Combination therapy with PD-1 blockade and radiofrequency ablation for recurrent hepatocellular carcinoma: a propensity score matching analysis

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ABSTRACT

Background: This study aimed to evaluate whether combined therapy with PD-1 blockade (anti-PD-1) and radiofrequency ablation (RFA) is 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 (n = 86) for recurrent HCC were included in this retrospective study. A matched cohort comprising 40 patients from each group was selected after propensity score matching analysis. Clinical data including post-RFA HCC recurrence (primary endpoint), overall survival (OS) (secondary endpoint), 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 32.5% and 10.0% after propensity score matching. There were statistically significant differences between the two groups in terms of the recurrence-free survival rate (p = 0.001) and OS rate (p = 0.016). Tumor number, tumor-node metastasis (TNM) stage, antiviral therapy, 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 included pleural effusions that require drainage and a mild or moderate increase in body temperature. Grade 3 or higher events related to anti-PD-1 treatment occurred in 12.8% (6) of patients and were infrequent.

Conclusions: Combination therapy with anti-PD-1 plus RFA was superior to RFA alone in improving survival in patients with recurrent HCC.

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Supplemental data for this article can be accessed here.

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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 approximately 10–20% of patients are diagnosed with early HCC and qualify for potentially surgical intervention [2]. Even after curative treatment, the recurrence rate is approximately 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 therapeutic option to prevent liver failure and preserve the hepatic parenchyma after surgical resection [5]. In addition, RFA exhibits a lower complication rate and is less invasive in the treatment of solitary and small HCCs than surgical resection [6]. However, the recurrence rate for HCC patients after RFA treatment is high [7], and such recurrent HCC appears to behave more aggressively than before RFA [8,9]. Thus, monotherapy with RFA is still unsatisfactory for patients with HCC.

Recently, a combination therapy strategy has been used to overcome the disadvantages of RFA treatment in the high incidence of recurrence and take full advantage of in HCC management. For example, recent efforts have focused on combining RFA with other anticancer approaches, including transarterial chemoembolization (TACE) and molecular targeted therapy [10]. TACE plus RFA exhibits better overall survival (OS) and recurrence-free survival (RFS) for patients with HCC <7 cm compared to RFA alone [11,12]. However, there were no statistically significant differences in OS rate between TACE plus RFA and RFA alone in the treatment of small HCC nodules (<3 cm) [13]. Sorafenib, a targeted molecular agent, is approved by the United States Food and Drug Administration (FDA) to treat patients with advanced HCC. Recently, some studies 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]. Therefore, the combined use of RFA with sorafenib is available for the treatment of HCC, but the reported response rates of sorafenib remain unsatisfactory, ranging from 2.3% to 9.2% [15]. In addition, angiotensin II receptor 1 blockers (sartans) significantly improved the time to recurrence and OS after RFA in patients with HCC [16]. Thus, the RFA-combined therapy strategy for the treatment of HCC may provide promising results.

In recent years, tumor immunotherapy has made significant progress in inhibiting tumor progression [17]. Among them, immune checkpoint inhibitors offer great promise for the approval of nivolumab by the FDA on 23 September 2017, based only on a phase I/II clinical trial for the treatment of patients with HCC [18]. Recently, a previous study showed that nivolumab treatment improved OS and progression-free survival and appears safe in patients with postoperative recurrence of malignant pleural mesothelioma [19]. Thus, it is a strong hint that immune checkpoint inhibitors may achieve good clinical efficacy in patients with recurrent HCC. Meanwhile, several studies have shown that RFA can induce massive necrotic cell death, thus releasing large amounts of tumor antigens that might activate immunity and the presentation of cryptic antigens to induce tumor-specific T cell response [20–22]. Moreover, the combined therapy of RFA and anti-PD-1 antibodies exhibited stronger antitumor immunity and prolonged survival by enhancing T cell immune responses in mouse tumor models [23]. These results suggest that immune checkpoint inhibitors plus RFA may provide promising results in patients with recurrent HCC. However, this has not been reported to date. 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.

Methods

Patients

This retrospective study was approved by the Ethics Committee of Southwest Hospital, Army Medical University (Ethical approval number: KY2021046). Written informed consent was obtained from all patients before RFA treatment alone or RFA plus anti-PD-1 treatment. According to the Milan criteria, the inclusion criteria for this study were as follows: (a) patients aged 18–75 years; (b) patients were diagnosed with recurrent intrahepatic HCC lesions after hepatic resection or RFA; (c) patients had a solitary intrahepatic tumor or multiple tumors with three or two nodules each ≤3 cm in size; (d) patients had no extrahepatic metastases; (e) patients without invasion of the portal vein, hepatic vein trunk or secondary branches, or the bile duct; (f) no other antitumor therapy received before treatment, including radiotherapy, TACE, and targeted drugs; (g) 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) 1 month after RFA in this study; (h) patients received PD-1 blockade therapy for at least 1 month after RFA in this study. Patients were excluded from our study if they met the following criteria: (a) had severe portal hypertension, a history of esophageal variceal hemorrhage, severe hypersplenism syndrome, or refractory ascites currently or in history; (b) had serious heart, kidney, and other organ dysfunction; (c) had autoimmune disease currently or in history; (d) had other malignant tumors currently or in history; (e) had serious adverse events after PD-1 blockade therapy; (f) received other treatments (stem cell therapy, or immune cell therapy) during the study period.

Patients diagnosed with recurrent HCC were recommended for RFA plus anti-PD-1 treatment in principle; however, some patients chose regular examination after RFA for economic problems or personal reasons rather than combined treatment. Patients were classified into the RFA + anti-PD-1 group if they received RFA combined with anti-PD-1 treatment or the RFA group if they received only RFA treatment. 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 between November 2013 and December 2019 at Southwest Hospital, Army Medical University. Follow-up data collection was terminated on 30 April 2017. Follow-up data collection was terminated on 31 December 2020.

**RFA procedure**

All the patients included in this study were treated percutaneously with an LDRF-120S radiofrequency ablation device (Lead Electron Corporation, Mianyang, China) following the guidelines of Chinese Expert Consensus Statement issued by the Chinese Society of Liver Cancer and the Chinese Society of Clinical Oncology [23,24]. The RFA procedures were performed by physicians with at least 5-year of experience in ultrasound-guided hepatic RFA. Before RFA, each lesion was confirmed using contrast-enhanced ultrasonography. After local or general anesthesia, the radiofrequency electrode was placed on the tumor lesions under ultrasound guidance. To eliminate residual tumor cells in the treatment area, the open cool-tip electrode was at least 1 cm larger than the maximum diameter of the tumor, and the open cluster electrode was rotated clockwise in situ by 15 °C after closing and reopening for another treatment. Each tumor lesion was treated with a single electrode. For patients with multiple tumors, all lesions were ablated using the same procedure. After RFA for 15 min, 24 h and 48 h, contrast-enhanced ultrasound was used to detect complete destruction of each lesion. Contrast-enhanced CT or gadoxetic acid-EOB-MRI was performed to confirm whether the residual tumor was detected 1 month after RFA. If a residual tumor was still detected, the lesions were retreated using the same procedure.

**PD-1 blockade management**

Patients in the RFA plus PD-1 blockade (RFA + anti-PD-1) treatment group received 200 mg of camrelizumab intravenously every 2 weeks or 200 mg of sintilimab intravenously every 2 weeks and the initial administration was within 72 h after RFA. Patients received continuous PD-1 blockade treatment until unacceptable toxic effects occurred or there was a 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 decline, 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 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 on CT or MRI 1 month after RFA. The recurrence of HCC was defined as the appearance of local and distant tumor progression. The local tumor recurrence was defined as the appearance of enhancing lesions at the edge of the ablation site (<2.0 cm from the edge of the ablation site) during follow-up imaging [25]. 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 performed by two independent diagnostic radiologists with at least 5-year of experience. The RFS time was defined as the time from complete RFA to recurrence of HCC. The OS time was defined as the time from complete RFA to death or the last follow-up. Patients who remained alive at the last follow-up were considered censored events in the statistical analysis. RFA-related complications were evaluated according to the Dindo–Clavien classification [26].

**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 conducted on 31 December 2020. When tumor recurrence was identified during follow-up, patients were treated with RFA, surgery, TACE, and targeted drugs, depending on the clinical presentation such as the characteristics of the recurrent tumor and hepatic function.

**Statistical analysis**

Statistical analyses were performed using SPSS software (version 25.0; SPSS, Chicago, IL). To reduce the degree of bias or confounding in our results, a propensity score matching analysis was performed [27]. Patients from both groups were paired in a 1:1 ratio, and the caliper was set to 0.100. Ten covariates, including age, sex, chronic hepatitis B, liver cirrhosis, tumor number, tumor size, tumor-node metastasis (TNM), antiviral therapy, hepatic resection, and RFA, were included for propensity score generation. Baseline characteristics of patients were compared using Fisher’s exact test, Mann–Whitney U test and the χ² test for categorical data. Survival curves were analyzed using the Kaplan–Meier method. The equivalence of the survival curves was evaluated using log-rank statistics. Risk factors of statistical significance in the log-rank statistical analysis were subjected to multivariate survival analysis. All statistical tests were two-tailed, and the statistical significance was set at p < 0.05.

**Results**

**Baseline characteristics of patients**

A total of 127 patients with recurrent HCC underwent RFA plus anti-PD-1 (n = 41) or RFA alone (n = 86) in this study (Figure 1). Detailed baseline patient characteristics are presented in Table 1. There were no significant differences in
Figure 1. Flow chart of the patients included in the study.

Table 1. Baseline patient characteristics in the unmatched cohort and the matched cohort.

| Characteristic                        | Unmatched cohort | Matched cohort | p-Value | p-Value |
|---------------------------------------|------------------|----------------|---------|---------|
|                                       | RFA + anti-PD-1 (n = 41) | RFA (n = 86) | RFA + anti-PD-1 (n = 40) | RFA (n = 40) |
| Age                                   | 56.24 ± 10.22    | 54.44 ± 10.89 | 0.376   | 0.928   |
| Sex                                    |                  |                | 0.753   | 0.330   |
| Male                                  | 37 (90.2%)       | 76 (88.4%)     |         |         |
| Female                                | 4 (9.8%)         | 10 (11.6%)     |         |         |
| Underlying liver disease              |                  |                |         |         |
| Chronic hepatitis B                   | 34 (82.9%)       | 80 (93.0%)     | 0.079   | 0.745   |
| No                                    | 7 (17.1%)        | 6 (7.0%)       |         |         |
| Liver cirrhosis                       |                  |                | 0.325   | 0.644   |
| Yes                                   | 25 (61.0%)       | 60 (69.8%)     |         |         |
| No                                    | 16 (39.0%)       | 26 (30.2%)     |         |         |
| Tumor characteristics at initial diagnosis |                  |                | 0.750   | 1.000   |
| Tumor number                          |                  |                |         |         |
| 1                                     | 33 (80.5%)       | 65 (75.6%)     | 0.127   | 0.820   |
| 2                                     | 5 (12.2%)        | 15 (17.4%)     |         |         |
| 3                                     | 3 (7.3%)         | 6 (7.0%)       |         |         |
| Maximal tumor size (cm)               | 0.127            | 0.820          |         |         |
| ≤ 1.8                                 | 25 (61.0%)       | 40 (46.5%)     | 0.538   | 1.000   |
| > 1.8 and ≤ 3.0                       | 16 (39.0%)       | 46 (53.5%)     |         |         |
| TNM                                   |                  |                |         |         |
| I                                     | 33 (77.3%)       | 65 (75.6%)     | 0.357   | 0.793   |
| II                                    | 8 (22.7%)        | 21 (24.4%)     |         |         |
| Treatment history before this study   |                  |                |         |         |
| Antiviral therapy                     |                  |                | 0.012*  | 0.256   |
| Yes                                   | 31 (70.7%)       | 71 (82.6%)     |         |         |
| No                                    | 10 (29.3%)       | 15 (17.4%)     |         |         |
| Hepatic resection                     |                  |                |         |         |
| Yes                                   | 15 (36.6%)       | 52 (60.5%)     | 0.495   | 0.091   |
| No                                    | 26 (63.4%)       | 34 (39.5%)     |         |         |
| RFA                                   |                  |                |         |         |
| Yes                                   | 31 (75.6%)       | 60 (69.8%)     |         |         |
| No                                    | 10 (24.4%)       | 26 (30.2%)     |         |         |

RFA: radiofrequency ablation; TNM: tumor-node metastasis. *p < 0.05.
age, sex, chronic hepatitis B, liver cirrhosis, tumor number, tumor size, TNM, or RFA treatment between the two treatment groups (Table 1). Both groups had a male predominance. Patients with chronic hepatitis B and patients with liver cirrhosis accounted for the majority of patients in both groups. A total of 67 patients underwent hepatic resection, with a significant difference between the two treatment groups (36.6% and 60.5% of the RFA + anti-PD-1 group and RFA alone groups, respectively, $p = 0.012$) (Table 1). However, the number of patients with recurrent HCC after monotherapy with hepatic resection in the RFA + anti-PD-1 group was not lower than in the RFA alone group (24.4% versus 30.2%, $p = 0.495$) (Supplementary Table 1). 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% versus 39.5%, $p = 0.012$) (Supplementary Table 1). A total of 31 (24.4%) patients underwent hepatic resection and RFA treatment: six (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). After propensity score matching, there were no significant baseline differences between the two groups.

### Complications or adverse events

As shown in Table 2, there was no RFA treatment-related inhospital mortality in either group in this study. There was no grade 3 or higher events in our study, and the overall complication rate was similar between the two groups ($p = 0.762$). Adverse events in the RFA + anti-PD-1 group were as follows: pleural effusions that require drainage (1/41, 2.4%) and a mild or moderate increase in body temperature (3/41, 7.3%). Adverse events in the RFA alone group were as follows: pleural effusion requiring drainage (2/86, 2.3%) and a mild or moderate increase in body temperature (5/86, 8.1%).

The anti-PD-1-related adverse events are shown in Table 3. The frequency of treatment-related adverse events (TRAEs) of any grade was 70.2% (33/47). The frequency of grade 3 or higher events was 12.8% (6/47). The most common TRAEs of any grade were fatigue and pruritus. Laboratory TRAEs were infrequent in this study. Approximately <10% of the patients experienced TRAEs of any grade and <3% experienced TRAEs of grade 3 or higher. In addition, the most commonly occurring TRAEs of any grade were skin, gastrointestinal, and hepatic events, and grade 3 or higher sTRAEs occurred in <3% of the patients. Six patients with grade 3 or higher TRAEs were excluded from this study as they met the exclusion criteria.

### Survival

The median RFS (mRFS) was 39.1 weeks (95% confidence interval [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 in the RFA alone group ($p = 0.002$) (Figure 2). Similar results were observed for OS: RFA + anti-PD-1 group, 51.0 weeks versus RFA alone group, 47.6 weeks ($p = 0.008$) (Figure 2). After tumor recurrence, 18 patients who met the conditions of RFA were treated with RFA plus regorafenib, and eight patients who were not suitable for RFA were treated with regorafenib plus TACE in the RFA + anti-PD-1 group. In addition, patients after tumor recurrence in the RFA + anti-PD-1 group continued camrelizumab or sintilimab treatment. In the RFA alone group, 20 patients who met the RFA treatment after tumor recurrence were treated with RFA plus sorafenib, six patients who met the conditions of RFA after tumor recurrence were treated with RFA plus regorafenib, four patients after tumor recurrence were treated with hepatic resection plus regorafenib, and six who were not suitable for RFA were treated with regorafenib plus TACE.

When only the propensity score-matched patients were considered, the mRFS was 38.6 weeks (95% CI, 26.6–50.5) in the RFA + anti-PD-1 group and 16.7 weeks (95% CI, 9.0–24.5) in the RFA alone group. RFS was significantly longer in the RFA + anti-PD-1 group than in the RFA alone group (35.2 weeks versus 22.2 weeks, $p = 0.001$), and the OS was significantly longer in the RFA + anti-PD-1 group than in the RFA alone group (50.9 weeks versus 47.3 weeks, $p = 0.016$) (Figure 3).

**Subgroup analysis**

The HR and 95% CI of each subgroup were calculated using a stratified Cox regression model. As shown in Supplementary Table 2, the RFA + anti-PD-1 treatment provided significant clinical benefit for RFS and OS in subgroups analyzed, although some patients had characteristics...
associated with poor prognosis such as chronic hepatitis B, liver cirrhosis, two or three tumors, larger tumor size (maximum diameter > 1.8 cm), and higher stage of TNM. Similar results were observed in the propensity score-matched patients (Table 4).

**Uni- and multivariate analyses**

Univariate Cox regression analysis and multivariate Cox regression analysis were used to evaluate the predictors of RFS and OS in propensity score-matched patients. As shown in Table 5 and Supplementary Table 5, tumor number (HR, 4.027; 95% CI, 1.956 – 8.292; p = 0.000), TNM stage (HR, 4.027; 95% CI, 1.956 – 8.292; p = 0.000), antiviral therapy (HR, 0.373; 95% CI, 0.167 – 0.836; p = 0.017), and anti-PD-1 treatment (HR, 0.345; 95% CI, 0.196 – 0.608; p = 0.000) were significantly correlated with RFS in the univariate Cox regression analysis. Multivariate Cox regression analysis showed that tumor number (HR, 3.060; 95% CI, 1.606 – 5.830; p = 0.001), TNM stage (HR, 3.060; 95% CI, 1.606 – 5.830; p = 0.001), antiviral therapy (HR, 0.418; 95% CI, 0.247 – 0.713; 95% CI, 0.167 – 0.713; p = 0.001), antiviral therapy (HR, 0.418; 95% CI, 0.247 – 0.713; 95% CI, 0.167 – 0.713; p = 0.001)

**Table 4.** Subgroup analysis of recurrence and overall survival by the stratified Cox regression model in the matched cohort.

| Characteristic | Progression-free survival | Overall survival |
|----------------|---------------------------|-----------------|
|                | HR (95% CI) | p-Value | HR (95% CI) | p-Value |
| Age            |             |          |             |          |
| ≤53            | 0.396 (0.180 – 0.873) | 0.022* | 0.169 (0.020 – 1.446) | 0.104 |
| >53            | 0.466 (0.237 – 0.916) | 0.027* | 0.201 (0.023 – 1.720) | 0.143 |
| Sex            |             |          |             |          |
| Male           | 0.366 (0.210 – 0.637) | 0.000** | 0.189 (0.041 – 0.877) | 0.033* |
| Female         | 0.947 (0.224 – 4.006) | 0.940 | 0.024 (0.000 – 5557142.998) | 0.667 |
| Underlying liver disease |             |          |             |          |
| Chronic hepatitis B (Positive versus Negative) | 0.422 (0.240 – 0.739) | 0.003** | 0.191 (0.042 – 0.874) | 0.033* |
| Liver cirrhosis | 0.423 (0.099 – 1.806) | 0.246 | – | – |
| Tumor characteristics at initial diagnosis | | | | |
| Tumor size     |             |          |             |          |
| ≤1.8           | 0.340 (0.172 – 0.671) | 0.002** | 0.123 (0.015 – 1.001) | 0.050 |
| >1.8 and < 3.0 | 0.573 (0.260 – 1.263) | 0.167 | 0.355 (0.037 – 3.411) | 0.369 |
| TNM            |             |          |             |          |
| I              | 0.400 (0.224 – 0.713) | 0.002** | 0.014 (0.000 – 0.624) | 0.171 |
| II             | 0.518 (0.162 – 1.654) | 0.267 | 0.609 (0.101 – 3.675) | 0.589 |
| Treatment history before study RFA | | | | |
| Antiviral therapy | 0.402 (0.220 – 0.732) | 0.003** | 0.106 (0.013 – 0.847) | 0.034 |
| Hepatic resection | 0.564 (0.202 – 1.580) | 0.276 | 0.659 (0.060 – 7.277) | 0.734 |
| RFA            |             |          |             |          |
| Yes            | 0.535 (0.242 – 1.185) | 0.123 | 0.345 (0.039 – 3.085) | 0.341 |
| No             | 0.382 (0.194 – 0.751) | 0.005** | 0.124 (0.015 – 1.032) | 0.053 |
| Maximal tumor size (cm) |             |          |             |          |
| ≤1.8           | 0.340 (0.172 – 0.671) | 0.002** | 0.123 (0.015 – 1.001) | 0.050 |
| >1.8 and < 3.0 | 0.573 (0.260 – 1.263) | 0.167 | 0.355 (0.037 – 3.411) | 0.369 |
| TNM            |             |          |             |          |
| I              | 0.400 (0.224 – 0.713) | 0.002** | 0.014 (0.000 – 0.624) | 0.171 |
| II             | 0.518 (0.162 – 1.654) | 0.267 | 0.609 (0.101 – 3.675) | 0.589 |

**Table 5.** Univariate and multivariate analysis of the relative risk of recurrence and overall survival in the matched cohort.

| Characteristic | Progression-free survival | Overall survival |
|----------------|---------------------------|-----------------|
|                | p-Value | HR(95% CI) | p-Value | HR(95% CI) | p-Value |
| Age            | 0.100 |          | 0.776 |
| Sex (male versus female) | 0.068 |          | 0.029* |
| Chronic hepatitis B (Positive versus Negative) | 0.974 |          | 0.974 |
| Liver cirrhosis (Positive versus Negative) | 0.164 |          | 0.164 |
| Tumor number (multiple versus solitary) | 0.000** | 3.060 (1.606 – 5.830) | 0.001** | 0.004** | 4.042 (1.279 – 12.776) | 0.017* |
| Tumor size (≥ 1.8 versus < 1.8) | 0.331 | 0.271 |
| TNM stage (II versus I) | 0* | 0* | 0* |
| Antiviral therapy (yes versus no) | 0.017* | 0.418 (0.235 – 0.745) | 0.003** | 0.057 |
| Hepatic resection (yes versus no) | 0.094 | 0.815 |
| RFA (yes versus no) | 0.072 | 0.876 |
| Anti-PD1-1 treatment (yes versus no) | 0.000** | 0.416 (0.248 – 0.698) | 0.001** | 0.006* | 0.186 (0.041 – 0.848) | 0.030* |

*TNM: tumor number. *Statistically significant (p < 0.05); **statistically significant (p < 0.01).
that RFA and after the propensity score matching analysis showed [28,29]. Therefore, all patients in our study had better phys-

However, all studies on single-agent checkpoint inhibitor chemotherapy tests have been approved by the US FDA to calcu-

PD-L1, PD-1 and CTLA-4 contribute to the inhibition of thermal induces by RFA led to a detectable antitumor reactivity in mouse models [23,31]. Previous studies have shown that PD-

Previous studies have shown that single-agent checkpoint inhibitors do not show a better survival outcome in patients with HCC [28,29]. In this study, there were two important differences between our study and previous studies. First, all patients included in this study underwent curative RFA before anti-PD-1 therapy. RFA has been shown to induce T cell immune responses, as well as PD-L1 expression, in synchronous colorectal cancer liver metastases and tumor-bearing mice [23,30]. In addition, T cell immune responses induced by RFA led to a detectable antitumor reactivity in mouse models [23,31]. Previous studies have shown that PD-L1, PD-1 and CTLA-4 contribute to the inhibition of thermal ablation-induced antitumor activity [23,32]. Positive PD-L1 expression in patients is associated with an objective response to anti-PD-1 therapy [33,34]. PD-L1 immunohistochemistry tests have been approved by the US FDA to calcu-

In this study, the stratified Cox regression model before and after the propensity score matching analysis showed that RFA + anti-PD-1 treatment provided significant clinical benefits for RFS and OS in all analyzed subgroups. A previous report showed that liver metastases in patients with advanced melanoma were associated with reduced ORR and PFS during anti-PD-1 therapy [38]. In addition, a recent report showed that patients with an ECOG performance status of one or more, bone metastases, and liver metastases had shorter 5-year OS in advanced melanoma, renal cell carcinoma, and non-small cell lung cancer [39]. Therefore, these results suggest that anti-PD-(L)1 therapy will provide a better clinical benefit for patients with early HCC. Our results sup-

Indeed, this study, the RFA treatment-related adverse events were pleural effusion requiring drainage and a mild or mod-

A recent report showed that liver metastases in patients with advanced melanoma were associated with reduced ORR and PFS during anti-PD-1 therapy [38]. In addition, a recent report showed that patients with an ECOG performance status of one or more, bone metastases, and liver metastases had shorter 5-year OS in advanced melanoma, renal cell carcinoma, and non-small cell lung cancer [39]. 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. The findings of this study showed that patients with one tumor experienced a better clinical benefit in RFS and OS than those with two or three tumors (RFS: HR = 0.400 versus HR = 0.518; OS: HR = 0.014 versus HR = 0.609) during anti-PD-1 therapy in the matched cohort. Similar results were found in patients with TNM stage I tumors and smaller tumor size (maximum diameter ≤ 1.8 cm). Although some studies have not shown a better sur-

In this study, the stratified Cox regression model before and after the propensity score matching analysis showed that tumor number, TNM stage, and anti-PD-1 treatment were independent prognostic factors for RFS and OS in the unmatched cohort. In addition, multivariate analysis showed that tumor number and anti-PD-1 treatment were independent prognostic factors for OS before and after the propensity score matching analysis. A possible reason is that neovascular invasion and extrahepatic dissemination occurs more easily in patients with HCC with multiple lesions [40].

In this study, the stratified Cox regression model before and after the propensity score matching analysis showed that tumor number, TNM stage, and anti-PD-1 treatment were also significant prognostic factors for OS (Supplementary Tables 4 and 6).

Discussion

In this retrospective study, we first reported the efficacy of anti-PD-1 therapy on RFS and OS outcomes in recurrent HCC after curative RFA treatment before and after the propensity score matching analysis. Our results showed that patients with recurrent HCC had significantly better RFS and OS outcomes in the RFA + anti-PD-1 group than in the RFA alone group before and after propensity score matching analysis. In addition, multivariate analysis showed that anti-PD-1 therapy was an independent prognostic factor for PFS and OS in recurrent HCC after curative RFA before and after propensity score matching analysis.

Previous studies have shown that single-agent checkpoint inhibitors do not show a better survival outcome in patients with HCC [28,29]. In this study, there were two important differences between our study and previous studies. First, all patients included in this study underwent curative RFA before anti-PD-1 therapy. RFA has been shown to induce T cell immune responses, as well as PD-L1 expression, in synchronous colorectal cancer liver metastases and tumor-bearing mice [23,30]. In addition, T cell immune responses induced by RFA led to a detectable antitumor reactivity in mouse models [23,31]. Previous studies have shown that PD-L1, PD-1 and CTLA-4 contribute to the inhibition of thermal ablation-induced antitumor activity [23,32]. Positive PD-L1 expression in patients is associated with an objective response to anti-PD-1 therapy [33,34]. PD-L1 immunohistochemistry tests have been approved by the US FDA to calcu-

A recent report showed that liver metastases in patients with advanced melanoma were associated with reduced ORR and PFS during anti-PD-1 therapy [38]. In addition, a recent report showed that patients with an ECOG performance status of one or more, bone metastases, and liver metastases had shorter 5-year OS in advanced melanoma, renal cell carcinoma, and non-small cell lung cancer [39]. 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. The findings of this study showed that patients with one tumor experienced a better clinical benefit in RFS and OS than those with two or three tumors (RFS: HR = 0.400 versus HR = 0.518; OS: HR = 0.014 versus HR = 0.609) during anti-PD-1 therapy in the matched cohort. Similar results were found in patients with TNM stage I tumors and smaller tumor size (maximum diameter ≤ 1.8 cm). Although some studies have not shown a better sur-

In this study, the stratified Cox regression model before and after the propensity score matching analysis showed that tumor number, TNM stage, and anti-PD-1 treatment were independent prognostic factors for RFS and OS in the unmatched cohort. In addition, multivariate analysis showed that tumor number and anti-PD-1 treatment were independent prognostic factors for RFS and OS before and after the propensity score matching analysis. A possible reason is that neovascular invasion and extrahepatic dissemination occurs more easily in patients with HCC with multiple lesions [40].

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findings. In addition, the follow-up time was only 1 year. Therefore, patients should be followed up for assessment of RFS and OS for at least 3–5 years in future studies.

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

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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