 group and 47.6 weeks (95% CI: 45.6–49.6) in the RFA alone group. The OS was significantly longer in the RFA + anti-PD-1 group than that in the RFA alone group ($P=0.008$) (Fig. 2).

Subgroup analysis

As shown in **Supplementary Table 2**, in patients with age ≤ 53 years, mRFS time was 44.7 weeks (95% CI: 21.4–68.1) in the RFA + anti-PD-1 group and 17.4 weeks (95% CI: 11.0–23.8) in the RFA alone group ($P=0.012$). mRFS time was 41.3 weeks (95% CI: 23.6–59.0) in the RFA + anti-PD-1 group and 19.3 weeks (95% CI: 14.9–23.7) in the RFA alone group ($P=0.003$) in male patients. In patients with chronic hepatitis B, mRFS time was 39.1 weeks (95% CI: 24.9–53.3) in the RFA + anti-PD-1 group and 18.6 weeks (95% CI: 14.3–22.8) in the RFA alone group ($P=0.002$). Among patients with liver cirrhosis, mRFS time was 44.7 weeks (95% CI: 27.5–61.9) in the RFA + anti-PD-1 group and 17.1 weeks (95% CI: 14.6–19.7) in the RFA alone group ($P=0.006$). In patients with one tumor, mRFS time was 44.7 weeks (95% CI: 32.6–56.8) in the RFA + anti-PD-1 group and 21.3 weeks (95% CI: 16.1–26.5) in the RFA alone group ($P=0.005$). In patients with maximal tumor size ≤ 1.8 cm, mRFS time was 49.6 weeks (95% CI: 29.5–69.6) in the RFA + anti-PD-1 group and 16.7 weeks (95% CI: 14.3–19.2) in the RFA alone group ($P<0.001$). In patients who underwent antiviral therapy before this study, mRFS time was 41.3 weeks (95% CI: 22.7–59.8) in the RFA + anti-PD-1 group and 19.3 weeks (95% CI: 15.3–23.3) in the RFA alone group ($P=0.001$). mRFS time was 38.6 weeks (95% CI: 27.0–50.2) in the RFA + anti-PD-1 group and 18.6 weeks (95% CI: 13.9–23.3) in the RFA alone group ($P=0.009$) in patients without underwent hepatic resection. Among patients who underwent RFA before this study, mRFS time was 38.6 weeks (95% CI: 27.8–49.3) in the RFA + anti-PD-1 group and 18.6 weeks (95% CI: 13.7–23.4) in the RFA alone group ($P=0.003$). The median OS in subgroup analysis was not achieved, because only twenty-four patients (18.9%) died during the follow-up period. Among subgroup patients with chronic hepatitis B, liver cirrhosis, one tumor, maximal tumor size > 1.8 and < 3.0 , and antiviral therapy, OS in the RFA + anti-PD-1 group were significantly longer than that in the RFA alone group ($P=0.042$, $P=0.010$, $P=0.014$, $P=0.041$ and $P=0.025$). As shown in Table 4, the stratified Cox regression model was used to calculate the HR and 95% CI for each subgroup. Our results showed that the RFA + anti-PD-1 group had significant RFS and OS benefits for all 20 subgroups (Table 4).

Table 4

Subgroup analysis of recurrence and overall survival by the stratified Cox regression model

Characteristic	Progression-free survival		Overall survival	
	HR (95% CI)	P value	HR (95% CI)	P value
<i>Age</i>				
≤ 53	0.429 (0.218–0.845)	0.014*	0.178 (0.023–1.358)	0.096
> 53	0.578 (0.313–1.067)	0.080	0.182 (0.023–1.438)	0.106
<i>Gender</i>				
Male	0.491 (0.303–0.795)	0.004**	0.200 (0.047–0.861)	0.031*
Female	0.631 (0.169–2.356)	0.493	0.028 (0.000–823.839)	0.495
<i>Underlying liver disease</i>				
<i>Chronic hepatitis B</i>				
Yes	0.467 (0.285–0.766)	0.003**	0.247 (0.057–1.066)	0.061
No	0.804(0.227–2.840)	0.734	0.008(0.000–40.680)	0.269
<i>Liver cirrhosis</i>				
Yes	0.462(0.262–0.815)	0.008**	0.113(0.015–0.844)	0.034*
No	0.568 (0.266–1.213)	0.144	0.530 (0.055–5.094)	0.582
<i>Tumor characteristics at initial diagnosis</i>				
<i>Tumor number</i>				
1	0.485(0.288–0.817)	0.007**	0.025(0.000–4.319)	0.160
2 or 3	0.628(0.249–1.587)	0.325	0.433(0.096–1.958)	0.277
<i>Maximal tumor size (cm)</i>				

RFA, radiofrequency ablation; TNM, tumor-node metastasis. * $P < 0.05$, ** $P < 0.01$.

Characteristic	Progression-free survival		Overall survival	
	HR (95% CI)	P value	HR (95% CI)	P value
≤ 1.8	0.338(0.182–0.628)	0.001**	0.260(0.031–2.159)	0.212
> 1.8 and < 3.0	0.730(0.371–1.436)	0.362	0.159(0.021–1.203)	0.075
<i>TNM</i>				
I	0.485(0.288–0.817)	0.007**	0.025(0.000–4.319)	0.160
II	0.628(0.249–1.587)	0.325	0.433(0.096–1.958)	0.277
<i>Treatment history before study</i>				
<i>RFA</i>				
<i>Antiviral therapy</i>				
Yes	0.438(0.260–0.737)	0.002**	0.139(0.018–1.049)	0.056
No	0.760(0.297–1.944)	0.566	0.196(0.024–1.597)	0.128
<i>Hepatic resection</i>				
Yes	0.580(0.290–1.159)	0.123	0.255(0.033–1.953)	0.189
No	0.447(0.241–0.830)	0.011*	0.134(0.017–1.056)	0.056
<i>RFA</i>				
Yes	0.459(0.272–0.775)	0.004*	0.275(0.062–1.219)	0.089
No	0.599(0.239–1.498)	0.273	0.029(0.000–12.337)	0.251

RFA, radiofrequency ablation; TNM, tumor-node metastasis. * $P < 0.05$, ** $P < 0.01$.

Uni- and Multivariate Analyses

Univariate Cox regression analysis and multivariate Cox regression analysis were used to evaluate the predictors of RFS and OS. As shown in Table 5 and **Supplementary Table 3**, tumor number (HR: 1.692, 95% CI: 1.040–2.753, $p = 0.034$), TNM stage (HR: 1.692, 95% CI: 1.040–2.753, $P = 0.034$) and anti-PD-1 treatment (HR: 0.494, 95% CI: 0.303–0.805, $P = 0.005$) significantly correlated with RFS in the univariate Cox regression analysis. Multivariate Cox regression analysis showed that tumor number (HR: 1.578, 95%

CI: 1.008–2.471, $P=0.046$), TNM stage (HR: 1.578, 95% CI: 1.008–2.471, $P=0.046$) and anti-PD-1 treatment (HR: 0.522, 95% CI: 0.331–0.823, $P=0.005$) were the independent predictors for RFS (Table 5 and **Supplementary Table 3**). Univariate Cox regression analysis showed that tumor number (HR: 4.187, 95% CI: 1.636–10.716, $P=0.003$), TNM stage (HR: 4.187, 95% CI: 1.636–10.716, $P=0.003$) and anti-PD-1 treatment (HR: 0.157, 95% CI: 0.033–0.733, $P=0.019$) were associated with OS (Table 5 and **Supplementary Table 4**). Multivariate Cox regression analysis showed that tumor number (HR: 4.234, 95% CI: 1.878–9.544, $P=0.001$), TNM stage (HR: 4.234, 95% CI: 1.878–9.544, $P=0.001$) and anti-PD-1 treatment (HR: 0.176, 95% CI: 0.041–0.753, $P=0.019$) were the significant prognostic factors for OS (Table 5 and **Supplementary Table 4**).

Table 5
Univariate and multivariate analysis of the relative risk of recurrence and overall survival.

	Progression-free survival			Overall survival		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
	P value	HR (95% CI)	P value	P value	HR (95% CI)	P value
Age (> 53 vs. ≤ 53)	0.745			0.104		
Gender (male vs. female)	0.947			0.989		
Chronic hepatitis B (Positive vs. Negative)	0.249			0.500		
Liver cirrhosis (Positive vs. Negative)	0.378			0.727		
Tumor number (multiple vs. solitary)	0.034*	1.578(1.008–2.471)	0.046*	0.003**	4.234 (1.878–9.544)	0.001**
Tumor size (≥ 1.8 vs. <1.8)	0.160			0.444		
TNM stage (II vs. I)	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Antiviral therapy (yes vs. no)	0.164			0.192	0.452 (0.191–1.068)	0.070
Hepatic resection (yes vs. no)	0.538			0.200		
RFA (yes vs. no)	0.209			0.130		

^aTNM = Tumor number, *Statistically significant ($P < 0.05$), **Statistically significant ($P < 0.01$).

	Progression-free survival		Overall survival			
	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis		
Anti-PD1-1 treatment	0.005**	0.522 (0.331– 0.823)	0.005**	0.019*	0.176 (0.041– 0.753)	0.019*
(yes vs. no)						
^a TNM = Tumor number, *Statistically significant ($P < 0.05$), **Statistically significant ($P < 0.01$).						

Discussion

In this retrospective study, we firstly reported the efficacy of anti-PD-1 therapy on RFS and OS outcome in recurrent HCC after curative RFA treatment. Our results showed that patients with recurrent HCC had significant better RFS and OS outcome in the RFA + anti-PD-1 group than in the RFA alone group.

Previous studies showed that single-agent checkpoint inhibitors did not show a better survival outcome in patients with HCC (26, 27). In this study, there were two important differences with the previous reports. First, all patients enrolled in this study underwent curative RFA treatment before anti-PD-1 therapy. It has been shown that RFA induced T cell immune responses as well as PD-L1 expression in synchronous colorectal cancer liver metastases and tumor-bearing mice (22, 28). In addition, T cell immune responses induced by RFA led to a detectable antitumor reactivity in mouse models (22, 29). Previous researches showed that PD-L1 as well as PD-1 and CTLA-4 contributed to the inhibition of thermal ablation-induced antitumor activities (22, 30). Positive PD-L1 expression in patients was associated with an objective response for anti-PD-1 therapy (31, 32). PD-L1 immunohistochemistry tests for calculating the efficacy of anti-PD-(L)1 therapy in NSCLC and several other cancers has been approved by the US Food and Drug Administration (33). Thus, RFA can provide a useful antigen source for the induction of antitumor immunity. Meanwhile, some preclinical studies have shown that combining of radiotherapy and PD-L1/PD-1 blockade synergistically enhance antitumor immunity (34, 35). These results suggested that local antitumor treatments by radiotherapy can elicit immune response, thereby providing a better opportunity for PD-1/PD-L1 blockade therapy. Second, patients with BCLC stage A, no extrahepatic metastases and no invasion of the portal vein, the hepatic vein trunk or secondary branches were enrolled in this study. However, all the studies about single-agent checkpoint inhibitors treatment evaluated patients with advanced HCC (26, 27). Thus, all patients in our study had a better physical condition compared with those in the above reports.

A previous report showed that liver metastases in patients with advanced melanoma were associated with reduced ORR and progression-free survival during anti-PD-1 therapy (36). In addition, one recent report showed that patients with an ECOG performance status of 1 or more, bone metastases, and liver metastases had a shorter 5-year OS in advanced melanoma, renal cell carcinoma, or non-small cell lung cancer (37). Therefore, these results suggest that anti-PD-(L)1 therapy will provide a better clinical benefit for patients with early HCC. Our results support this concept. Findings from the current study showed that tumor number, TNM stage and anti-PD-1 treatment were significant prognostic factors for RFS and OS, and patients with one tumor experienced better clinical benefit in RFS and OS than those with two or three tumors during anti-PD-1 therapy (RFS: HR = 0.501 vs. HR = 0.645; OS: HR = 0.025 vs. HR = 0.433). Similar results were found in patients with TNM stage I. Although some studies have failed to show a better survival outcome in patients with HCC during single-agent checkpoint inhibitors therapies, more reports and our results suggested that anti-PD-1 treatment could provide a better clinical benefit for early HCC patients pretreated with RFA.

In this study, the RFA treatment-related adverse events were pleural effusion requiring drainage and mild or moderate increase in body temperature, which is consistent with historical data in other trials (14, 23). The spectrum of adverse events observed with the anti-PD1 therapy were similar to those reported in previous studies (26, 38, 39). A total of 6 patients occurred grade 3 or higher TRAEs were excluded from this study because they met the exclusion criteria. In the analysis of complications with the anti-PD1 therapy, the above 6 patients were re-included. The frequencies of any grade TRAEs and grade 3 or higher events in this study were 70.2% (33) and 12.8% (6) respectively, which is not consistent with historical data in other trials of Pembrolizumab (any grade TRAEs: 98.2%, grade 3 or higher events:61.1%) or Atezolizumab (any grade TRAEs: 96.4%, grade 3 or higher events: 52.0%) for hepatocellular carcinoma (38, 39).The lower incidence of grade 3 or higher events in our study may be due to patients enrolled with BCLC stage A, no intrahepatic and extrahepatic metastases and no invasion of the portal vein, the hepatic vein trunk or secondary branches. The patients population enrolled in this study with a better physical condition well tolerated the toxicity caused by the anti-PD-1 therapy.

Our study as a retrospective research had several limitations. The baseline demographics were matched well between the two groups, but prospective randomized controlled trials are needed for further research. In addition, the number of patients enrolled in this study was limited (41 patients in the RFA + anti-PD-1 group vs. 86 patients in the RFA alone group). Thus, studies with a larger sample size are needed to confirm our findings. Meanwhile, the follow-up time is only one year. Therefore, patients should be followed up for assessment of RFS and OS at least 3–5 years in the future study.

In conclusion, RFA + anti-PD-1 is a safe and effective therapy for recurrent HCC patients with BCLC stage A, tumor diameters smaller than 3 cm and no more than three tumors, no extrahepatic metastases and no invasion of the portal vein, the hepatic vein trunk or secondary branches.

Declarations

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Authors' contributions: K.F., B.N. and K.S.M.: conceive and design the study. X.F.W. and S.C.: collect and analyze the data. X.F.W.: draft the manuscript. K.F., B.N. and K.S.M.: review and finalize the manuscript.

Compliance with ethical standards

Conflict of interest

Xiaofei Wang declared that no conflict of interest exists. Shu Chen declared that no conflict of interest exists. Huaqiang Bi declared that no conflict of interest exists. Feng Xia declared that no conflict of interest exists. Kai Feng declared that no conflict of interest exists. Kuansheng Ma declared that no conflict of interest exists. Bing Ni declared that no conflict of interest exists.

Ethics approval: This retrospective study was approved by the Ethical Committee of Southwest Hospital, Army Medical University (Ethical approval number: KY2021046). All patients provided written informed consent to participate in the study.

Consent to participate: Not applicable

Consent for publication: Not applicable

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Figures

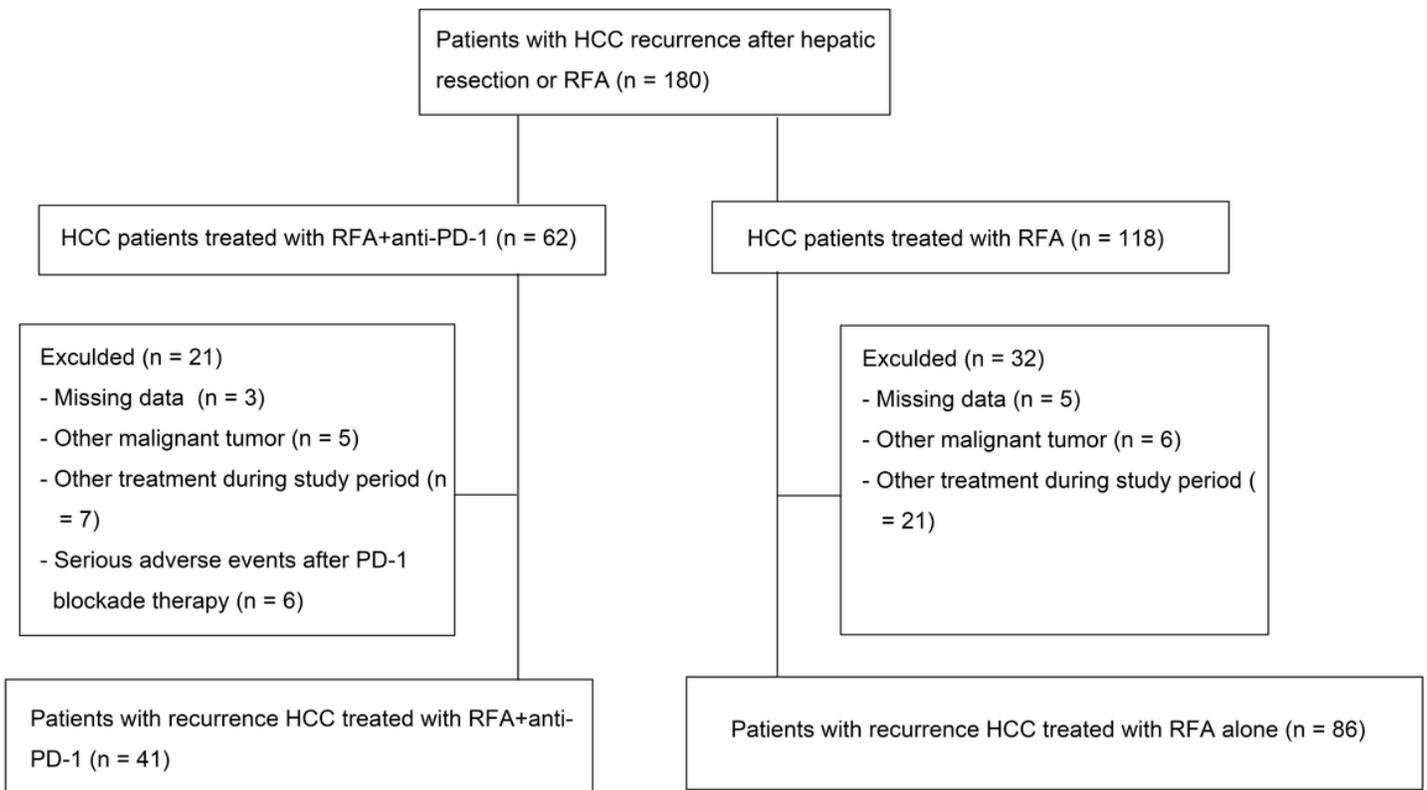
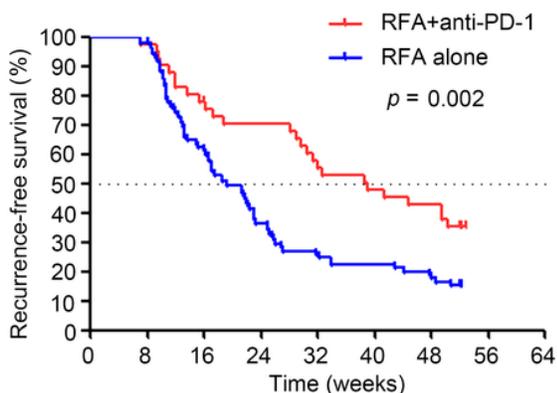


Figure 1

Flow chart of patients included in the study.

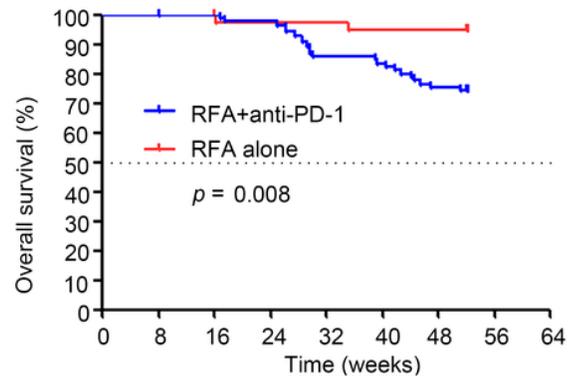
a



Patients at risk

RFA+anti-PD-1	41	40	31	28	22	19	17	14	14
RFA alone	86	83	52	31	22	19	15	13	13

b



Patients at risk

RFA+anti-PD-1	41	40	39	39	39	39	38	38	38
RFA alone	86	85	84	83	73	71	64	63	63

Figure 2

Kaplan-Meier curves show recurrence-free survival and overall survival in RFA+anti-PD-1 and RFA alone groups. (a) Recurrence-free survival analysis in patients with RFA+anti-PD-1 treatment and in patients with RFA alone treatment (log-rank test, $x^2 = 9.434$, $P = 0.002$). (b) Overall survival analysis in patients with RFA+anti-PD-1 treatment and in patients with RFA alone treatment (log-rank test, $x^2 = 7.114$, $P = 0.008$).

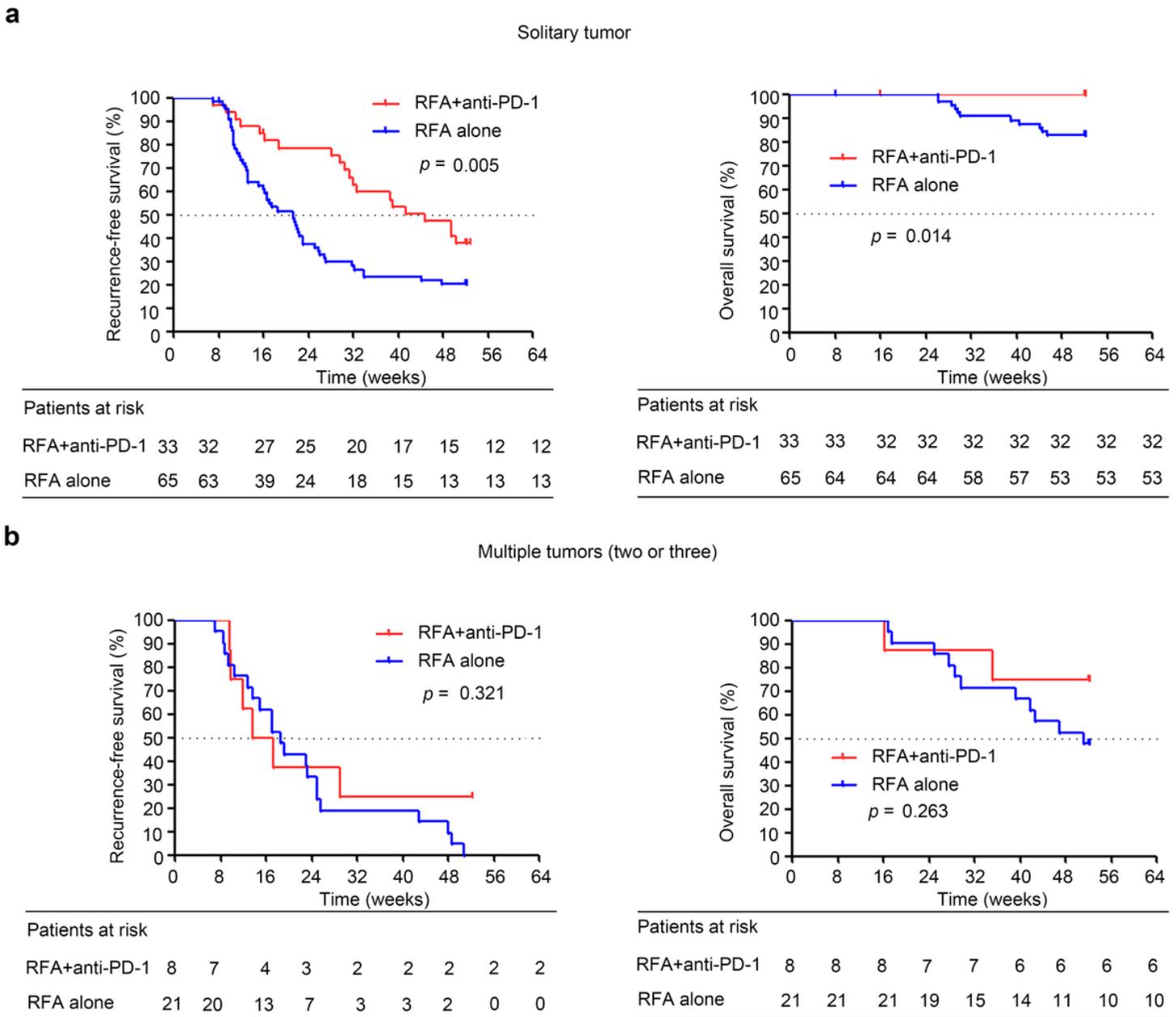


Figure 3

Recurrence-free survival and overall survival comparison between the RFA+anti-PD-1 and RFA alone groups for solitary tumor or multiple tumors (two or three tumors). (a) Recurrence-free survival and overall survival analyses in patients with RFA+anti-PD-1 treatment and in patients with RFA alone treatment for solitary tumor (log-rank test, $x^2 = 7.731$, $P = 0.005$; log-rank test, $x^2 = 5.981$, $P = 0.014$). (b) Recurrence-free survival and overall survival analyses in patients with RFA+anti-PD-1 treatment and in patients with

RFA alone treatment for multiple tumors (log-rank test, $x_2 = 0.986$, $P = 0.321$; log-rank test, $x_2 = 1.252$, $P = 0.263$).

Supplementary Files

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