Review

DOI: 10.5582/bst.2023.01275

The immune response of hepatocellular carcinoma after locoregional and systemic therapies: The available combination option for immunotherapy

Yuxin Duan[1,§], Hua Zhang[1,§], Tao Tan[2,§], Wentao Ye[1], Kunli Yin[1], Yanxi Yu[1], Meiqing Kang[1], Jian Yang[1], Rui Liao[1,*]

[1] Department of Hepatobiliary Surgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing, China;
[2] Chongqing Health Statistics Information Center, Chongqing, China.

SUMMARY Hepatocellular carcinoma (HCC) is associated with a highly heterogeneous immune environment that produces an immune response to various locoregional treatments (LRTs), which in turn affects the effectiveness of immunotherapy. Although LRTs still dominate HCC therapies, 50-60% of patients will ultimately be treated with systemic therapies and might receive those treatments for the rest of their life. TACE, SIRT, and thermal ablation can dramatically increase the immunosuppressive state of HCC, a condition that can be addressed by combination with immunotherapy to restore the activity of lymphocytes and the secretion of cellular immune factors. Immune treatment with locoregional and systemic treatments has dramatically changed the management of HCC. In this review, we examine the research on the changes in the immune microenvironment after locoregional or systemic treatment. We also summarize the regulation of various immune cells and immune factors in the tumor microenvironment and discuss the different infiltration degrees of immune cells and factors on the prognosis of HCC to better compare the efficacy between different treatment methods from the perspective of the tumor microenvironment. This information can be used to help develop treatment options for the upcoming new era of HCC treatment in the future.

Keywords Hepatocellular carcinoma, locoregional treatment, systemic treatment, immune therapy, immune microenvironment

1. Introduction

Hepatocellular carcinoma is one of the most common causes of cancer-related mortality worldwide, and it was also the sixth most common malignancy in 2020 (*1*). The stage, location, and comorbidities of the tumor largely determine the choice of an appropriate treatment including surgical resection, liver transplantation, percutaneous ablation, hepatic arterial infusion chemotherapy (HAIC), transarterial chemoembolization (TACE), and radiation embolization (*2*). Due to the high recurrence and metastasis rates of HCC, there are also certain limitations to regional therapy. Therefore, the advent of effective systemic therapies that have been used as preoperative neoadjuvant and postoperative adjuvant therapies may be beneficial in reducing recurrence (*3*). Nonetheless, in HCC, some specific immunotherapy attempts tended to result in short-term drug resistance and failed to dramatically improve efficacy and clinical outcome in HCC patients due to a strong intrinsic immune microenvironment. Excitedly, several high-level clinical studies have recently confirmed that immune checkpoint inhibitors (ICIs) are an effective and well-tolerated treatment option for patients with HCC (*4-6*). In particular, programmed cell death-1 (PD-1) and its ligand (PD-L1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), and others obtained early success in clinical trials of HCC. Recently, with advances in combination of immune therapy and local therapy or targeted systemic therapy, a growing number of patients with HCC have achieved longer survival. Based on the comprehensive guidelines for the management of HCC published worldwide, the main difference between the guidelines is the different staging systems which were used to select the best treatment option for patients with HCC. But the safety and efficacy of various combination immunotherapies requires further confirmation.

2. Immune response induced by locoregional treatments (LRTs)

Locoregional treatment (LRT) is an essential treatment for patients with HCC. Surgical resection, liver transplantation, and ablation techniques might also have a stimulatory effect on oncogenesis, related to the proliferation of HCC tumor cells, microenvironmental changes, and both angiogenetic and metastasis triggers of HCC. LRTs enable de novo immune priming by inducing the release of tumor-associated antigens after cell death. Therefore, LRTs, both thermal and nonthermal, and invasive and noninvasive, applied to HCC as well as to other conditions in the liver have been correlated with a more changeable immune microenvironment (Figure 1).

2.1. Surgical resection

Surgical resection (partial hepatectomy, PH) is one of the main radical treatments for HCC, but recurrence is still the main cause of death. It has been demonstrated that surgical resection may impact the liver regeneration microenvironment through suppression of the immune system, inflammation, and the vascular environment during HCC development (*7*). The immune response after surgical resection is mainly manifested in changes in the degree of infiltration of Tregs and the type and robustness of T-cell responses *via* an increase in the depletion of T cells in peripheral blood. As effector factors of liver innate immunity, liver Kupffer cells, natural killer (NK) cells, and natural killer T (NKT) cells can directly affect liver regeneration. After PH, the number of NKT cells and the proportion of $CD11b^+$ Kupffer cells/macrophages (Mφ) shows an increasing trend, and the consumption of NKT cells and NK cells can promote liver regeneration (*8,9*). Dendritic cells (DCs) and hepatic stellate cells (HSCs) have also been shown to promote liver regeneration and to have immunomodulatory effects in the inflammatory environment of the liver (*10,11*). Moreover, cold hepatic ischemia–reperfusion injury (IRI), an innate immunity-driven inflammatory response induced by hepatic vascular occlusion, is a major complication of liver resection and mainly manifests as damage to hepatic sinusoidal endothelial cells and destruction of the microcirculation (*12*). However, IRI is extremely complex, involving a large number of changes in cellular components, factors, and mediators. IRI can induce YAP/Hippo expression, amplified macrophage/neutrophil sequestration and increased the expression of cytokines and ischemia during PH activates Kupffer cells and adherent neutrophils to create reactive oxygen species (ROS) during the initial reperfusion period and. Which can stimulate the transcription factors NF-κB and activator protein-1 (AP-1) and enhance the expression of genes, such as tumor necrosis factor-α (TNF-α), TNF-α, Interleukin (IL)-1β, and IL-6, inducible nitric oxide (NO) synthase (iNOS), heme oxygenase-1, C-X-C motif ligand (CXC) chemokines, and adhesion molecules to inhibit HSC activation (*13,14*). In a mouse model, IRI triggered macrophage-specific T-cell immunoglobulin mucin-4 (TIM-4) activation by stressed hepatocellular phosphatidylserine (PS) presentation (*15*), which may provide us with an IRI therapeutic target to minimize innate inflammatory responses after liver resection. In patients following hepatectomy, C5L2 expression on monocytes and neutrophils decreases after liver resection, and there is a parallel decrease in the chemotactic response of neutrophils to C5a stimulation. C3a, C4a, and their des-Arg forms also showed significant distinct changes in plasma levels after liver resection (*16*). Therefore, after surgery, rapid and dramatic immune responses occur in the liver microenvironment, and postoperative adjuvant immunotherapy maybe necessary for high-risk recurrent patients with hepatocellular carcinoma (Figure 2).

2.2. Liver transplantation

Due to the special immune environment, HCC has a high recurrence rate after liver transplantation (LT). In numerous animal experiments that have investigated the changes in the immune microenvironment after LT, as a radical treatment method for HCC, the number and infiltration degree of immune cells in the HCC microenvironment had drastically changed after LT. As a predictor of HCC recurrence, the postoperative C-reactive protein (CRP) level is increased to varying degrees before, during, and after LT. However, the specificity and sensitivity of CRP have certain limitations. There are numerous reports suggesting that $CD4^+CD25^+$FoxP3 Tregs are elevated at the graft site at the time of rejection in clinical transplant recipients, which decreased to baseline numbers by day >100 in the population of cells within the liver allografts of long-surviving recipients (*17*). These results indicate that CD4+CD25+FoxP3 Tregs, as the most specific Tregs, are more capable of assessing the prognosis of transplantation than CRP. On the other hand, after liver transplantation, recipient NK cells exhibit tolerant phenotypes, with the downregulation of activating receptors and reduced cytotoxicity and cytokine production (*18*). In addition, the animal model of HCC showed that the levels of intragraft TGF-β in Day 35 liver allografts after LT increased markedly; however, they diminished by Day 100. In contrast, intragraft IL-10 mRNA is increased persistently in liver allografts (*17*). In addition to the changes in immune cells and immune factors, the changes in the infiltration degree of immune factors can also explain the changes in the immune microenvironment of the recipient and donor after LT to a certain extent. The lymphocyte-to-monocyte ratio (LMR) could reflect the immune status of the tumor microenvironment after LT, in particular, for HCC patients undergoing living donor liver transplantation (LDLT). Clear studies have indicated that the LMR values decreased within one month and increased again one year after surgery in almost all patients who received LDLT. The reduction in $CD3^+/CD68^+$ cells was greater in patients with a lower LMR (*19*). In addition, macrophages

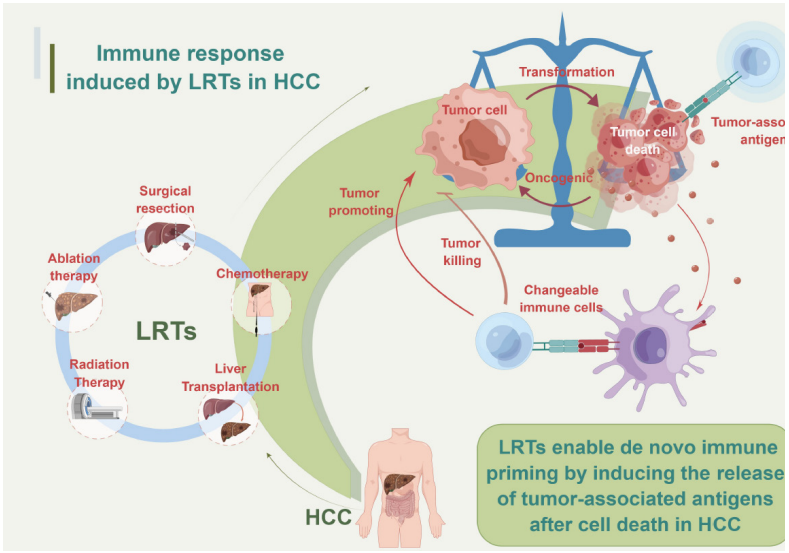

Figure 1. Overview of immune response induced by locoregional treatments via the release of tumor-associates antigens after cell death in hepatocellular carcinoma.

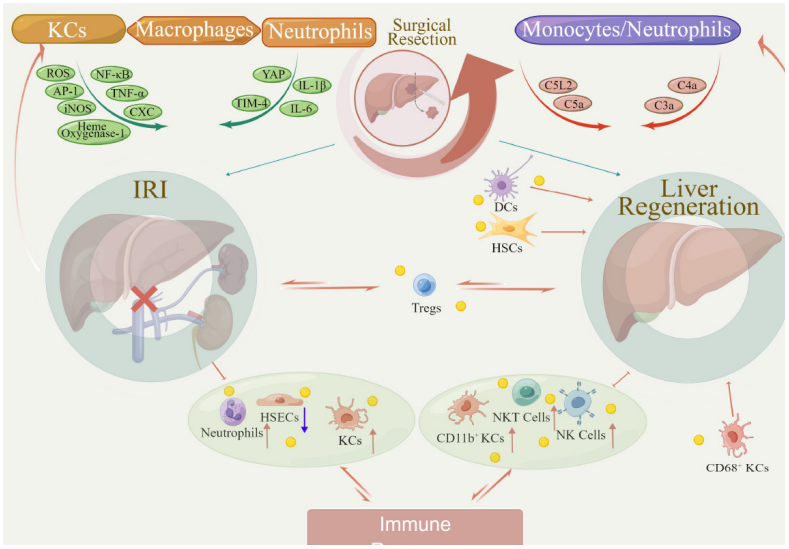

Figure 2. Surgical resection may trigger immune response during the liver regeneration and after ischemia-reperfusion in immune microenvironment of hepatocellular carcinoma.

and Kupffer cells are relevant to immune tolerance due to T-cell apoptosis through the factor-associated suicide/factor-associated suicide ligand (Fas/Fasl) pathway (*20*). In addition to the changes in immune cells, the nonimmune cells in the immune microenvironment after LT were also changed to varying degrees. Based on plasma metabolomics profiling, phosphatidylcholine (PC), nutriacholic acid, and 2-oxo-4-methylthiobutanoic acid were decreased in 122 patients undergoing LT (*21*). Controversially, there is no consensus on the use of postoperative immunosuppressants because the balance between rejection and tumor response is still an unresolved barrier after LT (Figure 3).

2.3. Transarterial chemotherapy

As the standard treatment for patients with intermediate-stage HCC, transarterial chemotherapy (TACE) is generally performed as the treatment for large, unresectable, or multinodular HCC in well-functioning patients. By collecting peripheral blood from liver cancer patients before and one month after TACE treatment, it was discovered that the expression of PD-L1 and PD-1 after TACE treatment was significantly higher in patients with poor TACE response. Moreover, PD-L1 mRNA expression was higher in peripheral blood monocytes, while the ratio of $CD4^+$ to $CD8^+$ cells decreased(*22*). In contrast, in research on tumor pathology in HCC patients, there was no difference in the expression of coinhibitory proteins, including PD-L1, Indoleamine2,3-dioxyge-1(IDO-1), lymphocyte activation gene-3 (LAG-3), TIM-3, or CTLA-4 corepressor proteins, in HCC tissue samples treated with TACE(*23*). On the other hand, using high-throughput sequencing technologies to verify the changes in gene expression within the TME, the results showed evidence of the significant upregulation of a number of proinflammatory genes in TACE-pretreated samples (*23*). Other studies support this conclusion. Cytometric bead immunoassays were used to simultaneously measure cytokines in the sera of patients with HCC after TACE, which revealed that the inflammatory cytokines IL-6 and IL-22 significantly increased early and that Th2

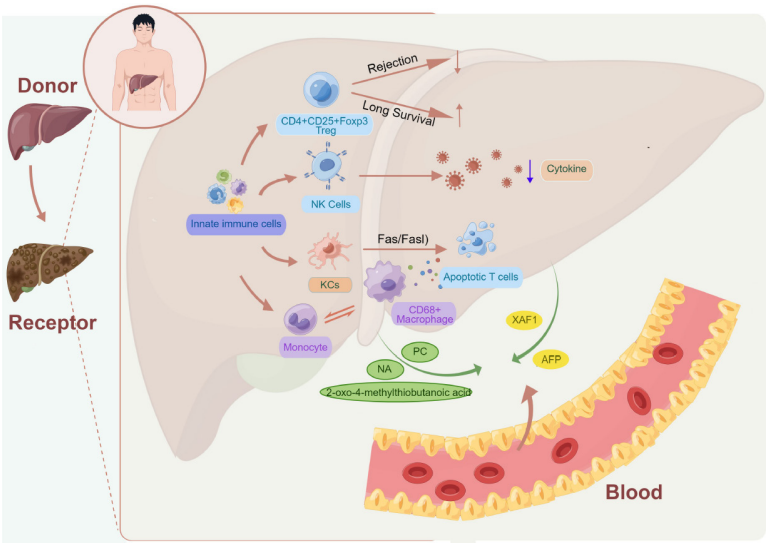

Figure 3. The changes in the immune microenvironment after liver transplantation summarized in this review.

cytokines (IL-4, IL-5, or the suppressive cytokine IL-10) were also increased in the late phase (*24*). In addition, evidence from other studies suggests that the levels of several cytokines, including IL-1β, IL-2R, IL-6, IL-22, and IL-8, were all increased after TACE (*25*). Regarding the changes in the tumor microenvironment of HCC patients caused by TACE, retrospective studies have proven that patients who received TACE were found to experience peritumoral portal lipiodol enhancement (*26*). Additionally, hypoxia regulators such as vascular endothelial growth factor (VEGF), which are induced by HIF-1α, also underwent dynamic changes after TACE in HCC patients (*27,28*). Researchers evaluated the levels of VEGF and tryptase serum concentrations from 71 unresectable HCC patients before and after hepatic TACE and obtained higher serum VEGF levels and lower tryptase levels (*28*). In addition to VEGF, the levels of AST, ALT, and lactate dehydrogenase (LDH) in the sera of patients with HCC who underwent TACE reached peak values within 1 day, while basic fibroblast growth factor (bFGF) and TNF-α levels exhibited mild increases during the 1st week. Conversely, angiogenin, epidermal growth factor (EGF), and TGF-β levels decreased following TACE (*29*).

Whereas hepatic arterial infusion chemotherapy (HAIC) entails infusing chemotherapeutic agents directly into hepatic tumors, which can avoid the first-pass effect and provide a higher intratumoral concentration of chemotherapeutic agents.Theoretically, compared with TACE, HAIC yields less hepatocellular injury and greater treatment efficacy. In a study involving 36 HCC patients treated with HAIC, the frequency of TAA-specific T cells and Tregs and myeloid-derived suppressor cells (MDSCs) was measured by interferon-gamma enzyme-linked immunospot assays and multicolor fluorescence-activated cell sorting analysis (*30*). In 22.2% of patients, after HAIC, the frequency of TAA-specific T cells increased. However, the frequency of Tregs decreased. Patients with a low MDSC frequency were also found to have a longer overall survival time. In addition to the levels of various immune cells, and similar to the changes in cytokines after TACE, for example, the increase in serum cytokine levels, chemokines, and other growth factors in the HCC microenvironment (*31*), the combination of the neutrophil-to-lymphocyte ratio and the ratio of early des-carboxyprothrombin changes (*32*) can also be a useful predictor of HAIC. Among 90 patients from a retrospective study evaluating AFP and des-gamma-carboxyprothrombin (DCP) levels after half a course of HAIC, 8 (8.9%), 19 (21.1%), and 63 (70.0%) had an elevated level of AFP, elevated level of DCP, or elevated level of both of these tumor markers, respectively (*33*). In recent studies, the changes in 20 serum cytokines and growth factors were proven to be associated with prognosis in HCC patients who were treated and underwent HAIC, such as von Willebrand factor (VWF) and VEGF (*34*). The serum level of VEGF was significantly decreased after HAIC, and it is an important predictive factor for therapeutic effect and survival in patients with advanced HCC undergoing HAIC. In addition, the ADAMTS13 activity-VWF levels and serum cell-derived factor 1 levels in HCC patients with stable disease and partial response to HAIC treatment were significantly higher than those in patients with progressive disease and lower serum hepatocyte growth factor (HGF) and IL-4 levels than nonresponders (*34*). Nonetheless, cytokines have limited roles in predicting HCC responses in patients who receive HAIC. Because HAIC mainly targets cancer epithelial cells in HCC, the exposed important biomarkers have greater predictability in advanced HCC patients (Figure 4).

2.4. Radiation therapy

The importance of transarterial radioembolization (TARE) in the treatment of patients with unresectable HCC cannot be ignored. Clinical studies have suggested that higher doses of radiation to the tumor increase the likelihood of

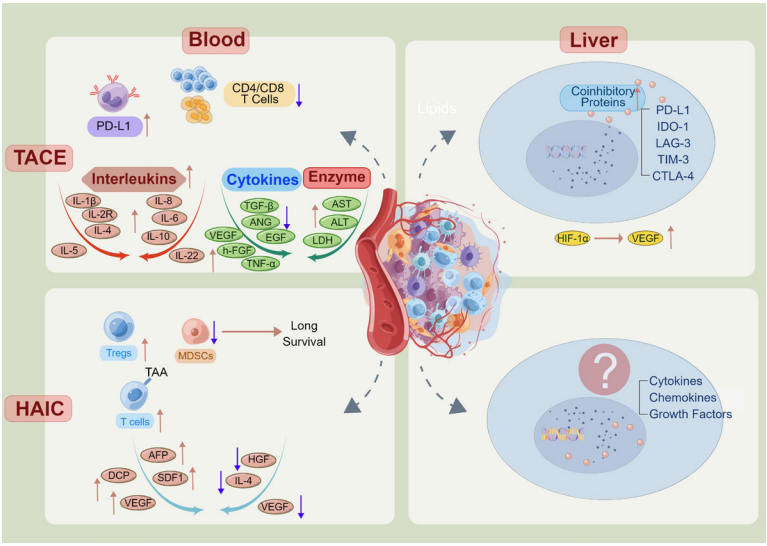

Figure 4. Various immune cells, cytokines, chemokines and growth factors emerging in the blood and liver tissues after trans-arterial chemotherapy and hepatic arterial infusion chemotherapy, respectively.

inducing a positive immune response (*35*). A retrospective assessment of the dynamic changes in lymphocytes following TARE of HCC and its association with normal liver dose (NLD) of TARE found a moderate negative association between the NLD and lymphocyte count at 1 month posttreatment that was most significant at 3 months posttreatment (*36*). In an early prospective clinical study, after TARE, proinflammatory (IL-6 and IL-8) and oxidative stress (malondialdehyde) markers continued to increase, endothelial damage markers (vW factor and PAI-1) and coagulation cascades (factor VIII, PAI-1, and D-dimer) were induced, and a significant increase in factors associated with liver regeneration (FGF-19 and HGF) was observed (*36*).

TARE with yttrium 90 is one kind of palliative lobar therapy for HCC patients with advanced disease or for those in whom other therapies have failed; it delivers local high-dose radiation to tumors through microembolic microspheres, preserving blood flow to promote radiation injury to the tumor. An earlier follow-up trial of the hepatic artery involving (yttrium)-Y-90 microspheres showed that lymphocyte subsets except for NK cells were significantly (> 50% from pretherapy values), promptly (as early as 24 h) and persistently (up to 30 months) depleted post-(90)Yttrium microsphere therapy (*37*), which may lead to increased levels of IL-10 and IP-10 (*38*). ILs isolated after Y90-RE showed signs of local immune activation: elevated granzyme B expression and infiltration of $CD8^+$ T cells, $CD56^+$ NK cells, and $CD8^+$ $CD56^+$ NKT cells.

Stereotactic body radiotherapy (SBRT) is a treatment option for advanced HCC patients who are not candidates for local therapies such as surgery that uses high doses of radiation per fraction in fewer fractions with high precision and accuracy to achieve local tumor control. In preclinical studies, SBRT has been shown to induce tumor cell death by causing tumor-associated endothelial cell death and vascular damage (*39*). SBRT could preserve lymphocytes and increase the expression of various immunostimulatory cytokines within the irradiated tumor microenvironment and the activation of antitumor T cells, thereby eliciting enhanced antitumor immunity. The principle of SBRT in HCC, however, differs partially from that of other radiotherapy methods. The inflammatory environment can be modified to become suitable for HCC after undergoing SBRT. To determine the changes in circulating levels of a panel of soluble cytokine receptors and liver-secreted proteins in HCC patients during SBRT, Sylvia S *et al*. (*40*) assessed the plasma levels of these soluble factors following one to two fractions of SBRT. The researchers found that in patients with HCC after one to two fractions of SBRT, those who developed liver toxicity had significantly higher levels of soluble tumor necrosis factor receptor II and lower levels of soluble CD40 ligand (sCD40L) and chemokine CXCL1 compared to levels in those who did not develop liver toxicity. In addition, plasma sCD40L levels are positively associated with platelet number, and the low platelet and sCD40L levels in HCC patients contribute in part to a decrease in liver immune function (*41*). On the other hand, with the development of immuno-oncology, increasing evidence has suggested that the changes in the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and cytokines can respond to antitumor therapies. Several retrospective studies collected peripheral blood cell count, NLR, and PLR data before and after SBRT and found that circulating blood cell, total leukocyte, neutrophil, lymphocyte, and platelet counts decreased significantly after SBRT (*42,43*). While no significant difference was observed for hemoglobin levels, it was found that the NLR and PLR were significantly increased post-SBRT compared with pre-SBRT and were complementary predictors of OS in HCC patients treated with SBRT (*42*) (Figure 5).

2.5. Ablation therapy

2.5.1. Radiofrequency ablation (RFA)

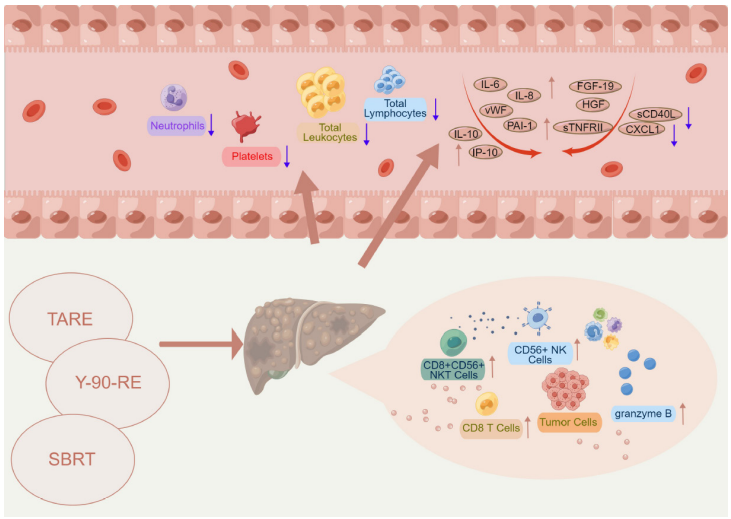

Figure 5. The immune response induced by radiation therapy including trans-arterial radioembolization (TARE), TARE with yttrium 90 (Y-90-RE) and stereotactic body radiotherapy.

RFA is the most validated and widely used technique in the early stages of HCC, and it is the most commonly used technique for local ablation of HCC tumors smaller than 5 cm in diameter. Both local and systemic immune responses induced by RFA have been extensively documented. RFA is performed by using frictional heat generated by the high-frequency alternating current to induce HCC cell death, and the available studies clearly demonstrate that there is a greater release of immunogenic intracellular substrates in areas subjected to heat-induced cell necrosis (*44*).

Thermal ablation induced by RFA can induce a large number of changes in the expression of immune cytokines in HCC tissues, especially in incompletely ablated tissues. One study found that the changes in Th1/Th2 cytokines in HCC patients after RFA exhibit clear upregulation and downregulation trends (*45*). After RFA, the levels of Th1 cytokines, including TNF-α, IFN-γ, and IL-2, were significantly upregulated, and the levels of Th2 cytokines, including IL-4, IL-6, and IL-10, were markedly downregulated, while the serum level of AFP decreased, which indicates that RFA could change the expression of immune cytokines, promoting tumor antigen presentation and activating T lymphocytes. In addition to Th1/Th2 cytokines, after RFA, there is a release of circulating histones, including myeloperoxidase (*46*), and an increase in tumor-specific antibodies, $CD4^+$ T cells (*47*), $CD8^+$ T cells, and NK cells (*48*). Apart from T helper cells, earlier studies have demonstrated that the number of tumor-associated antigen-specific T cells after RFA was inversely correlated with the frequency of $CD14^+$ HLA-DR/low myeloid-derived suppressor cells (MDSCs) (*49*).

On the other hand, RFA can not only effectively kill HCC tumor cells but can also release tumor antigens to induce an immune response or trigger an inflammatory response, resulting in the accumulation of a large number of antigen-presenting cells (APCs). For example, the nuclear proteins high mobility group B1 and heat shock proteins (HSPs) may induce antitumor immune responses by activating dendritic cells (DCs). In particular, the expression levels of HSP70 and HSP90 showed the most pronounced increasing trend after RFA, with 8-fold and 1.2-fold increases, respectively (*50*). In serological studies, three proteins, namely, CLU, Ficolin-3, and RBP4, were also identified as having significantly altered expression, especially Ficolin-3, which showed marked overexpression affected by thermal ablation (*51*). Palliative RFA (pRFA) has also been shown to accelerate residual tumor progression by increasing tumor-infiltrating MDSCs and reducing the T-cell-mediated antitumor immune response (*52*).

Although it is well established that thermal radiation can alter the expression of various immune cells and cytokines in the microenvironment of HCC, the impact of these changes on HCC progression and recurrence remains to be confirmed in further studies. Many available studies have noted that the main actors of RFA immune dynamics are innate immune cells (*e.g.*, NK cells and DCs) and that they are closely linked to HCC recurrence, and the specific mechanisms involved are a hot topic for future studies.

2.5.2. Cryoablation

The safety and feasibility of cryoablation as a new nonthermal locoregional treatment have been verified. Cryoablation is used to promote tumor cell death indirectly or directly through repeated cycles of freezing, and the host immune system can use tumor antigens to trigger the activation of innate and adaptive immunity against tumor antigens. The process of repeated freezing and thawing and cell membrane lysis can promote the release of cellular antigens and trigger a cryoimmunological response.

In a rat liver model, cryoablation induces inflammation and coagulation, and the production by splenocytes of tumor necrosis factor TNF-α, interferon INF-γ, and the interleukins IL-4, IL-6 and IL-10 increased significantly after cryoablation (*53*). In addition, there were increases in WBCs and decreases in platelets

and hemoglobin. Cryoablation also has an effect on angiogenesis, with upregulation of VEGF expression in tumor tissue and a significant increase in angiogenesis in the residual tumor (*54*).

In addition to rat liver models, similar results have been found in clinical studies. A study using flow cytometry to measure the Treg frequency in the peripheral blood of 111 patients with liver cancer showed that the numbers of $CD8^+$ $CD4^+$ and $FoxP3^+$ cells were significantly decreased after cryoablation cycles (*55*). In addition, it has been shown that the PD-1 and PD-L1 receptors on activated T cells and B cells are also altered. The expression of PD-1/PD-L1 decreased after cryoablation but was elevated at the time of tumor recurrence (*56*). The argon-helium cryosurgery system (AHCS) has now been clearly demonstrated to be effective in killing tumor cells and maximizing the protection of normal liver tissue. By monitoring the percentage of $CD4^+$ and $CD8^+$ T cells and NK cells in HCC patients after AHCS, it was found that the percentage of $CD4^+$ T cells and NK cells was significantly higher compared to pretreatment, but the percentage of $CD8^+$ T cells was significantly lower (*57*). These studies can preliminarily demonstrate an excellent synergistic effect between cryoablation and immunotherapy in the treatment of HCC.

2.5.3. Microwave ablation (MWA)

MWA, similar in immune action to RFA, delivers a microwave oscillating electric field through a needle that greatly increases the temperature in the targeted cancer tissue. The effect of MWA on immune cells is well established, with several demonstrations of altered numbers of T cells, B cells and NK cells following MWA. For example, there was a significant increase in Th17 cell levels and a significant increase in $CD3^+$ cells and $CD4^+$ cells 1 month after MWA (*58*). In addition, the percentage of immune cell subsets was also affected by MWA, with the frequency of effector memory T cells decreasing at 7 days after MWA, the percentage of plasmablasts increasing at Day 7 after treatment, and NK cells consistently increasing after MWA treatment. In particular, a significant increase in subsets of activated T and B cells was observed in patients with long survival times (*59*).

As in RFA, in addition to the alteration of immune cells, corresponding immune cytokines were also altered by ablation, such as a significant increase in the frequency of IFN-y and IL-5 responses in patients with long-term remission relative to patients in early relapse and a significant enhancement of IL-12 and a significant decrease in IL-4 and IL-10 1 month after MWA (*59,60*).

2.5.4. High-Intensity focused ultrasound

High-intensity focused ultrasound (HIFU), as a noninvasive medical technique, is safe and well tolerated and has a significant survival advantage compared with other ablation treatments. It is a kind of hyperthermia and ultrasound therapy that can produce mechanical and thermal effects. The mechanical effect is caused by the negative pressure of ultrasound that forms cavitation to destroy the tumor tissue. The thermal effect induced by the ultrasound beam causes coagulative necrosis of the tissue.

As early as a decade ago, studies were conducted to compare the changes in circulating levels of all measured immunosuppressive cytokines in patients with malignant tumors before and after high-intensity focused ultrasound (HIFU) treatment. The results showed that the levels of serum immunosuppressive cytokines decreased after HIFU treatment, especially VEGF, TGF-β1 and TGF-β2, which were all significantly reduced (*61*). Regarding the changes in cellular immune factors in a short period of time after HIFU treatment, an ultrasound-guided HIFU study from Germany showed that tumor ablation with HIFU induced early sterile inflammation and an increase in leukocytes, CRP, and LDH within the first 20 h after HIFU (*62*). However, a major issue with HIFU is that it is difficult to achieve complete tumor ablation. The decrease in the levels of HIFs, including HIF-1α and HIF-2α, are the result of HIFU, and the levels of these factors increased significantly in residual tumor tissue following HIFU treatment. In addition, a high antigen-specific T-cell response was observed after 2 weeks and did not decrease, even after 10 months (*63*).

2.5.5. Laser ablation

Laser ablation (LA) is an efficient and safe novel treatment for HCC. The technology mainly causes photochemical damage to biological tissue with the formation of radicals and inflammation and causes protein denaturation with heat damage (*64*).

In existing animal studies, the moderate heat stress induced by LA could induce the expression of growth factors in HCC cells and hepatocytes, including heparin-binding growth factor, fibroblast growth factor 21, and nerve growth factor (*65*). In addition to immune cytokines, temperature can induce alterations in the tissue constituents and their structural organization, thus resulting in a measurable change in tissue optical properties. Hyperspectral imaging (HSI) has potential for diagnostic purposes such as the detection of cancers, and it can also provide valid support for therapy and surgery guidance. The thermal response in porcine hepatic tissue induced by laser ablation was assessed based on the spectral and spatial information provided by HSI. It was found that methemoglobin (MetHb) and deoxyhemoglobin (Hb) decreased with increasing temperature and then gradually reached a plateau phase with an increase in temperature > 80°C (*66*). However, the effect of LA on the microenvironmental changes and

immune response of HCC prognosis needs to be further studied.

2.5.6. Irreversible Electroporation (IRE)

Irreversible electroporation (IRE), as a new nonthermal ablation technique, is unlikely to damage cancer tissue by thermal effects, which are found with RFA, MWA, HIFU, and LA. Because of this feature, IRE can be used for tumor ablation in special sites such as those adjacent to bile ducts and blood vessels without destroying the adjacent structures (*67*).

IRE can affect many immune factors in the HCC microenvironment to varying degrees. Animal experimental models of IRE in recent years have illustrated the advantages and disadvantages of this ablation method in terms of the postoperative inflammatory response and the degree of immune cell infiltration. On Day 1 after IRE, activated T cells and NK cells increased, and Treg cells and circulating CD4 T-cell subsets (but not CD8) decreased (*68*). Furthermore, a significant increase in the infiltration of cytotoxic CD8 T cells was observed in post-IRE tumors in mice. The serum IFN-γ level was also significantly increased after IRE in rats (*68*). Moreover, the results from the animal model indicated that IRE could induce antitumor adaptive immunity dominated by the infiltration of cytotoxic $CD8^+$ T cells into the tumors, accompanied by reduced Tregs. IRE can evoke $CD8^+$ T-cell immunity by inducing cell necrosis and significant release of risk-related molecular patterns, including ATP, high mobility group box 1, and calreticulin, helping to prevent HCC progression after ablation (*69*). However, studies on the prognostic value of IRE in HCC in clinical patients are limited, and most of the current studies are focused on comparing the survival rate of patients after IRE treatment or on comparing the effects of different ablation modalities in mouse models. Therefore, the changes in the tumor microenvironment when IRE is applied to human HCC need to be further discussed and verified (Figure 6).

3. Immune response induced by systemic therapy

Systemic therapy for HCC has changed drastically since the combination of atezolizumab and bevacizumab was approved by the FDA and shown to improve overall survival relative to sorafenib. Many clinical randomized trials have demonstrated that combined immunotherapy, such as atezolizumab plus bevacizumab and apatinib plus camrelizumab (*70*), can improve prognosis in all aspects compared with sorafenib monotherapy. However, changes in the immune microenvironment of HCC after systemic therapy remain a major cause of resistance and recurrence, and the mechanisms underlying the altered immune response to systemic therapy due to the robust immunosuppression state involve many stromal cells, humoral mediators, and inhibitory checkpoint molecules, which need to be further explored.

3.1. Tyrosine kinase inhibitors (TKI)

Currently, tyrosine kinase inhibitors (TKIs) are used as first-line therapy for HCC. There are clinical shreds of evidence that suggest that the acquisition of somatic mutations can lead to TKI resistance. Since the adaptive immunity of HCC can inhibit tumor recurrence, TKIs can act on multiple tumor-activated signaling pathways, such as KIT, RET, vascular endothelial growth factor receptor (VEGFR), PDGFR, and fibroblast growth factor receptor (FGFR), thus showing the universality and persistence of efficacy.

Sorafenib can facilitate apoptosis, mitigate angiogenesis and inhibit tumor cell proliferation. To explain the effects of RFA alone and in combination with sorafenib, growth factor measurement in a standing tumor in a two-tumor rat model of HCC revealed that sorafenib treatment decreased HGF levels and microvessel density, whereas VEGF, macrophages, T cells and IL-10 levels were increased by sorafenib (*71*). Macrophages serve as an important component of the immune system and are the key for antitumor activity in HCC. In contrast, clinical

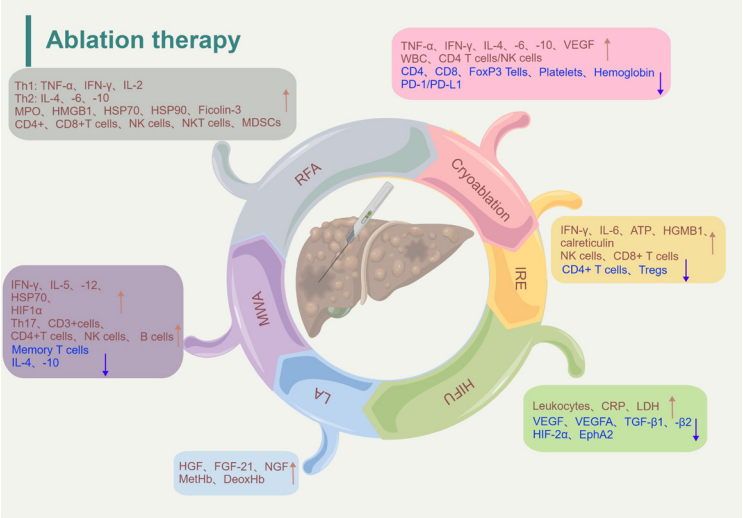

Figure 6. The immune response induced by various ablation therapies summarized in this review.

trials have proven that when dendritic cells are inhibited by sorafenib, macrophages are reduced or activated by altered polarization (*72*).

Lenvatinib has potent antiangiogenic activity, which suppresses VEGFR 1–3, FGFR 1–4, platelet-derived growth factor receptor (PDGFR)-α, and the proto-oncogenes RET and KIT (*73*). Currently, lenvatinib has been approved as a first-line treatment for HCC in Japan, the United States, and China. In addition to the angiogenic effects due to the inhibition of kinases, it also has a regulatory effect on immune function. Lenvatinib reduced tumor PD-L1 levels, Tregs, and the proportion of monocyte and macrophage populations and increased the proportion of CD8 T-cell populations (*74*).

Donafenib is a novel multikinase inhibitor that is similar to sorafenib. As a second-line treatment for patients with HCC, donafenib is superior to sorafenib in terms of improved survival and safety tolerance. Serum cytokines, including IL-6, TNF-α and IFN-γ, were strongly upregulated in a rabbit VX2 liver tumor model after treatment with donafenib (*75*).

Apatinib selectively blocks VEGFR2 by occupying its binding site, thereby preventing the formation of new blood vessels in tumor tissues. In an immunodeficient mouse xenograft model of HCC, apatinib was shown to cause metabolomic changes, with a significant increase in 3-hydroxybutyric acid (3-HB) in serum, tumor tissue, and liver(*76*). In an *in vitro* study on apatinib inhibiting the invasion and metastasis of HCC, the expression of tissue inhibitors of metalloproteinases (TIMPs)-3 and TIMP-4 was upregulated, while the expression of matrix metalloproteinases (MMPs)-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, and MMP-16 was downregulated by apatinib treatment (*77*). However, there is also much evidence that apatinib can cause immune and hematological adverse effects, mainly leukopenia, granulocytopenia and thrombocytopenia (*76*).

As an orally available multitargeted TKI, regorafenib has better efficacy than sorafenib. It can prevent the progression of HCC by reducing cell proliferation, invasion and metastasis; inducing cell death and autophagy; and exerting great antitumor activity. Most importantly, similar to other TKI inhibitors, it can reduce the expression and secretion of the metastasis-related markers MMP-2 and MMP-9. Regorafenib also decreased the levels and secretion of angiogenesis-related proteins, including VEGF, TNF-α, IL-1β and IL-6 (*78*). In addition, regorafenib can regulate tumor-associated macrophages to enhance the body's antitumor immunity, and it can induce T-cell activation and M1 macrophage polarization (*79*).

A growing number of clinical studies have demonstrated that, regardless of the stage of HCC, corresponding TKI therapy can support other treatments for HCC patients, which can help patients achieve better efficacy or improve the prognosis and tumor recurrence to a certain extent.

3.2. Immune checkpoint inhibitor (ICI)

In the application of ICIs in HCC, pembrolizumab and nivolumab, anti-PD-1 humanized antibodies, were approved by the US FDA as a second-line treatment for HCC patients. The main antitumor mechanism of immune checkpoint inhibitors is the reversal of anti-PD-1/anti-PD-L1 CD8 T-cell failure. The major suppressed inhibitory immune checkpoint receptors include PD-1, CTLA4, LAG3 and TIM3, which play an important role in maintaining self-tolerance. Mechanistically, PD-1 binds to T-cell receptors upon binding to its ligand PD-L1, leading to broad dephosphorylation of T-cell-activating kinases and resulting in apoptosis of T cells (*80*). Both in mouse model of primary HCC and in clinical trials, a reduction in PD-L1 and TGF-β expression and Treg infiltration, a significant increase in circulating CD8+ T cell activity, and downregulation of neutrophil-related markers were found during pembrolizumab treatment (*80,81*). More specifically, in a recent study evaluating the efficacy of PD-1 immunotherapy based on single-cell sequencing, patients treated with ICIs were identified as having an increase in B cells and a decrease in dendritic cells, regulatory T cells, and NK cells (especially those overexpressing CD16, CD38, and CD11c)(*82*).

Anti-CTLA-4 is most strongly expressed on Tregs, so the effect of anti-CTLA-4 antibodies may be related to the inhibition of Treg activity. The two most common anti-CTLA-4 antibody drugs are ipilimumab and tremelimumab. A result from survival analyses and an immune monitoring study of tremelimumab therapy showed that $CD3^+$ T-cell infiltration and PD-1 expression increased in the tumor tissue, and $CD4^+$-HLA-DR^+, $CD4^+PD\text{-}1^+$, $CD8^+$HLA-DR^+, $CD8^+PD\text{-}1^+$, $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells in the peripheral blood also increased after tremelimumab therapy (*83*). Similar results were obtained in a study in which CTLA-4 blockade suppressed the progression of tumors in a subcutaneous murine hepatoma model. IHC showed that the expression of CD4+ and CD8+ T cells and the level of IFN-γ were increased in tumor tissues treated with an anti-CTLA-4 antibody alone compared with untreated tumor tissues (*84*). In other words, anti-CTLA-4 antibodies might exert antitumor effects by depleting the Treg cell population in the tumor microenvironment.

According to numerous former studies on the poor prognosis of anti-CTLA-4 antibodies, the adverse effects of CTLA-4 inhibition occur mainly after activation of T cells in lymphoid organs (*85*). These insights may also provide evidence for why anti-CTLA-4 antibodies should be used in combination with other immunotherapies.

In addition to anti-PD-1, anti-PD-L1 and anti-CTLA-4 antibodies that have been widely used in the clinical treatment of HCC, Tim-3, as a newly discovered immune checkpoint molecule, also shows antitumor effects in the targeted treatment of HCC. In contrast to the limited expression of PD-1 on depleted T cells,

Tim-3 is an immune checkpoint molecule that is widely expressed in humans. Previous research has established that there are biointerfacing antagonizing T-cell inhibitory nanoparticles (BAT NPs) for HCC, which were developed by cloaking the platelet membrane on the PLGA microsphere surface to load T-cell immunoglobulin domain and mucin domain-3 antibodies (anti-TIM-3) as well as PD-L1. This therapeutic effect could subsequently activate effector T lymphocytes and the polarization of M1-type macrophages as well as antigen presentation by dendritic cells (*86*). Finally, the relationship between the high expression of Tim-3 and the poor prognosis of HCC has been clearly confirmed, and it can regulate the microenvironment of stem cells and affect the regulation of the biological behavior of HCC. Although the development of anti-TIM-3 antibodies in HCC is still relatively new, it must be a potential strategy for HCC immunotherapy. The immune response of hepatocellular carcinoma induced by systemic therapies is summarized in Table 1.

4. Combination of LRTs and immunotherapy

One of the theoretical bases of combination therapy is that LRTs can induce an immune response and inform the immunosuppressive microenvironment of HCC. The combination of LRTs and immunotherapy has synergistic antitumor effects. Previous studies have shown that LRTs such as TACE, SIRT, and thermal ablation can increase tumor immunogenicity by inducing inflammation and releasing more tumor-associated antigens, thereby increasing tumor invasion cytotoxicity and inducing systemic antitumor immune responses. Immunotherapy can address these immunosuppressive states by modulating the activity of lymphocytes and the secretion of cellular immune factors, helping patients achieve longer survival; for example, the combination of TACE and sorafenib has both efficacy and safety benefits due to the use of either treatment alone (*87*). The combination therapy of RFA and adoptive cell immunotherapy has shown excellent clinical efficacy, and RFA and RetroNectin activated killer cells effectively increase the proportion of $CD3^+/CD8^+$ cells and decrease the ratio of $CD4^+/CD8^+$ cells (*88*). Additionally, a phase I clinical study evaluating the safety of MWA combined with adoptive immunotherapy in HCC patients showed a reduction in the percentage of $CD4^+CD25^{high}$ Treg cells and an increase in $CD8^+CD28^-$ effector cells after 1 month (*89*).

As mentioned earlier, treatment with anti-CTLA-4 antibody alone may produce cytotoxicity and adverse effects, and combination therapy with anti-CTLA-4 antibody with other LRTs is able to achieve a better prognosis. In a clinical trial of tremelimumab in combination with ablation, six-week tumor biopsies following treatment demonstrated a clear increase in CD8 T cells in patients showing clinical benefit (*90*). To demonstrate the efficacy of the combination of RFA and anti-CTLA-4 antibody, Zhang *et al*. divided forty mice with tumors established on their right flanks into four groups: control group (no treatment), RFA group (insufficient RFA alone), anti-CTLA-4 group (anti-CTLA-4 monotherapy), and RFA + anti-CTLA-4 group (insufficient RFA + anti-CTLA-4). The IFN-γ concentration and $CD4^+$ and $CD8^+$ lymphocyte expression in the mice of the RFA + anti-CTLA-4 group were significantly higher than those of the other three groups (*84*).

Nevertheless, there are still clinical findings that run counter to the points mentioned above. As mentioned earlier, sorafenib can affect VEGF and HGF levels in the tumor environment, but sorafenib combined with arterial infusion chemotherapy was more likely to cause adverse events including neutropenia and thrombocytopenia than sorafenib alone. In conclusion, to achieve the best outcome of immunotherapy, the specific implementation of LRTs and immunotherapies needs to be further verified. Table 2 reviews the clinical trials of the combinations of LRTs and immunotherapy in HCC.

5. Combination of systemic therapy and immunotherapy

Although monotherapy for advanced HCC did not show a statistically significant change in efficacy, the combination of immunotherapies has shown an advantage in various survival assessment efficacy values. The combination of an inhibitor of VEGF and PD-1 or its ligand PD-L1 is a standard of care for patients with advanced HCC. Recently, the combination of atezolizumab (anti-PD-L1 antibody) plus bevacizumab (an anti-VEGF monoclonal antibody) demonstrated significantly longer OS and PFS than sorafenib in patients who were not previously treated (*91*). In orthotopic-grafted or induced-murine models of HCC, combination therapy with anti-PD-1 and anti-VEGFR-2 increased cluster $CD8^+$ cytotoxic T-cell infiltration and activation, shifted the M1/M2 ratio of TAMs and reduced Treg and chemokine (C-C motif) receptor 2-positive monocyte infiltration in HCC tissue (*92*). Furthermore, under anti-PD-1 therapy, CD4+ cells promote normalized vessel formation in the face of antiangiogenic therapy with anti-VEGFR-2 antibody (*92*). Compared with PD-1 antibody monotherapy, the combination therapy enhanced T-cell infiltration, improved the efficacy of the PD-1 antibody and prolonged survival. Mechanistically, Peg-IFNα promotes the cytotoxic CD8+ T-cell infiltration microenvironment by inducing the secretion of chemokine CCL4, and the PD-1 antibody was able to restore the cytotoxic capacity of CD8+ T cells by inhibiting the IFNα-IFNAR1-JAK1-STAT3 signaling pathway (*93*).

A series of recent studies involving multiplex IHC, suspension mass cytometry (CyTOF), and Imaging Mass Cytometry™ (*94*) were performed to elucidate both

Table 1. Summary of the immune response of hepatocellular carcinoma induced by systemic therapies

Systemic Therapy	Immune Cell Response		Regulation of Cytokine and Chemokine		Ref
	Increased	Decreased	Up	Down	
TKI	macrophage, T-cells,NK cell		VEGF and IL-10 levels	HGF levels and microvessel density (MVD),growth factor receptor (VEGFR)1–3, fibroblast growth factor receptor (FGFR) 1–4, platelet-derived growth factor receptor (PDGFR)-α	*71,72*
	CD8 T cell	Treg, and the proportion of monocyte and macrophage populations		PD-L1	*73*
			serum cytokines including IL-6, TNF-α and IFN-γ, 3-hydroxybutyric acid (3-HB)		*74*
			TIMPs -3 and TIMP-4	MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-11, and MMP-16	*76*
		leukopenia, granulocytopenia and thrombocytopenia		VEGF, TNF-α, IL-1β and IL-6	
	T cell activation and M1 macrophage				*78*
Immune checkpoint inhibitor (ICI)					
PD-1 PD-L1		T cells		PD-L1	*80*
	cytotoxic T-cell and CD8+ T cells	Tregs		neutrophil-associated markers	*84*
	B cells	DCs, regulatory T cells, and NK cells(over-expressing CD16, CD38, and CD11c)	CXCL9	TGF-β	*81*
CTLA-4	CD3+ T cells (in tumor tissue)		PD-1 expression		
	CD4+-HLA-DR+, CD4+PD-1+, CD8+HLA-DR+, CD8+PD-1+, CD4+ICOS+ and CD8+ICOS+ T cells (in the peripheral blood)				*82*
	CD4+ and CD8+T cell		IFN-γ		*83*
	T cells (in lym-phoid organs)	Tregs			*84*
Tim-3	T lymphocytes and polarization of M1-type macrophages				*86*

Abbreviation: TKI: Tyrosine kinase inhibitors; VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; NK: natural killer cell; VEGFR: growth factor receptor; FGFR: fibroblast growth factor receptor; PDGFR: platelet-derived growth factor receptor; PD-L1: Programmed cell death 1 ligand 1; TNF-α: tumor necrosis factor-α; IFN-γ: interferon-γ; 3-HB: 3-hydroxybutyric acid; TIMP: tissue inhibitor of metalloproteinases; MMP: matrix metalloproteinase; CXCL chemokine (C-X-C motif) ligand; TGF-β1: tansforming growth factor-β: CTLA-4: cytotoxic T lymphocyte-associated antigen-4.

systemic and local immune responses to the combination with cabozantinib and nivolumab, and the preliminary findings indicated that cabozantinib and nivolumab promote T-cell-mediated antitumor immunity locally and systemically (*95*). Specifically, the tumor tissue samples from HCC patients with a better response exhibited a greater presence of $CD8^+$ and $CD4^+$ T cells. In addition to the difference in the lymphocyte profile, the combination treatment also promoted the differentiation of macrophage clusters with low CD163 and arginase-1 expression, which was associated with higher plasma levels of CXCL9/10/11, CCL2 and CCL26 (*96*). In conclusion, combination therapy resulted in a sustained twofold increase in response rates, with a complete response rate of ~5% and encouraging survival beyond 18 months (*97*).

Table 2. Clinical trials registered on NIH investigating combinations of LRTs and immunotherapy in HCC

Trials ID	Regimen	Target Population	Status	Phase	Enrollment number	Study Period
	ICI-based Combination therapy					
NCT02851784	Cellular Immunotherapy+Microwave Ablation	HCC	Completed	II/III	40	2021/3/1-2009/12/1
NCT03914352	PD-1 Antiboty + Hepatic Resection	HCC Combined With PVTT After Hepatic Resection	Unknown status	NA	40	2020/1/31-2019/4/1
NCT04521153	Camrelizumab+Apatinib Mesylate	Perioperative of Resectable HCC	Recruiting	NA	290	2026/3/1-2021/3/25
NCT04220944	Immunotherapy +Locoregional Treatment	Unresectable HCC.	Recruiting	I	45	2022/9/30-2020/1/1
NCT05546619	Tislelizumab+Hyperthermic Intraperitoneal Chemotherapy	High-risk HCC After R0 Resection	Active, not recruiting	NA	30	2025/7/31-2022/8/1
NCT05407519	Tislelizumab + Sitravatinib	High Risk of Recurrence After Curative Resection	Recruiting	II	40	2026/6/30-2022/7/25
NCT03867084	Pembrolizumab(MK-3475)+Surgical Resection or Local Ablation	HCC	Active, not recruiting	III	950	2029/8/31-2019/5/28
NCT04682210	Sintilimab Plus Bevacizumab+Curative Resection		Not yet recruiting	III	246	2024/12/1-2020/12/1
NCT03937830	Durvalumab, Bevacizumab, Tremelimumab+TACE	HCC or Biliary Tract Carcinoma	Recruiting	II	39	2023/12/31-2021/3/10
NCT03630640	Nivolumab+Electroporation	HCC	Active, not recruiting	II	43	2023/11/30-2018/10/11
NCT02960594	hTERT Immunotherapy+IL-12 DNA+Electroporation	Adults With Solid Tumors at High Risk of Relapse	Completed	I	93	2018/11/9-2014/12/1
NCT05240404	Toripalimab+Curative-intent Ablation	Recurrent HCC	Recruiting	II	116	2024/7/31-2020/7/1
NCT03753659	Pembrolizumab+IMMULAB - Immunotherapy+Local Ablation	HCC	Active, not recruiting	II	30	2024/6/1-2019/5/9
NCT05027425	Durvalumab (MEDI4736)+Tremelimumab	HCC in Patients Listed for a Liver Transplant	Recruiting	II	30	2030/12/7-2021/12/7
NCT05609695	Immune Checkpoint Inhibitor+Molecular Targeted Drugs / Locoregional Therapy	HCC	Not yet recruiting	NA	100	2025/9/1-2023/3/1
		HCC	Unknown status	II	40	2022/12/31-2019/2/12
NCT03510871	Nivolumab+Ipilimumab	Advanced Liver Cancer	Active, not recruiting	I/II	659	2024/12/29-2012/10/30
NCT01658878	Nivolumab+Other Agents	Advanced Hepatopancreabiliary Tumors (BLOCKED)	Not yet recruiting	II	62	2028/10/1-2023/3/1
NCT05451043	Durvalumab+Tremelimumab+Propranolol+Chemotherapy	HCC	Suspended	II	150	2028/5/1-2023/5/1
NCT05063565	Durvalumab+Tremelimumab	Hepatocellular Carcinom	Not yet recruiting	II/III	574	2026/4/1-2023/2/1
NCT05665348	Ipilimumab+Atezolizumab+Bevacizumab	Advanced/Metastatic HCC	Recruiting	I	20	2024/3/31-2021/10/30
NCT05031949	Camrelizumab+Hyperbaric Oxygen	HCC	Recruiting	I/II	32	2023/12/1-2019/3/1
NCT03682276	Ipilimumab+Nivolumab+Liver Resection	High-risk HCC	Not yet recruiting	II	27	2028/12/5-2023/3/1
NCT05194293	Regorafenib+Durvalumab (MEDI4736)	Advanced HCC	Completed	NA	124	2023/3/9-2021/5/1
NCT04862949	Atezolizumab+Bevacizumab	Resectable Liver Cancer	Completed	II	30	2022/9/14-2017/9/28
NCT03222076	Nivolumab+Ipilimumab	Advanced Liver Cancer	Unknown status	II	27	2022/8/1-2020/8/30
NCT04523662	Carrelizumab+Apatinib Mesylate+Radiotherapy	Advanced Liver Cancer	Recruiting	II	88	2025/1/31-2022/2/11
NCT05211323	Chemotherapy+Bevazicumab+Atezolizumab	Resectable Liver Cancer	Recruiting	II	30	2027/12/31-2021/2/10
NCT04721132	Atezolizumab+Bevacizumab Before Surgery	Treatment of Liver Cancer After Progression on Prior PD-1 Inhibition	Recruiting	II	30	2027/2/28-2022/2/4
NCT04430452	Hypofractionated Radiotherapy+Durvalumab+Tremelimumab	Unresectable, Locally Advanced, or Metastatic Liver Cancer	Recruiting	II	122	2026/12/31-2022/5/27
NCT05168163	Atezolizumab+Multi-Kinase Inhibito	Advanced Refractory Liver Cancer	Active, not recruiting	II	12	2022/12/31-2016/12/9
NCT02940496	Pembrolizumab+Elbasvir/Grazoprevir+Ribavirin	Advanced Solid Tumors	Recruiting	1	230	2023/12/29-2021/2/3
NCT04580485	INCB106385+Immunotherapy	Advanced HCC	Active, not recruiting	II	433	2023/12/29-2015/10/19
NCT02519348	Durvalumab/Tremelimumab+Durvalumab +Tremelimumab/ Bevacizumab	HCC	Recruiting	II	134	2025/3/1-2022/4/12
NCT05359861	Atezolizumab+Bevacizumab+SRF388	Fibrolamellar HCC	Recruiting	I	56	2027/3/1-2020/4/20
NCT04248569	Nivolumab+Ipilimumab+DNAJB1-PRKACA Fusion Kinase Peptide Vaccine	in Advanced HCC Patients Who Have Progressed on First Line Atezolizumab + Bevacizumab	Recruiting	II	40	2027/1/31-2023/1/19
NCT05199285	Nivolumab+Ipilimumab					

Abbreviations: NIH: National Institutes of Health; LRTs: locoregional treatments; HCC: Hepatocellular Carcinoma; NA: Not Applicable;

Table 2. Clinical trials registered on NIH investigating combinations of LRTs and immunotherapy in HCC (continued)

Trials ID	Regimen	Target Population	Status	Phase	Enrollment number	Study Period
NCT05269381	Pembrolizumab+Personalized Neoantigen Peptide-Based Vaccine	Advanced Solid Tumors	Recruiting	I	36	2025/2/24-2022/3/31
NCT05431270	PD-1 Inhibitor+PT199 (an Anti-CD73 mAb)	HCC	Recruiting	I	41	2024/1/1-2022/6/23
NCT03836352	Low Dose Cyclophosphamide & Pembrolizumab+Immunotherapeutic, DPX-Survivac	Selected Advanced & Recurrent Solid Tumors	Active, not recruiting	II	184	2023/12/31-2018/12/21
NCT03544723	Immune Checkpoint Inhibitors+p53 Gene Therapy	Solid Tumors	Unknown status	II	40	2022/12/31-2018/10/1
NCT03638141	Durvalumab+Tremelimumab+CTLA-4 /PD-L1 Blockade Following Transarterial Chemoembolization (DEB-TACE)	Intermediate Stage of HCC	Recruiting	II	30	2023/11/1-2019/10/2
NCT04785287	Nivolumab+Anti-CTLA4-NF mAb (BMS986218)+Stereotactic Body RadiationTherapy	Metastatic Solid Malignancies	Active, not recruiting	I/II	13	2024/5/27-2021/3/29
NCT02886897	Anti-PD-1+D-CIK Immunotherapy	In Refractory Solid Tumors	Unknown status	I/II	50	2019/10/1-2016/7/1
	TKI-based Combination therapy					
NCT05535998	TKI+TACE-HAIC +Immunotherapy	Hepatocellular Carcinoma With PVTT	Completed	NA	743	2022/6/30-2021/1/1
NCT05135364	TKI+HAIC+Camrelizumab	Unresectable Hepatocellular Carcinoma After TACE Failure	Recruiting	II	48	2024/12/5-2021/11/5
NCT04518852	Sorafenib+TACE+PD-1 Monoclonal Antibody	HCC	Unknown status	II	60	2023/1/31-2020/9/14
NCT04229355	Sorafenib+DEB-TACE+Lenvatinibor PD-1 Inhibitor	Unresectable Hepatocellular Carcinoma	Unknown status	III	90	2022/12/30-2021/2/2
NCT02989870	Sorafenib+Bavituximab+SBRT	Unresectable Hepatocellular Carcinoma	Withdrawn	1	0	2018/10/15-2017/3/27
NCT05286320	Lenvatinib+Pembrolizumab+SBRT	HCC Patients With Portal Vein Thrombosis.	Not yet recruiting	I/II	27	2026/9/30-2023/3/1
NCT03841201	Lenvatinib+Immunotherapy With Nivolumab	Advanced Stage Hepatocellular Carcinoma	Active, not recruiting	II	50	2023/3/1-2019/6/12
NCT02562755	Sorafenib+Vaccinia Virus	HCC	Completed	III	459	2020/7/1-2015/10/1
NCT04273100	Lenvatinib+TACE+PD-1 Monoclonal Antibody	HCC	Unknown status	II	56	2021/6/30-2019/11/14
NCT04627012	Lenvatinib+Anti-PD1 Antibody	Advanced Hepatocellular Carcinoma	Completed		600	2022/7/1-2018/1/1
NCT02988440	Sorafenib+PDR001 local treatment-based Immunotherapy	Advanced Hepatocellular	Completed	I	20	2020/2/27-2017/4/20
NCT01749865	CIK+Radical Resection	HCC	Completed	III	200	2014/12/1-2008/10/1
NCT04658147	Nivolumab+Relatlimab	Potentially Resectable Hepatocellular Carcinoma	Recruiting	I	20	2026/6/1-2021/5/28
NCT03755739	Trans-Artery/Intra-Tumor Infusion of ICI+Chemodrug	Advanced Solid Tumors	Recruiting	II/III	200	2033/11/1-2018/11/1
NCT03299946	Cabozantinib+Nivolumab (CaboNivo)+Resection	Patients With Locally Advanced Hepatocellular Carcinoma	Completed	I	15	2021/10/1-2018/5/14
NCT03966209			Unknown status	I	20	2022/10/31-2019/5/1
NCT05411926	PD-1 /PD-L1 Inhibitor Therapy+Liver Transplantation	Acute Rejection After Liver Transplantation in Patients With Hepatocellular Carcinoma	Recruiting	NA	30	2023/9/1-2021/3/17
NCT01828762	Autologous Immune Cell Therapy+Resection+TACE Therapy	Primary Hepatocellular Carcinoma	Completed	NA	8	1900/1/0-2012/12/1
NCT04564313	Camrelizumab+Liver Transplantation	Recurrent HCC After Liver Transplantation	Recruiting	I	20	2023/7/1-2020/9/21
NCT03575806	Autologous Tcm Immunotherapy+TACE	HCC With MVI After Radical Resection	Completed	II	52	2019/10/31-2017/1/9
NCT02638857	Immunotherapy Using Precision T Cells Specific to Multiple Common Tumor-Associated Antigen+Transcatheter Arterial Chemoembolization	Advanced Hepatocellular Carcinoma	Unknown status	I/II	60	2017/9/1-2015/9/1
NCT05475613	HAIC+Targeted Therapy and Immunotherapy	Down-stage Therapy of liver transplantation	Not yet recruiting	II	75	2028/7/30-2022/8/1
NCT04988945	TACE+SBRT+Double Immunotherapy	for Downstaging Hepatocellular Carcinoma	Recruiting	II	33	2026/12/1-2020/12/1
NCT05613478	Camrelizumab+Apatinib Mesylate+TACE	Perioperative Treatment of Hepatocellular Carcinoma	Recruiting	III	130	2027/11/1-2022/11/1
NCT04522544	Durvalumab (MEDI4736)+Tremelimumab +Y-90 SIRT/TACE	Intermediate Stage HCC	Recruiting	II	84	2024/9/30-2020/12/15
NCT05198609	Camrelizumab, Apatinib+HAIC	HCC With Portal Vein Invasion	Recruiting	III	214	2026/1/1-2022/1/17
NCT05313282	Hepatic Arterial Infusion+Apatinib+Camrelizumab	C-staged Hepatocellular Carcinoma in BCLC Classification	Recruiting	III	140	2025/6/1-2022/6/15

Abbreviations: NIH: National Institutes of Health; LRTs: locoregional treatments; HCC: Hepatocellular Carcinoma; NA: Not Applicable;

Table 2. Clinical trials registered on NIH investigating combinations of LRTs and immunotherapy in HCC (continued)

Trials ID	Regimen	Target Population	Status	Phase	Enrollment number	Study Period
NCT03817736	TACE+SBRT+Immuno Therapy	Downstaging HCC for Hepatectomy	Active, not recruiting	II	33	2023/1/31-2019/3/1
NCT04945720	Durvalumab +HAIC	Advanced Hepatocellular Carcinoma	Recruiting	II	30	2023/12/30-2022/4/11
NCT04981665	TACE+Tislelizumab	HCC at High Risk of Recurrence After Curative Resection	Recruiting	II	50	2024/12/1-2021/11/8
NCT04796025	Sintilimab+Bevacizumab Biosimilar+TACE	Hepatocellular Carcinoma (BCLC-C Stage)	Recruiting	II	34	2024/8/31-2021/9/23
NCT05701488	SIRT+Tremelimumab+Durvalumab	Resectable HCC	Not yet recruiting	I	20	2025/10/1-2023/5/1
NCT02487017	DC-CIK+TACE	Hepatocellular Carcinoma	Unknown status	II	60	2018/12/1-2015/5/1
NCT04268888	Nivolumab+TACE/TAE	Intermediate Stage HCC	Recruiting	II/III	522	2026/6/1-2019/5/8
NCT04547452	Sintilimab+Stereotactic Body Radiotherapy	Advanced Metastatic HCC	Recruiting	II	84	2023/7/1-2020/7/1
NCT03086564	Hepatitis B Virus (HBV)-Specific Antigen Peptides+HepG2 Cell Lysate Co-activated Dendritic Cells+TACE	HBV-related HCC Treatment	Completed	I/II	70	2020/10/29-2017/5/1
NCT01853618	Tremelimumab+Chemoembolization/Ablation	Liver Cancer	Completed	I/II	61	2017/6/7-2013/5/2
NCT04191889	Hepatic Arterial Infusion+Apatinib+Camrelizumab	C-staged Hepatocellular Carcinoma in BCLC	Recruiting	II	47	2025/12/31-2020/4/13
NCT04167293	Sintilimab+Stereotactic Body Radiotherapy	Hepatocellular Carcinoma	Unknown status	II/III	116	2022/10/31-2019/11/16
NCT03864211	Thermal Ablation+Immunotherapy	HCC	Active, not recruiting	I/II	145	2023/5/30-2019/6/15
NCT05809869	Immunotherapy+Radioembolisation	Metastatic Hepatocellular Carcinoma	Recruiting	II	25	2026/6/30-2023/2/15
NCT02678013	RFA+Highly-purified CTL	Recurrent HCC	Unknown status	III	210	2022/1/1-2016/2/1
NCT03067493	RFA/Surgical Resection+Neo-MASCT	Primary HCC	Recruiting	II	98	2023/3/31-2017/7/25
NCT03939975	Anti-PD-1therapy+Thermal Ablation	Advanced HCC	Completed	II	50	2019/7/31-2019/6/1
NCT04724226	Camrelizumab and Apatinib+Cryoablation	Advanced Hepatocellular Carcinoma	Recruiting	II	34	2024/8/31-2021/9/1
NCT03812562	Nivolumab+Yttrium-90	Patients With Liver Cancer Undergoing Surgical Resection	Unknown status	I	2	2022/12/1-2019/2/7
NCT05327738	Atezolizumab+ Cabozantinib+Yttrium Y 90 Glass Microspheres	Unresectable or Locally Advanced Hepatocellular Carcinoma	Withdrawn	II	0	2027/12/4-2022/12/10
NCT03008343	Irreversible Electroporation+NK Immunotherapy	Recurrent Liver Cancer	Completed	I/II	20	2019/7/1-2016/12/1

Abbreviations: NIH: National Institutes of Health; LRTs: locoregional treatments; HCC: Hepatocellular Carcinoma; NA: Not Applicable;

6. Conclusion and prospect

The immune microenvironment of HCC is a system composed of hepatocytes, immune cell subsets, immune receptors and ligands, cytokines and chemokines, extracellular matrix, and other elements (*98,99*). From the above studies, we know that local therapy, systemic therapy, or immunotherapy can affect the immune microenvironment of HCC. Immunotherapy is an increasingly recognized and used method in clinical practice, and its combination with LRTs and systemic therapy is also increasingly used in the clinical treatment of HCC. However, there is great individual variability in combination therapy, which is affected by the tumor size, location, sequence and duration of treatment, and frequency of treatment. More clinical trials are needed to explore the specific time and regimen of immune combination therapy and to continue to optimize the development of the most accurate treatment.

Acknowledgements

Thanks 'HOME for Researchers' and 'Figdraw' for the graphic assistance.

Funding: This research was supported by the Natural Science Foundation of Chongqing (No. CSTB2022NSCQ-MSX0112); Science and Health Joint Research Project of Chongqing Municipality (2020GDRC013); Program for Youth Innovation in Future Medicine, Chongqing Medical University (W0087).

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

Availability of data and materials: The datasets generated during the current study are available in the *https://clinicaltrials.gov/repository*

References

1. Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A. Hepatocellular carcinoma. Lancet. 2022; 400:1345-1362.
2. Wang H, Li W. Recent update on comprehensive therapy for advanced hepatocellular carcinoma. World Journal of Gastrointestinal Oncology. 2021; 13:845-855.
3. Horrow MM, Huynh MHL, Callaghan MM, Rodgers SK. Complications after Liver Trans-plant Related to Preexisting Conditions: Diagnosis , Treatment, and Prevention. Radiographics. 2020; 40:895-909.
4. Bai J, Liang P, Li Q, Feng R, Liu J. Cancer Immunotherapy - Immune Checkpoint Inhibitors in Hepatocellular Carcinoma. Recent Pat Anticancer Drug Discov. 2021; 16:239-248.
5. Feng GS, Hanley KL, Liang Y, Lin X. Improving the Efficacy of Liver Cancer Immunotherapy: The Power of Combined Preclinical and Clinical Studies. Hepatology. 2021; 73 Suppl 1:104-114.
6. Sharma R, Motedayen Aval L. Beyond First-Line Immune Checkpoint Inhibitor Therapy in Patients With Hepatocellular Carcinoma. Front Immunol. 2021; 12:652007.
7. Li H, Zhang L. Liver regeneration microenvironment of hepatocellular carcinoma for prevention and therapy. Oncotarget. 2017; 8:1805-1813.
8. Yin S, Wang H, Bertola A, Feng D, Xu MJ, Wang Y, Gao B. Activation of invariant natural killer T cells impedes liver regeneration by way of both IFN-γ- and IL-4-dependent mechanisms. Hepatology. 2014; 60:1356-1366.
9. Nishiyama K, Nakashima H, Ikarashi M, Kinoshita M, Nakashima M, Aosasa S, Seki S, Yamamoto J. Mouse CD11b+Kupffer Cells Recruited from Bone Marrow Accelerate Liver Regeneration after Partial Hepatectomy. PloS one. 2015; 10:e0136774-e0136774.
10. Almeda-Valdes P, Aguilar Olivos NE, Barranco-Fragoso B, Uribe M, Méndez-Sánchez N. The Role of Dendritic Cells in Fibrosis Progression in Nonalcoholic Fatty Liver Disease. Biomed Res Int. 2015; 2015:768071.
11. Schon HT, Bartneck M, Borkham-Kamphorst E, Nattermann J, Lammers T, Tacke F, Weiskirchen R. Pharmacological Intervention in Hepatic Stellate Cell Activation and Hepatic Fibrosis. Front Pharmacol. 2016; 7:33.
12. Wang H, Xi Z, Deng L, Pan Y, He K, Xia Q. Macrophage Polarization and Liver Ischemia-Reperfusion Injury. International journal of medical sciences. 2021; 18:1104-1113.
13. Wang H, Xi Z, Deng L, Pan Y, He K, Xia Q. Macrophage Polarization and Liver Ischemia-Reperfusion Injury. Int J Med Sci. 2021; 18:1104-1113.
14. Liu Y, Lu T, Zhang C, Xue Z, Busuttil R, Kupiec-Weglinski J, Ji H. Activation of YAP Attenuates Hepatic Damage and Fibrosis in Liver Ischemia-Reperfusion Injury: From Bench to Bedside. American Journal of Transplantation. 2020; 20:735-735.
15. Ji H, Liu Y, Zhang Y, Shen XD, Gao F, Busuttil RW, Kuchroo VK, Kupiec-Weglinski JW. T-cell immunoglobulin and mucin domain 4 (TIM-4) signaling in innate immune-mediated liver ischemia-reperfusion injury. Hepatology. 2014; 60:2052-2064.
16. Strey CW, Siegmund B, Rosenblum S, Marquez-Pinilla RM, Oppermann E, Huber-Lang M, Lambris JD, Bechstein WO. Complement and neutrophil function changes after liver resection in humans. World J Surg. 2009; 33:2635-2643.
17. Fujiki M, Esquivel CO, Martinez OM, Strober S, Uemoto S, Krams SM. Induced tolerance to rat liver allografts involves the apoptosis of intragraft T cells and the generation of CD4(+)CD25(+)FoxP3(+) T regulatory cells. Liver Transpl. 2010; 16:147-154.
18. Jamil KM, Hydes TJ, Cheent KS, Cassidy SA, Traherne JA, Jayaraman J, Trowsdale J, Alexander GJ, Little AM, McFarlane H, Heneghan MA, Purbhoo MA, Khakoo SI. STAT4-associated natural killer cell tolerance following liver transplantation. Gut. 2017; 66:352-361.
19. Mano Y, Yoshizumi T, Yugawa K, Ohira M, Motomura T, Toshima T, Itoh S, Harada N, Ikegami T, Soejima Y, Maehara Y. Lymphocyte-to-Monocyte Ratio Is a Predictor of Survival After Liver Transplantation for Hepatocellular Carcinoma. Liver Transpl. 2018; 24:1603-1611.
20. Chen G-S, Qi H-Z. Effect of Kupffer cells on immune tolerance in liver transplantation. Asian Pacific Journal of Tropical Medicine. 2012; 5:970-972.

21. Lu D, Yang F, Lin Z, Zhuo J, Liu P, Cen B, Lian Z, Xie H, Zheng S, Xu X. A prognostic fingerprint in liver transplantation for hepatocellular carcinoma based on plasma metabolomics profiling. European Journal of Surgical Oncology. 2019; 45:2347-2352.
22. Guo JJ, Wang SX, Han YJ, Jia ZY, Wang RC. Effects of transarterial chemoembolization on the immunological function of patients with hepatocellular carcinoma. Oncology Letters. 2021; 22.
23. Pinato DJ, Murray SM, Forner A, *et al*. Trans-arterial chemoembolization as a loco-regional inducer of immunogenic cell death in hepatocellular carcinoma: implications for immunotherapy. J Immunother Cancer. 2021; 9.
24. Kim MJ, Jang JW, Oh BS, Kwon JH, Chung KW, Jung HS, Jekarl DW, Lee S. Change in inflammatory cytokine profiles after transarterial chemotherapy in patients with hepatocellular carcinoma. Cytokine. 2013; 64:516-522.
25. Lin W, Wang H, Zhong M, Yu S, Zhao S, Liang S, Du J, Cheng B, Gu W, Ling C. Effect and Molecular Mechanisms of Jiedu Recipe on Hypoxia-Induced Angiogenesis after Transcatheter Arterial Chemoembolization in Hepatocellular Carcinoma. Evid Based Complement Alternat Med. 2021; 2021:6529376-6529376.
26. Nyman SS, Creusen AD, Johnsson U, Rorsman F, Vessby J, Barbier CE. Peritumoral portal enhancement during transarterial chemoembolization: a potential prognostic factor for patients with hepatocellular carcinoma. Acta Radiol. 2021;2841851211041832.
27. Liu K, Min XL, Peng J, Yang K, Yang L, Zhang XM. The Changes of HIF-1α and VEGF Expression After TACE in Patients With Hepatocellular Carcinoma. J Clin Med Res. 2016; 8:297-302.
28. Ranieri G, Ammendola M, Marech I, Laterza A, Abbate I, Oakley C, Vacca A, Sacco R, Gadaleta CD. Vascular endothelial growth factor and tryptase changes after chemoembolization in hepatocarcinoma patients. World J Gastroenterol. 2015; 21:6018-6025.
29. Raoul JL, Forner A, Bolondi L, Cheung TT, Kloeckner R, de Baere T. Updated use of TACE for hepatocellular carcinoma treatment: How and when to use it based on clinical evidence. Cancer Treat Rev. 2019; 72:28-36.
30. Mizukoshi E, Yamashita T, Arai K, Terashima T, Kitahara M, Nakagawa H, Iida N, Fushimi K, Kaneko S. Myeloid-derived suppressor cells correlate with patient outcomes in hepatic arterial infusion chemotherapy for hepatocellular carcinoma. Cancer Immunology, Immunotherapy. 2016; 65:715-725.
31. Matsui D, Nagai H, Mukozu T, Ogino YU, Sumino Y. VEGF in patients with advanced hepatocellular carcinoma receiving intra-arterial chemotherapy. Anticancer Res. 2015; 35:2205-2210.
32. Tsunematsu S, Suda G, Yamasaki K, *et al*. Combination of neutrophil-to-lymphocyte ratio and early des--carboxyprothrombin change ratio as a useful predictor of treatment response for hepatic arterial infusion chemotherapy against advanced hepatocellular carcinoma. Hepatology Research. 2017; 47:533-541.
33. Saeki I, Yamasaki T, Tanabe N, Iwamoto T, Matsumoto T, Urata Y, Hidaka I, Ishikawa T, Takami T, Yamamoto N, Uchida K, Terai S, Sakaida I. A new therapeutic assessment score for advanced hepatocellular carcinoma patients receiving hepatic arterial infusion chemotherapy. PloS one. 2015; 10:e0126649-e0126649.
34. Takaya H, Namisaki T, Moriya K, *et al*. Association between ADAMTS13 activity-VWF antigen imbalance and the therapeutic effect of HAIC in patients with hepatocellular carcinoma. World J Gastroenterol. 2020; 26:7232-7241.
35. Filatenkov A, Baker J, Mueller AM, Kenkel J, Ahn GO, Dutt S, Zhang N, Kohrt H, Jensen K, Dejbakhsh-Jones S, Shizuru JA, Negrin RN, Engleman EG, Strober S. Ablative Tumor Radiation Can Change the Tumor Immune Cell Microenvironment to Induce Durable Complete Remissions. Clin Cancer Res. 2015; 21:3727-3739.
36. Young S, Ragulojan R, Chen T, Owen J, D'Souza D, Sanghvi T, Golzarian J, Flanagan S. Dynamic Lymphocyte Changes Following Transarterial Radioembolization: Association with Normal Liver Dose and Effect on Overall Survival. J Hepatocell Carcinoma. 2022; 9:29-39.
37. Carr BI, Metes DM. PERIPHERAL BLOOD LYMPHOCYTE DEPLETION AFTER HEPATIC ARTERIAL (YTTRIUM)-Y-90 MICROSPHERE THERAPY FOR HEPATOCELLULAR CARCINOMA. International Journal of Radiation Oncology Biology Physics. 2012; 82:1179-1184.
38. Liu CA, Lee IC, Lee RC, Chen JL, Chao YE, Hou MC, Huang YH. Prediction of survival according to kinetic changes of cytokines and hepatitis status following radioembolization with yttrium-90 microspheres. Journal of the Formosan Medical Association. 2021; 120:1127-1136.
39. Song CW, Kim MS, Cho LC, Dusenbery K, Sperduto PW. Radiobiological basis of SBRT and SRS. Int J Clin Oncol. 2014; 19:570-578.
40. Ng SSW, Zhang H, Wang L, Citrin D, Dawson LA. Association of pro-inflammatory soluble cytokine receptors early during hepatocellular carcinoma stereotactic radiotherapy with liver toxicity. NPJ Precis Oncol. 2020; 4:17.
41. Cuneo KC, Devasia T, Sun Y, Schipper MJ, Karnak D, Davis MA, Owen D, Feng M, El Naqa I, Bazzi L, Ten Haken R, Lawrence TS. Serum Levels of Hepatocyte Growth Factor and CD40 Ligand Predict Radiation-Induced Liver Injury. Transl Oncol. 2019; 12:889-894.
42. Hsiang CW, Huang WY, Yang JF, Shen PC, Dai YH, Wang YF, Lin CS, Chang WC, Lo CH. Dynamic Changes in Neutrophil-to-Lymphocyte Ratio are Associated with Survival and Liver Toxicity Following Stereotactic Body Radiotherapy for Hepatocellular Carcinoma. Journal of Hepatocellular Carcinoma. 2021; 8:1299-1309.
43. Zhuang Y, Yuan BY, Hu Y, Chen GW, Zhang L, Zhao XM, Chen YX, Zeng ZC. Pre/Post-Treatment Dynamic of Inflammatory Markers Has Prognostic Value in Patients with Small Hepatocellular Carcinoma Managed by Stereotactic Body Radiation Therapy. Cancer Management and Research. 2019; 11:10929-10937.
44. Kang TW, Rhim H. Recent Advances in Tumor Ablation for Hepatocellular Carcinoma. Liver Cancer. 2015; 4:176-187.
45. Ji L, Gu J, Chen L, Miao D. Changes of Th1/Th2 cytokines in patients with primary hepatocellular carcinoma after ultrasound-guided ablation. Int J Clin Exp Pathol. 2017; 10:8715-8720.
46. Duan XH, Li H, Han XW, Ren JZ, Li FY, Ju SG, Chen PF, Kuang DL. Upregulation of IL-6 is involved in moderate hyperthermia induced proliferation and invasion of hepatocellular carcinoma cells. European Journal of Pharmacology. 2018; 833:230-236.
47. Yu ZS, Li GW, Yu H, Asakawa T. Changes of immune cells in patients with hepatocellular carcinoma treated by

radiofrequency ablation and hepatectomy, a pilot study. Open Life Sciences. 2021; 16:1002-1009.
48. Rochigneux P, Nault JC, Mallet F, *et al*. Dynamic of systemic immunity and its impact on tumor recurrence after radiofrequency ablation of hepatocellular carcinoma. Oncoimmunology. 2019; 8.
49. Mizukoshi E, Yamashita T, Arai K, Sunagozaka H, Ueda T, Arihara F, Kagaya T, Yamashita T, Fushimi K, Kaneko S. Enhancement of tumor-associated antigen-specific T cell responses by radiofrequency ablation of hepatocellular carcinoma. Hepatology. 2013; 57:1448-1457.
50. Schueller G, Kettenbach J, Sedivy R, Stift A, Friedl J, Gnant M, Lammer J. Heat shock protein expression induced by percutaneous radiofrequency ablation of hepatocellular carcinoma *in vivo*. Int J Oncol. 2004; 24:609-613.
51. Shen S, Peng H, Wang Y, Xu M, Lin M, Xie X, Peng B, Kuang M. Screening for immune-potentiating antigens from hepatocellular carcinoma patients after radiofrequency ablation by serum proteomic analysis. BMC Cancer. 2018; 18:117.
52. Wu H, Li SS, Zhou MJ, Jiang AN, He YN, Wang S, Yang W, Liu HM. Palliative Radiofrequency Ablation Accelerates the Residual Tumor Progression Through Increasing Tumor-Infiltrating MDSCs and Reducing T-Cell-Mediated Anti-Tumor Immune Responses in Animal Model. Frontiers in Oncology. 2020; 10.
53. Urano M, Tanaka C, Sugiyama Y, Miya K, Saji S. Antitumor effects of residual tumor after cryoablation: the combined effect of residual tumor and a protein-bound polysaccharide on multiple liver metastases in a murine model. Cryobiology. 2003; 46:238-245.
54. Pimentel CB, Moraes AM, Cintra ML. Angiogenic effects of cryosurgery with liquid nitrogen on the normal skin of rats, through morphometric study. An Bras Dermatol. 2014; 89:410-413.
55. Zhou L, Fu JL, Lu YY, Fu BY, Wang CP, An LJ, Wang XZ, Zeng Z, Zhou CB, Yang YP, Wang FS. Regulatory T cells are associated with post-cryoablation prognosis in patients with hepatitis B virus-related hepatocellular carcinoma. Journal of Gastroenterology. 2010; 45:968-978.
56. Zeng Z, Shi F, Zhou L, *et al*. Upregulation of circulating PD-L1/PD-1 is associated with poor post-cryoablation prognosis in patients with HBV-related hepatocellular carcinoma. PLoS One. 2011; 6:e23621.
57. Huang M, Wang X, Bin H. Effect of Transcatheter Arterial Chemoembolization Combined with Argon–Helium Cryosurgery System on the Changes of NK Cells and T Cell Subsets in Peripheral Blood of Hepatocellular Carcinoma Patients. Cell Biochemistry and Biophysics. 2015; 73:787-792.
58. Zhou Y, Xu X, Ding J, Jing X, Wang F, Wang Y, Wang P. Dynamic changes of T-cell subsets and their relation with tumor recurrence after microwave ablation in patients with hepatocellular carcinoma. J Cancer Res Ther. 2018; 14:40-45.
59. Leuchte K, Staib E, Thelen M, *et al*. Microwave ablation enhances tumor-specific immune response in patients with hepatocellular carcinoma. Cancer Immunol Immunother. 2021; 70:893-907.
60. Zhang H, Hou X, Cai H, Zhuang X. Effects of microwave ablation on T-cell subsets and cytokines of patients with hepatocellular carcinoma. Minim Invasive Ther Allied Technol. 2017; 26:207-211.
61. Zhou Q, Zhu XQ, Zhang J, Xu ZL, Lu P, Wu F. Changes in circulating immunosuppressive cytokine levels of cancer patients after high intensity focused ultrasound treatment. Ultrasound Med Biol. 2008; 34:81-87.
62. Tonguc T, Strunk H, Gonzalez-Carmona MA, *et al*. US-guided high-intensity focused ultrasound (HIFU) of abdominal tumors: outcome, early ablation-related laboratory changes and inflammatory reaction. A single-center experience from Germany. International Journal of Hyperthermia. 2021; 38:65-74.
63. Yi Y, Han J, Fang Y, Liu D, Wu Z, Wang L, Zhao L, Wei Q. Sorafenib and a novel immune therapy in lung metastasis from hepatocellular carcinoma following hepatectomy: A case report. Molecular and clinical oncology. 2016; 5:337-341.
64. Vogl TJ, Mack MG, Balzer JO, Engelmann K, Straub R, Eichler K, Woitaschek D, Zangos S. Liver metastases: neoadjuvant downsizing with transarterial chemoembolization before laser-induced thermotherapy. Radiology. 2003; 229:457-464.
65. Jondal DE, Thompson SM, Butters KA, Knudsen BE, Anderson JL, Carter RE, Roberts LR, Callstrom MR, Woodrum DA. Heat Stress and Hepatic Laser Thermal Ablation Induce Hepatocellular Carcinoma Growth: Role of PI3K/mTOR/AKT Signaling. Radiology. 2018; 288:730-738.
66. De Landro M, Espíritu García-Molina I, Barberio M, Felli E, Agnus V, Pizzicannella M, Diana M, Zappa E, Saccomandi P. Hyperspectral Imagery for Assessing Laser-Induced Thermal State Change in Liver. Sensors (Basel). 2021; 21.
67. Lu DS, Kee ST, Lee EW. Irreversible electroporation: ready for prime time? Tech Vasc Interv Radiol. 2013; 16:277-286.
68. Guo X, Du F, Liu Q, Guo Y, Wang Q, Huang W, Wang Z, Ding X, Wu Z. Immunological effect of irreversible electroporation on hepatocellular carcinoma. BMC Cancer. 2021; 21:443.
69. Dai ZH, Wang ZR, Lei K, Liao JB, Peng ZW, Lin MX, Liang P, Yu J, Peng S, Chen SL, Kuang M. Irreversible electroporation induces CD8(+) T cell immune response against post-ablation hepatocellular carcinoma growth. Cancer Letters. 2021; 503:1-10.
70. Ju S, Zhou C, Yang C, Wang C, Liu J, Wang Y, Huang S, Li T, Chen Y, Bai Y, Yao W, Xiong B. Apatinib Plus Camrelizumab With/Without Chemoembolization for Hepatocellular Carcinoma: A Real-World Experience of a Single Center. Front Oncol. 2021; 11:835889.
71. Erös de Bethlenfalva-Hora C, Mertens Joachim C, Piguet A-C, Kettenbach J, Schmitt J, Terracciano L, Weimann R, Dufour J-F, Geier A. Radiofrequency ablation suppresses distant tumour growth in a novel rat model of multifocal hepatocellular carcinoma. Clinical Science. 2013; 126:243-252.
72. Deng YR, Liu WB, Lian ZX, Li XS, Hou X. Sorafenib inhibits macrophage-mediated epithelial-mesenchymal transition in hepatocellular carcinoma. Oncotarget. 2016; 7:38292-38305.
73. Tohyama O, Matsui J, Kodama K, Hata-Sugi N, Kimura T, Okamoto K, Minoshima Y, Iwata M, Funahashi Y. Antitumor activity of lenvatinib (e7080): an angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. J Thyroid Res. 2014; 2014:638747.
74. Torrens L, Montironi C, Puigvehí M, *et al*. Immunomodulatory Effects of Lenvatinib Plus Anti-Programmed Cell Death Protein 1 in Mice and Rationale for Patient Enrichment in Hepatocellular Carcinoma. Hepatology. 2021; 74:2652-2669.

75. Shi Q, Li T, Huang S, Bai Y, Wang Y, Liu J, Zhou C, Chen Y, Xiong B. Transcatheter Arterial Embolization Containing Donafenib Induces Anti-Angiogenesis and Tumoricidal CD8(+) T-Cell Infiltration in Rabbit VX2 Liver Tumor. Cancer Manag Res. 2021; 13:6943-6952.
76. Feng S, Wang H, Wang Y, Sun R, Xie Y, Zhou Z, Wang H, Aa J, Zhou F, Wang G. Apatinib induces 3-hydroxybutyric acid production in the liver of mice by peroxisome proliferator-activated receptor α activation to aid its antitumor effect. Cancer Sci. 2019; 110:3328-3339.
77. He X, Huang Z, Liu P, Li Q, Wang M, Qiu M, Xiong Z, Yang S. Apatinib Inhibits the Invasion and Metastasis of Liver Cancer Cells by Downregulating MMP-Related Proteins *via* Regulation of the NF-κB Signaling Pathway. Biomed Res Int. 2020; 2020:3126182.
78. Liu YC, Wu RH, Wang WS. Regorafenib diminishes the expression and secretion of angiogenesis and metastasis associated proteins and inhibits cell invasion *via* NF-κB inactivation in SK-Hep1 cells. Oncol Lett. 2017; 14:461-467.
79. Ou DL, Chen CW, Hsu CL, Chung CH, Feng ZR, Lee BS, Cheng AL, Yang MH, Hsu C. Regorafenib enhances antitumor immunity *via* inhibition of p38 kinase/Creb1/Klf4 axis in tumor-associated macrophages. J Immunother Cancer. 2021; 9.
80. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. J Exp Med. 2012; 209:1201-1217.
81. Feun LG, Li YY, Wu C, Wangpaichitr M, Jones PD, Richman SP, Madrazo B, Kwon D, Garcia-Buitrago M, Martin P, Hosein PJ, Savaraj N. Phase 2 study of pembrolizumab and circulating biomarkers to predict anticancer response in advanced, unresectable hepatocellular carcinoma. Cancer. 2019; 125:3603-3614.
82. Shi J, Liu J, Tu X, Li B, Tong Z, Wang T, Zheng Y, Shi H, Zeng X, Chen W, Yin W, Fang W. Single-cell immune signature for detecting early-stage HCC and early assessing anti-PD-1 immunotherapy efficacy. J Immunother Cancer. 2022; 10.
83. Agdashian D, ElGindi M, Xie C, *et al*. The effect of anti-CTLA4 treatment on peripheral and intra-tumoral T cells in patients with hepatocellular carcinoma. Cancer Immunol Immunother. 2019; 68:599-608.
84. Zhang L, Wang J, Jiang J, Zhang M, Shen J. CTLA-4 Blockade Suppresses Progression of Residual Tumors and Improves Survival After Insufficient Radiofrequency Ablation in a Subcutaneous Murine Hepatoma Model. Cardiovasc Intervent Radiol. 2020; 43:1353-1361.
85. Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, Korman AJ. Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. Cancer Immunol Res. 2013; 1:32-42.
86. Wu H, Zhu JQ, Xu XF, Xing H, Wang MD, Liang L, Li C, Jia HD, Shen F, Huang DS, Yang T. Biointerfacing Antagonizing T-Cell Inhibitory Nanoparticles Potentiate Hepatocellular Carcinoma Checkpoint Blockade Therapy. Small. 2021; 17:e2105237.
87. Kudo M, Ueshima K, Ikeda M, *et al*. Randomised, multicentre prospective trial of transarterial chemoembolisation (TACE) plus sorafenib as compared with TACE alone in patients with hepatocellular carcinoma: TACTICS trial. Gut. 2020; 69:1492-1501.
88. Ma H, Zhang Y, Wang Q, Li Y, He J, Wang H, Sun J, Pan K, Chen M, Xia J. Therapeutic safety and effects of adjuvant autologous RetroNectin activated killer cell immunotherapy for patients with primary hepatocellular carcinoma after radiofrequency ablation. Cancer Biol Ther. 2010; 9:903-907.
89. Zhou P, Liang P, Dong B, Yu X, Han Z, Xu Y. Phase I clinical study of combination therapy with microwave ablation and cellular immunotherapy in hepatocellular carcinoma. Cancer Biol Ther. 2011; 11:450-456.
90. Duffy AG, Ulahannan SV, Makorova-Rusher O, *et al*. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. J Hepatol. 2017; 66:545-551.
91. Catalano M, Casadei-Gardini A, Vannini G, Campani C, Marra F, Mini E, Roviello G. Lenvatinib: established and promising drug for the treatment of advanced hepatocellular carcinoma. Expert Review of Clinical Pharmacology. 2021; 14:1353-1365.
92. Shigeta K, Datta M, Hato T, *et al*. Dual Programmed Death Receptor-1 and Vascular Endothelial Growth Factor Receptor-2 Blockade Promotes Vascular Normalization and Enhances Antitumor Immune Responses in Hepatocellular Carcinoma. Hepatology. 2020; 71:1247-1261.
93. Zhu Y, Chen M, Xu D, Li TE, Zhang Z, Li JH, Wang XY, Yang X, Lu L, Jia HL, Dong QZ, Qin LX. The combination of PD-1 blockade with interferon-α has a synergistic effect on hepatocellular carcinoma. Cell Mol Immunol. 2022; 19:726-737.
94. Qi X, Yang M, Ma L, Sauer M, Avella D, Kaifi JT, Bryan J, Cheng K, Staveley-O'Carroll KF, Kimchi ET, Li G. Synergizing sunitinib and radiofrequency ablation to treat hepatocellular cancer by triggering the antitumor immune response. Journal for immunotherapy of cancer. 2020; 8.
95. Mi H, Ho WJ, Yarchoan M, Popel AS. Multi-Scale Spatial Analysis of the Tumor Microenvironment Reveals Features of Cabozantinib and Nivolumab Efficacy in Hepatocellular Carcinoma. Front Immunol. 2022; 13:892250.
96. Ho WJ, Zhu Q, Durham J, *et al*. Neoadjuvant Cabozantinib and Nivolumab Converts Locally Advanced HCC into Resectable Disease with Enhanced Antitumor Immunity. Nat Cancer. 2021; 2:891-903.
97. Sangro B, Sarobe P, Hervas-Stubbs S, Melero I. Advances in immunotherapy for hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2021; 18:525-543.
98. Fu Y, Liu S, Zeng S, Shen H. From bench to bed: the tumor immune microenvironment and current immunotherapeutic strategies for hepatocellular carcinoma. J Exp Clin Cancer Res. 2019; 38:396.
99. Lawal G, Xiao Y, Rahnemai-Azar AA, Tsilimigras DI, Kuang M, Bakopoulos A, Pawlik TM. The Immunology of Hepatocellular Carcinoma. Vaccines (Basel). 2021; 9:1184.

Received October 20, 2023; Revised November 11, 2023; Accepted November 16, 2023.

[§]These authors contributed equally to this work.
**Address correspondence to:*
Rui Liao, Department of Hepatobiliary Surgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China.
E-mail: liaorui99@163.com

Released online in J-STAGE as advance publication November 19, 2023.