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# Clinical Research Progress on Radiofrequency Ablation Combined with Immunotherapy for Hepatocellular Carcinoma

**Ruiqi Liu, Xiujuan Liu***

Department of Radiology, Zhuhai Clinical Medical College of Jinan University (Zhuhai People's Hospital, The Affiliated Hospital of Beijing Institute of Technology), Zhuhai, China
Email: liuruiqi8253@163.com, *liuxiujuan2005@126.com

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## Abstract

Hepatocellular carcinoma is one of the leading causes of cancer-related deaths worldwide. As an effective curative treatment for early-stage hepatocellular carcinoma, radiofrequency ablation is widely used due to its minimally invasive nature, simplicity, and repeatability, however, its postoperative recurrence rate remains relatively high. Immunotherapy, particularly immune checkpoint blockade, has made significant progress in recent years, offering new treatment options for cancer. Combined immunotherapy is considered an important strategy for reducing recurrence and delaying progression after radiofrequency ablation. This review provides a systematic summary of the immunological basis of radiofrequency ablation, combined strategies of radiofrequency ablation with immunotherapy, recent clinical research progress, and discusses the challenges in this field. It aims to provide theoretical and practical references for the comprehensive treatment of hepatocellular carcinoma.

## Keywords

Hepatocellular Carcinoma, Radiofrequency Ablation, Immunotherapy, Interventional Radiology

## 1. Introduction

Primary liver cancer is the third leading cause of cancer-related death worldwide [1]. Among these, hepatocellular carcinoma (HCC) accounts for 75% - 85% of cases. Surgical resection remains the first-line curative option for HCC, but it is only suitable for early-stage patients with adequate hepatic functional reserve. For

those ineligible for surgery, ablation therapy offers an alternative curative approach [2] [3]. Common ablation modalities include radiofrequency ablation (RFA), microwave ablation (MWA), high-intensity focused ultrasound (HIFU), cryoablation, and irreversible electroporation (IRE). Among these, RFA is the most established and widely utilized. RFA induces coagulative necrosis by heating tumor tissue with high-frequency electrical currents, offering the advantages of minimal invasiveness, rapid recovery, and preservation of liver function. It is especially effective for small HCC (e.g., tumor diameter < 3 cm), achieving outcomes comparable to surgical resection [4]. However, RFA has limitations: its ablation zone is restricted and not recommended for larger tumors (e.g., tumor diameter > 5 cm), where recurrence rates exceed those of surgical resection [5]. Effects are also suboptimal for tumors adjacent to major blood vessels [6].

Therefore, enhancing RFA efficacy and reducing postoperative recurrence of HCC have become focal points in clinical practice. In recent years, the success of immunotherapy, especially immune checkpoint blockade (ICB) therapy, in cancer treatment has spurred interest in combining RFA with immunotherapy for HCC. RFA induces immunogenic cell death (ICD), creating a tumor microenvironment favorable for immune activation. By combining RFA with immunotherapy, it is possible to sustain and amplify this immune response, thereby reducing the risk of recurrence and metastasis. This review systematically summarizes the immunological basis of RFA, the combined strategies of RFA with immunotherapy, recent clinical research progress, and discusses the challenges in this field, aiming to provide guidance for the treatment of HCC.

## 2. Immunological Basis of RFA

Numerous studies have demonstrated that RFA is not merely a physical therapy but also exerts positive immunomodulatory effects in tumor treatment. RFA induces tumor cell death via thermal injury, primarily causing coagulative necrosis and apoptosis, thereby leading to ICD and the release of various damage-associated molecular patterns (DAMPs) such as high-mobility group box 1, adenosine triphosphate, and calreticulin, as well as tumor-associated antigens (TAAs). These molecules act as "danger signals" that activate the immune system and trigger a series of immune responses [7].

ICD induced by RFA serves as the starting point of immune activation, promoting the activation and infiltration of various immune cells. In terms of innate immunity, RFA can promote the polarization of M1-type macrophages at the early stage after treatment, which usually exert antitumor effects, as reflected by the increased number of intratumoral $CD86^+$ macrophages [8]; RFA enhances the infiltration and activation of antigen-presenting cells, particularly dendritic cells (DCs) [9], whose antigen-presenting capacity is markedly improved, stimulating T-cell activation and proliferation and activating natural killer (NK) cells [10]; RFA also enhances NK cell cytotoxicity to stimulate innate antitumor immunity, with a significant increase in peripheral NK cell proportion after ablation, accom-

panied by upregulation of interferon-$\gamma$ and tumor necrosis factor-$\alpha$ levels, as well as enhanced expression of NK cell activating receptor NKG2D on HCC cells, which is critical for NK cell recognition and targeting of tumor cells [11] [12]. In terms of adaptive immunity, the abundance of $CD4^+$ and $CD8^+$ T cells significantly increases in both peritumoral regions and peripheral blood after RFA, inducing tumor-specific cytotoxic T cell responses with significantly enhanced cytolytic activity of $CD8^+$ T cells [13] [14]; in addition, RFA can trigger a "systemic immune response" and induce antitumor effects in distant, non-ablated tumors, known as the "abscopal effect," suggesting that RFA may act as an "in situ vaccine" that stimulates the immune system to combat both primary tumors and distant metastases [15].

However, the immune responses induced by RFA alone are often weak and short-lived, mainly due to dynamic changes in the tumor microenvironment after RFA. The total numbers of $CD4^+$ and $CD8^+$ T cells, particularly effector and memory T cells, gradually decrease over time after RFA, accompanied by downregulation of cytotoxicity-related genes and reduced expression of CD5 and CD161 in T-cell subsets, indicating that RFA may induce T-cell "inactivation" or exhaustion [16]. Moreover, after RFA, the numbers of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) gradually increase, and macrophages polarize from the immunostimulatory M1 phenotype to the immunosuppressive M2 phenotype, further impairing $CD8^+$ T-cell activity [17] [18]. Meanwhile, the expression of immune checkpoint molecules is upregulated in the tumor microenvironment after RFA, mainly observed in tumor cells and immunosuppressive stromal cells such as MDSCs and macrophages, suggesting potential immune evasion mechanisms [18] [19]. RFA also leads to increased expression of immunosuppressive cytokines such as interleukin-10 and transforming growth factor-$\beta$ in the peritumoral region, which further weakens immune activation after RFA [20]. Meanwhile, RFA alone yields a weak abscopal effect that is insufficient to elicit adequate immune activation in distant metastases [21].

In summary, RFA can create a tumor microenvironment favorable for immune activation, but RFA alone is insufficient to sustain durable and effective antitumor immunity. Therefore, it is necessary to combine RFA with immunotherapy to maintain and enhance immune activation, suppress local residual tumor, amplify abscopal effects to eradicate distant micrometastases and reduce the risk of postoperative recurrence and metastasis.

## 3. Combined Strategies of RFA and Immunotherapy

Currently, immunotherapy has become an important systemic treatment modality for HCC, changing the therapeutic landscape of HCC. Tumor cells often possess immune evasion capabilities, primarily manifested as T-cell exhaustion and immunosuppression [22]. ICD induced by RFA provides the antigen "fuel" for immune activation, promoting tumor antigen presentation and T-cell priming.

Immunotherapy, especially ICB therapy, aims to relieve tumor microenvironment-mediated T-cell suppression, restoring T-cell recognition and cytotoxicity against tumor cells. RFA and immunotherapy play complementary roles in immune activation and show enormous potential. In recent years, the use of immunotherapy in early-stage HCC as adjuvant treatment to RFA has become a research hotspot, with main strategies including ICB therapy, cell therapy, and tumor vaccines.

### 3.1. ICB Therapy Combined with RFA

Immune checkpoints are receptors or ligands expressed on T cells, tumor cells, and antigen-presenting cells that maintain immune tolerance by inhibiting T-cell activity [23]; however, their overexpression on tumor cells often leads to immune evasion [24]. ICB therapy employs immune checkpoint inhibitors (ICIs) to disrupt these receptor-ligand interactions and restore antitumor immunity. The programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) axis is the most frequently targeted checkpoint, and monoclonal antibodies against PD-1 or PD-L1—such as nivolumab, pembrolizumab, and atezolizumab—have been approved for treatment of HCC. Nonetheless, objective response rates to PD-1/PD-L1 monotherapy remain modest [25], and combination regimens have been explored to enhance efficacy.

Currently, the combination of atezolizumab and bevacizumab (a VEGF inhibitor) serves as the first-line systemic therapy for advanced HCC, as demonstrated in the IMbrave150 trial [26]. IMbrave050 was the first positive Phase III trial for postoperative adjuvant therapy in HCC: 668 high-risk patients post-ablation (RFA or MWA) or resection were randomized (334 per arm) to receive adjuvant atezolizumab plus bevacizumab or active surveillance. At 12 months, recurrence-free survival (RFS) was 78% in the combination arm versus 65% in the surveillance arm, corresponding to a 28% reduction in the risk of recurrence or death (hazard ratio (HR) 0.72, adjusted 95% confidence interval (CI) 0.53 - 0.98; P = 0.012). This study first confirmed the regimen's efficacy as postoperative adjuvant therapy in HCC, advancing immunotherapy into earlier settings. However, treatment-related adverse events occurred in 98% of combination-treated patients (vs. 62% in controls), including immune-related toxicities in 63% (vs. 18%), indicating that optimizing administration strategies may help balance efficacy and safety [27]. Wen *et al.* evaluated 48 patients with recurrent HCC and found that RFA plus toripalimab yielded a lower tumor progression rate than RFA alone (45% vs. 80%) and higher 18-month RFS (48.7% vs. 18.8%) [28]. Wang *et al.*'s retrospective analysis of 127 recurrent HCC patients demonstrated that RFA combined with PD-1 inhibitors (camrelizumab or sintilimab) significantly prolonged 1-year RFS (32.5% vs. 10.0%), median RFS (38.6 vs. 16.7 weeks, P = 0.001) and median overall survival (OS) (50.9 vs. 47.3 weeks, P = 0.016) [29]. Zhou *et al.* conducted a multicenter, randomized Phase I/II trial. In the first stage, 48 patients with previously treated, unresectable HCC were randomized to RFA or MWA plus toripalimab

versus toripalimab alone. The combination arm achieved a higher objective response rate (ORR) than monotherapy, and initiating toripalimab on day 3 post-ablation (Schedule D3) yielded better responses than starting on day 14 (Schedule D14). In the second stage, 98 eligible patients were randomized to Schedule D3 versus toripalimab alone. Schedule D3 significantly improved median progression-free survival (PFS) (7.1 vs. 3.8 months; HR = 0.57; P < 0.001) and median OS (18.4 vs. 13.2 months; HR = 0.58; P = 0.005) compared with monotherapy [30]. Lyu *et al.* reported that combining anti-PD-1 therapy (nivolumab or pembrolizumab) with RFA or MWA markedly increased ORR and extended both PFS and OS in advanced HCC, with manageable toxicity [31]. These studies indicate that combining ICB therapy with RFA exerts a more favorable antitumor effect in HCC than monotherapy. Moreover, several large Phase III trials are underway to validate adjuvant PD-1/PD-L1 inhibition post-RFA in HCC, including CheckMate 9DX (NCT03383458; nivolumab) [32], KEYNOTE-937 (NCT03867084; pembrolizumab) [33], and EMERALD-2 (NCT03847428; durvalumab ± bevacizumab) [34].

Another key target is cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which inhibits T-cell activation by competitively binding CD80/CD86 [35]. Its inhibitors, such as ipilimumab and tremelimumab, are clinically approved. Sangro *et al.* were the first to conduct relevant research. They treated 21 HCC patients with chronic HCV using tremelimumab, successfully inducing antiviral immune responses and reducing viral loads [36]. Duffy *et al.* further combined tremelimumab with RFA in 32 BCLC B/C HCC patients; on day 36 ± 96 hours, they performed subtotal RFA or cryoablation, with transarterial chemoembolization (TACE) for BCLC B. They observed partial responses in 5 patients, significant HCV viral load reductions in 12, and increased intratumoral $CD8^+$ TILs in responders [37]. Agdashian *et al.* used a similar design in 39 refractory advanced HCC patients, administering tremelimumab followed by subtotal RFA/ cryoablation/TACE on day 35; median OS was 10.9 month, and peripheral activated T-cell subsets rose markedly ($CD4^+HLA\text{-}DR^+$ + 65%, $CD8^+HLA\text{-}DR^+$ + 106%, $CD4^+ICOS^+$ + 444%, $CD8^+ICOS^+$ + 528%; all P < 0.0001), while $CD3^+$ tumor-infiltrating lymphocytes increased from 0.78% to 2.45% (P = 0.012) [38]. These data highlight CTLA-4 blockade's immunomodulatory potential, but its later development and higher systemic toxicity have limited its clinical use compared with PD-1/PD-L1 inhibitors [39], and large Phase III results are still pending.

### 3.2. Cell Therapy

Cell therapy involves ex vivo activation and expansion of immune cells, which are then reinfused to precisely target tumor cells. While cell therapy has achieved remarkable success in hematologic malignancies, its application in solid tumors, including HCC, has expanded in recent years, and its combination with RFA has drawn considerable interest.

DC-based therapy is a major focus in this context. Kitahara *et al.* enrolled 30

HCV-related HCC patients who had undergone RFA. 14 received infusions of DCs activated with the immunostimulant OK-432 (OK432-DC), while 16 received unmodified "baseline" DCs as controls. The OK432-DC group exhibited significantly longer RFS (24.8 vs. 13.0 months; P = 0.003), although OS did not differ between groups (67.8 vs. 73.4 months; P = 0.780). Among 17 HLA-A24$^+$ patients, 35.3% mounted an IFN-$\gamma$ response to at least one TAA peptide, indicating that DC infusion aids in the generation of tumor-associated antigen-specific cytotoxic T lymphocytes [40]. Building on ex vivo DC activation, DC vaccines can be further loaded with TAAs to generate specificity. Peng *et al.* performed whole-exome sequencing on tumor specimens from 10 HCC patients treated by resection or RFA, synthesized personalized neoantigen peptides, and manufactured a personalized DC vaccine plus neoantigen-activated T cells. Patients received 18 cycles of DC vaccine + adoptive T-cell therapy, yielding encouraging results: 70% developed novel neoantigen-specific T-cell responses, and 71.4% of responders remained recurrence-free at 2 years [41]. Conversely, Lee *et al.* reported that DC vaccines loaded with TAAs reduced recurrence in non-RFA patients but paradoxically increased recurrence in RFA-treated patients—possibly due to more rapid DC exhaustion in the RFA setting. The underlying mechanisms remain to be elucidated [42].

Another notable cell therapy is cytokine-induced killer (CIK) cell therapy, in which peripheral blood mononuclear cells are stimulated ex vivo with cytokines to generate highly cytotoxic effectors bearing both T-cell and NK-cell characteristics, then reinfused to target tumor cells. Lee *et al.* conducted a Phase III multicenter randomized trial in 230 postoperative HCC patients (after RFA, resection, or percutaneous ethanol injection), randomizing 115 to CIK and 115 to control. The CIK arm achieved significantly longer median RFS (44.0 vs. 30.0 months; HR 0.63; 95% CI 0.43 - 0.94; P = 0.010) and prolonged OS (HR 0.21; 95% CI 0.06 - 0.75; P = 0.008). Treatment-emergent adverse events were predominantly grade 1 - 2, and no treatment-related deaths occurred in the CIK group [43]. Yoon *et al.* compared 59 Phase I/II HCC patients (post-resection or RFA), finding that median RFS was not reached in the CIK arm versus 29.8 months in controls (HR 0.42; P = 0.006). Safety was favorable, with no ≥ grade 3 AEs reported [44]. Ji *et al.* evaluated CIK combined with RFA + TACE in HCC patients. The RFA + TACE + CIK group achieved a mean OS of 42.1 ± 5.6 months versus 37.8 ± 4.8 months in the RFA + TACE only arm; 5-year OS rates were 29.3% versus 13.8% (log-rank P = 0.045) [45].

Emerging cell-based therapies such as T cell receptor-engineered T cells and chimeric antigen receptor T cells have shown preliminary efficacy in HCC immunotherapy, but none have been investigated in combination with RFA [46]-[48].

### 3.3. Tumor Vaccine

The core mechanism of tumor vaccines is to deliver TAAs to elicit tumor-specific T-cell immunity. Vaccine platforms include protein/peptide, nucleic acid, cellu-

lar, viral-vector vaccines, etc. In HCC, the glypican-3 (GPC3) peptide vaccine is among the most well-studied: as a TAA highly expressed in HCC, GPC3 peptides can induce HCC-specific immune responses [49]. Taniguchi *et al.* treated 35 post-operative HCC patients with the GPC3 peptide vaccine and observed that the 1-year recurrence rate was 25.7% versus 42.4% in controls; 5-year OS was 70.6% versus 57.6%; and 8-year OS was 67.1% versus 38.9%. Among GPC3 IHC-positive patients, 60.0% mounted a specific CTL response versus 16.7% of IHC-negative patients [50]. In a Phase I/II trial, Sawada *et al.* showed that adjuvant GPC3 vaccination after resection or RFA reduced the 1-year recurrence rate in GPC3-positive patients from 48% in controls to 24% in the vaccine arm ($P = 0.047$), with robust induction of tumor-specific immunity [51] [52]. Other vaccine approaches are also in development. Löffler *et al.* conducted a multicenter Phase I/II study in 22 HLA-matched, early- to intermediate-stage HCC patients (including six post-RFA). After low-dose cyclophosphamide preconditioning, patients received subcutaneous IMA970A, a 17-peptide HCC TAA vaccine, combined with the CV8102 RNA adjuvant. Among 19 evaluable patients, this regimen induced HLA-I peptide responses in 37%, HLA-II responses in 53%, and vaccine-specific T-cell responses in 68.4% [53]. DC-based vaccines also constitute a form of tumor vaccination; their progress has been detailed above under cell-based therapies.

## 4. Discussion

RFA combined with immunotherapy has demonstrated substantial therapeutic potential, but several critical challenges remain to be addressed.

### 4.1. Incomplete RFA

The greatest limitation of RFA is incomplete RFA (iRFA), a major driver of increased local recurrence. This stems from RFA's reliance on tissue conductivity: as surrounding tissue heats and chars, its conductivity drops sharply, constraining the ablation zone, a phenomenon known as the roll-off effect [54]. When tumors abut large intrahepatic vessels, convective heat loss further restricts the ablation margin, a phenomenon known as the heat-sink effect [55]. Together, these effects limit the efficacy of RFA for larger lesions (typically >3 - 5 cm). iRFA not only fails to eradicate the tumor but may also enhance the aggressiveness of residual disease, leading to tumor progression [56]-[58]. Even more concerning, iRFA induces persistent local inflammation and accumulation of immunosuppressive cells, amplifying post-RFA immunosuppressive factors, creating a suppressive immune microenvironment and hindering subsequent immunotherapy. For example, Zhang *et al.* showed that although iRFA transiently activated antitumor immunity, $CD4^+$ and $CD8^+$ T-cell and DC infiltrates precipitously declined thereafter, while immunosuppressive tumor-associated macrophages rose to $1.92 \pm 0.50$-fold and $4.12 \pm 0.64$-fold of baseline at days 3 and 8 post-iRFA, respectively [59]. Similarly, Shi *et al.* found that Tregs, MDSCs, and M2 macrophages continued to

accumulate after iRFA, suppressing T-cell function and abrogating PD-1 blockade—thereby establishing a vicious cycle in which incomplete ablation leads to immunosuppression, which in turn drives accelerated tumor progression [19].

To enlarge the effective ablation zone and achieve complete necrosis, strategies beyond conventional saline infusion (which temporarily enhances conductivity) have been explored. Mocan *et al.* developed PEG-coated gold nanoparticles that are injected directly into the ablation zone via the electrode; their superior conductivity and thermal diffusivity substantially mitigate the roll-off effect compared with saline infusion [60]. Combining TACE with RFA has also been investigated to counteract heat-sink effects near large vessels. Zhang *et al.* compared TACE + RFA versus surgical resection for small recurrent HCC and found that, although OS was similar, the TACE + RFA arm had a significantly lower complication rate [61]. A meta-analysis further demonstrated that TACE + RFA yields superior recurrence-free and OS compared with RFA alone [62]. With the advent of various new technologies, the roll-off and heat-sink effects of RFA are expected to be mitigated.

### 4.2. Immune-Related Adverse Events

Another major challenge is treatment-related toxicity, especially immune-related adverse events (irAEs) triggered by ICIs. Common irAEs include hepatotoxicity, rash, pneumonitis, colitis, etc. In the IMbrave050 study of high-risk HCC patients after curative resection or ablation, any-grade adverse events occurred in 98% of patients treated with atezolizumab plus bevacizumab versus 62% under active surveillance; grade 3 - 4 events occurred in 41% versus 13% and grade 5 events in 2% versus < 1%. 88% of events were deemed treatment-related, including proteinuria in 46%, hypertension in 38%, thrombocytopenia in 20%, elevated AST in 16%, ALT in 14% and hypothyroidism in 14%—all substantially higher than in the control arm [26]. Because many HCC patients have underlying liver disease such as cirrhosis, their hepatic reserve is limited and hepatotoxic irAEs (for example, immune-mediated hepatitis) are particularly dangerous. A meta-analysis of thirty real-world studies encompassing 3867 HCC patients treated with atezolizumab plus bevacizumab found AST and ALT elevations in 35 percent and 20 percent of patients respectively—rates exceeding those observed in IMbrave050 [63]. This heightened liver toxicity may reflect checkpoint blockade disrupting intrahepatic immune-tolerance pathways, leading to excessive T-cell activation and acute inflammatory damage [64].

The ASCO guideline classifies irAEs into four grades by severity. Grade 1 (mild) generally does not require treatment interruption. Grade 2 (moderate) warrants holding immunotherapy and initiating low-dose corticosteroids. Grade 3 (severe) should prompt permanent discontinuation of the ICI, administration of systemic high-dose steroids and a slow taper once symptoms improve. Grade 4 (life-threatening) necessitates permanent ICI cessation and consideration of additional immunosuppressive agents alongside steroids [65].

### 4.3. Immunological Exploration of Other Ablation Methods

Aside from RFA, other ablation methods, such as MWA, cryoablation, HIFU and IRE, also elicit antitumor immune responses.

MWA uses electromagnetic energy to heat and destroy tumor cells; like RFA, it relies on percutaneous electrodes and is subject to both roll-off and heat-sink effects. Clinical trials have shown no significant differences between MWA and RFA in local efficacy, complication rates, or long-term survival [66]. By thermally destroying tumor cells and releasing TAAs and DAMPs, MWA can activating antitumor immunity [67]. MWA combined with immunotherapy in HCC has also emerged as a major focus of current clinical research [68] [69].

Cryoablation employs cycles of rapid freezing and thawing to form intracellular ice crystals, disrupting cell membranes and inducing apoptosis or necrosis. Its low-temperature mechanism better preserves intact antigen structures and can more effectively provoke antitumor immunity compared with RFA or MWA [70] [71]. Despite this promise, the complexity and cost of cryoablation equipment have limited its adoption in HCC.

HIFU is a noninvasive technique that focuses ultrasonic energy to generate localized thermal and mechanical damage, releasing TAAs and DAMPs to trigger immune activation [72]. However, obstruction of ultrasound transmission to the liver by the rib cage prevents HIFU's effective use in HCC [73]; it is thus not included in HCC treatment guidelines, and its main current applications are in uterine fibroids and adenomyosis.

IRE is a newer, nonthermal ablative method that delivers brief high-voltage pulses to create permanent nanopores in cell membranes, inducing programmed cell death without heat [74]. IRE avoids heat-sink effects and can effectively ablate tumors adjacent to major vessels. Preclinical studies demonstrate that IRE elicits even stronger immune activation than RFA, and may surpass cryoablation in this regard [71] [75]. Nonetheless, IRE's application in HCC remains exploratory, and its technical complexity and cost have so far limited widespread clinical adoption.

Future research should further explore the clinical application of cryoablation, IRE, and HIFU in HCC, elucidating their immunologic mechanisms and working to reduce cost and procedural complexity. It should also compare RFA with these alternative ablation methods in combination with immunotherapy, assessing both immunogenicity and clinical efficacy. Patient-specific factors, such as tumor size, location, and liver function, must guide the choice of the optimal ablation-immunotherapy regimen. Such comparative, personalized studies will be essential to tailor multimodal strategies for HCC.

### 4.4. Other Challenges

The spatiotemporal dynamics of RFA-induced immune responses remain poorly defined, and the optimal sequencing of immunotherapy with RFA requires further investigation. To date, most studies have evaluated immunotherapy as an adjuvant post-RFA [27]-[31] [40]-[45] [50]-[53], although some have explored its

use in a neoadjuvant setting, administering RFA during the course of immunotherapy [37] [38]. In clinical practice, immunotherapy for HCC must be tailored and precise, based on disease stage. For intermediate- or advanced-stage patients who are not candidates for curative resection, neoadjuvant immunotherapy can be used to downsize tumors before RFA, thereby reducing overall tumor burden. Conversely, in early-stage patients with a solitary lesion, upfront RFA followed by adjuvant immunotherapy may eradicate microscopic residual disease and lower the risk of recurrence or metastasis.

Moreover, to date no single biomarker has been endorsed by major guidelines for accurately selecting HCC patients most likely to benefit from immunotherapy. Traditional tissue biomarkers, such as PD-L1 expression, tumor mutational burden (TMB), and microsatellite instability-high (MSI-H), have limited use in HCC due to inconsistent predictive value or low prevalence [76]-[78]. In contrast, liquid biopsy approaches exemplified by circulating tumor DNA (ctDNA) are garnering attention for their noninvasive nature and ability to provide dynamic monitoring [79], while routine serum markers like alpha-fetoprotein (AFP) and the albumin-bilirubin (ALBI) score offer important prognostic information despite limited specificity [80]. Concurrently, studies of the gut microbiome and resistance-related pathways such as Wnt/$\beta$-catenin are yielding mechanistic insights into differential treatment responses [81] [82]. It is foreseeable that integrated, multidimensional biomarker models will enable a robust predictive framework for immunotherapy response in HCC and thus facilitate personalized treatment optimization. Progress toward this goal is underway, but further foundational research and clinical validation remain essential.

Emerging therapies, such as tumor vaccines and cell therapy, combined with RFA have shown early promise in reducing recurrence rates. However, these trials have generally been small and nonrandomized, limiting their ability to inform clinical practice. Moreover, the high cost and technical complexity of these advanced immunotherapies restrict their application. For example, Peng *et al.*'s personalized DC vaccine approach involves whole-exome sequencing and custom synthesis of neoantigen peptides, which under current technology can only be evaluated in small cohorts [40]. Future Large-scale, randomized controlled trials are needed to define the ideal combination strategies, identify the patient subgroups most likely to benefit, and establish long-term efficacy and safety.

## 5. Summary

As a minimally invasive therapy for HCC, RFA not only directly ablates tumor tissue but also stimulates antitumor immunity. However, its standalone immunologic efficacy is limited and readily counteracted by immunosuppressive elements within the tumor microenvironment. Consequently, combination strategies represent the future of HCC management. Although RFA plus immunotherapy faces several challenges, its therapeutic potential in HCC merits continued and in-depth exploration.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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