

A well-matched marriage of immunotherapy and radiofrequency ablation to reduce the relapse and progression of hepatocellular carcinoma

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SUMMARY Hepatocellular carcinoma (HCC) remains a health challenge with increasing incidence worldwide. Radiofrequency ablation (RFA) is a potentially curative option for patients with early-stage HCC. However, the high rate of tumor recurrence limits long-term survival when the tumors are larger than 2 cm and undergoing insufficient RFA (iRFA). Notably, in situ tumor necrosis due to thermal ablation is assumed to be a source of antigens that induce antitumor immunity. Therefore, mounting studies and trials have attempted to provide a rational and effective therapeutic strategy combining RFA and immunotherapy to treat HCC. Nowadays, many controversies and challenges with this combined therapeutic strategy remain to be resolved, such as the indications for adjuvant immunotherapy along with RFA in early HCC, the sequence of the two treatments in advanced HCC, and the optimal timing of immunotherapy before or after RFA. In addition, individualized treatment strategies need to be perfected for patients with HCC.

Keywords immunotherapy, radiofrequency ablation, hepatocellular carcinoma, recurrence, metastasis

Hepatocellular carcinoma (HCC) is one of the most common malignancies with typical immunogenic tumor characteristics, and it develops most frequently in the context of chronic hepatitis virus infection and cirrhosis (1). Although surgical resection and liver transplantation are potentially curative options for patients with early-stage HCC, the high rate of tumor recurrence limits long-term survival (2). A point worth noting is that treatment with radiofrequency ablation (RFA) is widely accepted as a first-line therapeutic approach for early HCC, and its advantages are a high level of efficacy, a low incidence of complications, and low cost. Compared to surgery, RFA alone has a discouraging 5-year survival rate, with a high rate of HCC recurrence as the main problem post-ablation, and particularly for tumors more than 2-3 cm in size (3). To resolve this dilemma, recent efforts have focused on a combination of RFA and immunotherapy as a multimodal treatment strategy. So far, there is a lack of reliable comparative clinical data as to whether immunotherapy could really reduce additional sessions of RFA to treat HCC and halt cancer progression. Therefore, the current recommendations for combined strategies involving RFA and distinct

immunotherapies need to be devised for this deadly disease, and the long-term effects of this promising approach need to be validated further.

1. Use of RFA to treat HCC

RFA is a common minimally invasive therapeutic technique to destroy HCC through a Joule effect induced by the generation of a high-frequency alternating current and local heat from an electrode tip inserted into neoplastic tissues. RFA can produce tissue hyperthermia to achieve tumor necrosis at 375-480 kHz. Currently, heat-ablated lesions are surmised to consist of three zones: a central zone (> 60°C), a transitional zone (43-50°C), and the surrounding tissue unaffected by ablation (4). In the transitional zone, a tumor could undergo sublethal injury and result in tumor dissemination after insufficient RFA (iRFA). International clinical practice guidelines on HCC do not recommend the use of RFA for tumors larger than 5 cm due to the high risk of residual tumors (2). Aside from tumor size, multiple studies (5,6) have confirmed several clinical characteristics associated with a risk

of local recurrence, such as multiple tumor nodules, poorly defined HCC margins, and the location of the tumor near major intrahepatic blood vessels. As well as local recurrence, iRFA was also found to be significantly related to distant recurrence (7). Based on HCC guidelines, RFA is not a curative treatment option for patients with cancer in the intermediate (BCLC B) or advanced (BCLC C) stage. That said, if treating HCC with transarterial chemoembolization (TACE) and systemic therapies yields no better efficacy, then this task would be an insurmountable mountain. Fortunately, thanks to the advent of the genomic era as well as an in-depth understanding of the immune response generated directly by RFA in the tumor microenvironment, the combination of RFA and immunotherapy seems to be a potential therapeutic strategy for decreasing the recurrence and metastasis of HCC.

2. Immune response induced by RFA when treating HCC

Research has firmly established that HCC is not a local disease even in the early stages. In fact, ablation treatments physically eliminate local tumors but also play a considerable role in distant lesions through an immune response, named the "abscopal effect". One possible rationale is that thermally induced in situ tumor necrosis can constantly give rise to antigens that induce antitumor immunity (8). In 2003, Wissniowski *et al.* (9) reported the first study to identify an RFA-mediated antitumor response and they found a potential immunological effect on tumor growth due to a tumor-specific T-cell response elicited 2 weeks after RFA treatment. A recent study involving a mouse model revealed that iRFA enhanced the immunosuppressive environment, increasing CD11b⁺CD15⁺ polymorphonuclear-myeloid-derived suppressor cells (PMN-MDSCs) and decreasing CD8⁺ T cells, and it subsequently promoted tumor growth and metastasis (10). However, mounting evidence has revealed that dendritic cell (DC), natural killer (NK) cell, and CD4⁺ and CD8⁺ T-cell responses to tumors and systemic immune variations increased significantly following RFA (11,12). The prevailing view posits that tumor-specific immune responses facilitate an increased innate immune response and reduced immunosuppression after RFA. However, inconsistent findings are constantly emerging. For example, CD4⁺ and CD8⁺ T cells played a temporary antitumor role in RFA-treated mice that was quickly tampered by active immune suppression responses and then followed by a higher regulatory T-cell to CD8⁺ cell ratio and increased PD-L1/PD-1 expression (13). A clinical observation of patients with HCC receiving RFA revealed that the memory phenotype and survival time of enhanced tumor-associated antigen-specific T-cells following RFA were not sufficient to impede HCC recurrence (14). Hence, these results

are thought provoking because anti-tumor immune responses initiated by RFA are not strong enough to eliminate all tumor cells, potentially leading to the recurrence of HCC.

3. Current status of and challenges with this combination therapy

In clinical practice, there is contradictory evidence regarding which population is eligible for RFA and immunotherapy. On one hand, RFA is recommended for patients with very early and early stage HCC who could not be suitable for immunotherapy. On the other hand, RFA is considered to be a curative treatment in early HCC instead of an adjuvant approach like immunotherapy for patients with HCC in the intermediate or advanced stage. The rationale for this novel combined therapeutic strategy is based on the positive results of basic studies and pilot trials. The promising results of immunotherapy in patients with advanced HCC imply that adjuvant treatment with RFA is a possible option in early to intermediate stage HCC. Notably, the STORM trial noted no benefit in terms of recurrence-free survival (RFS) in patients with HCC receiving the adjuvant sorafenib, an oral multityrosine kinase inhibitor, post-resection or ablation (15). Undoubtedly because of this, the combined therapy strategy is also attracting the attention of clinicians who want to know whether enhanced anti-tumor immunity may optimize RFA to treat HCC.

In a multicenter, randomized, open-label, phase 3 trial conducted in South Korea, Lee *et al.* (16) reported that adjuvant immunotherapy with activated cytokine-induced killer (CIK) cells increased recurrence-free and overall survival (OS) in patients with HCC who underwent curative treatment with surgical resection, RFA, or percutaneous ethanol injection. A combined treatment involving RFA and cellular immunotherapy (CIT) yielded similar results in patients with HCC (17). In this trial, autologous mononuclear cells inducing NK cells, $\gamma\delta$ T cells, and CIK cells were infused intravenously to patients within 8-11 days of RFA. The preliminary results implied that a combination of sequential CIT and RFA may prevent the recurrence of HCC in patients after RFA. In another clinical trial, Kitahara *et al.* also demonstrated that RFA + administration of OK432-stimulated DCs to a necrotic tumor improved RFS in patients with HCC (18). Based on the results of CheckMate-040 and KEYNOTE-224, nivolumab and pembrolizumab, two immune checkpoint inhibitors (ICIs) causing a PD-1 blockade, were approved as second-line therapy for patients with HCC after sorafenib failure. Interestingly, a propensity score matching analysis of recurrent HCC revealed that combination therapy with anti-PD-1 inhibitors (initially administered within 72 hours of RFA) and RFA resulted in longer RFA and OS than RFA alone (19). Greten *et*

al. reported a survival analyses and immune monitoring data via anti-CTLA4 (tremelimumab) treatment prior to subtotal RFA or chemoablation in patients with advanced HCC (20). In contrast to previous studies, the median OS rate of patients treated with RFA was 9.2 months (95% CI: 6.6–11.2 months). They also demonstrated the safety and feasibility of anti-CTLA4 treatment plus RFA and they observed the clear activation of T cell responses. Table 1 reviews clinical trials involving combinations of RFA and immunotherapy to treat HCC.

4. Conclusions and perspectives

Nowadays, many controversies and challenges remain to be resolved as various proof-of-concept clinical trials are underway. First, would adjuvant immunotherapy for early HCC beyond the Milan criterion have a further survival benefit for completely ablated HCC according to imaging? Second, which treatment should be used first in advanced HCC since both are adjuvant therapies?

The frequency of ablation and immunotherapy still needs to be specified. Third, current clinical trials have no satisfactory answer regarding the optimal timing of immunotherapy to produce a synergistic effect with RFA. Most importantly, ablative immunotherapy has yielded better outcomes in some studies but the objective response rate has yet to improve, so the combination therapy could be administered to all patients with HCC. Consequently, individualized treatment strategies need to be taken into consideration in combined therapy for HCC. Nonetheless, immunotherapy is an option to compensate for the deficiencies of RFA in treating HCC. Rational and standardized clinical use of this therapy is still a long way away.

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Table 1. Clinical trials registered with the NIH investigating combinations of RFA and immunotherapy to treat HCC

Study Registrant	Trial ID (Phase)	Number of Patients	Clinical Title	Treatment	Study Period
Greten TF, <i>et al.</i>	NCT01853618 (Phase I/II)	61	A Pilot Study of Tremelimumab - A Monoclonal Antibody Against CTLA-4 in Combination with Trans-Arterial Catheter Chemoembolization (TACE), Radiofrequency Ablation (RFA), or Cryoablation in Subjects with Hepatocellular Carcinoma (HCC) or Biliary Tract Carcinomas (BTC)	RFA/TACE/Cryoablation + Tremelimumab	5/2013-6/2017
Lai K, <i>et al.</i>	NCT03124498 (Phase I/II)	55	A Study to Evaluate Autologous CIK Cells in Patients with Hepatocellular Carcinoma After TACE, PEIT or RFA	RFA/TACE/PEIT + CIK	11/2017-6/2019
Zhao M, <i>et al.</i>	NCT03939975 (Phase II)	50	A Prospective Study of Anti-PD-1 Inhibitor Therapy in Combination with Incomplete Thermal Ablation in Patients with Advanced Hepatocellular Carcinoma	Thermal Ablation + PD-1	6/2019-7/2019
Peng ZW, <i>et al.</i>	NCT02678013 (Phase III)	210	Radiofrequency Ablation Combined with Highly-purified CTL <i>vs.</i> Radiofrequency Ablation Alone for Recurrent HCC	RFA + CTL	1/2016-1/2022
Kuansheng Ma, <i>et al.</i>	NCT05277675 (NA)	160	A Prospective Study of Radiofrequency Ablation Combined with Systematic Neoadjuvant Therapy in the Treatment of Recurrent Hepatocellular Carcinoma	RFA + Tislelizumab/Sintilimab + Lenvatinib/Bevacizumab	1/12/2021-30/10/2023
Zhou JX, <i>et al.</i>	NCT05162898 (Phase II)	90	Radiofrequency Ablation Combined with Toripalimab and Lenvatinib in the Treatment of Short-term Recurrent Hepatocellular Carcinoma	RFA + Toripalimab + Lenvatinib	1/2022-12/2025
Renier W, <i>et al.</i>	NCT04727307 (Phase II)	202	Neoadjuvant Atezo, Adjuvant Atezo + Beva Combined with RF Ablation of Small HCC: A Multicenter Randomized Phase II Trial	RFA + the Neoadjuvant Atezolizumab, and the Adjuvants Atezolizumab + Bevacizumab	1/2022-7/2027
Chen MS, <i>et al.</i>	NCT04652440 (Phase II)	30	Phase II Study of Ablation Combined With PD-1 Antibody in Patients with Hepatocellular Carcinoma	RFA/MWA + PD-1	12/2020-12/2023
Kuang M, <i>et al.</i>	NCT03067493 (Phase II)	98	RFA or Surgical Resection Combined with Neo-MASCT for Primary HCC: A Phase II Trial	RFA/Surgical Resection + Neo-MASCT	1/2022-

Abbreviations: NIH: National Institutes of Health; RFA: radiofrequency ablation; HCC: hepatocellular carcinoma; CTLA-4: cytotoxic T lymphocyte-associated protein-4; TACE: trans-arterial catheter chemoembolization; PEIT: percutaneous ethanol injection treatment; CIK: cytokine-induced killer; PD-1: programmed cell death protein-1; CTL: cytotoxic T lymphocyte; NA: not applicable; MWA: microwave ablation; MASCT: multiple antigen-stimulating cellular therapy.

Conflict of Interest: The authors have no conflicts of interest to disclose.

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