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Immunotherapy for advanced or recurrent hepatocellular carcinoma

Immunotherapy for HCC

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Abstract

Hepatocellular carcinoma (HCC) is associated with high morbidity and mortality, and is prone to intra-and extrahepatic metastasis owing to the anatomical and functional characteristics of the liver. Due to the complexity and high relapse rate associated with radical surgery or radiofrequency ablation, immune checkpoint inhibitors (ICIs) are increasingly being used to treat HCC. Several immunotherapeutic agents, along with their combinations, have been clinically approved to treat advanced or recurrent HCC. This review discusses the leading ICIs in practice and those currently undergoing randomized phase I– III trials as monotherapy or combination therapy. Furthermore, we summarize the rapidly developing alternative strategies such as chimeric antigen receptor-engineered T cell therapy, tumor vaccines, and oncolytic viruses. Combination therapy is a promising potential treatment option. These immunotherapies are also summarized in this review, which provides insights into the advantages, limitations, and novel angles for future research in establishing viable and alternative therapies against HCC.

Key Words: Recurrent HCC; Immunotherapy; Immune checkpoint inhibitor; CAR-T; Oncolytic Viruse; Tumor vaccines

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Core Tip: The high recurrence rate of hepatocellular carcinoma (HCC) following radical treatment remains challenging; therefore, immune checkpoint inhibitors (ICIs) are increasingly being used to treat HCC. Herein, we discuss the ICIs in practice and those undergoing trials, and summarize the alternative strategies such as chimeric antigen receptor-engineered T cell therapy and tumor vaccines. Combination therapy is also a promising potential treatment option. We believe our study significantly contributes to the literature as it addresses the current state of immunotherapy against HCC and

provides insights into the advantages and limitations, thereby facilitating future research.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy (75-85% of cases), sixth most diagnosed cancer, and the third most common cause of cancer-related deaths worldwide in 2020 [1]. The incidence and main risk factors for HCC vary from area to area. Traditionally, the highest epidemic of HCC is mainly in East and South-East Asia; however, the incidence of HCC has increased in the United States and Europe [2]. The key risk factors of HCC include chronic HBV or HCV infection, aflatoxin-contaminated foods, excessive drinking, obesity, and smoking [3, 4].

Hepatic resection is the best method for treating early-stage HCC [5-7]. Radiofrequency ablation (RFA) is also considered a radical treatment in many patients with small HCC and is the recommended treatment for patients with a single tumor < 2 cm or two–three nodules of ≤ 3 cm [5-8]. Less than 30% of patients with HCC can be treated with surgery and RFA due to distant metastases, anatomical location limitations, hepatic insufficiency, and neurovascular invasion [9, 10]. Besides, patients with HCC who receive radical treatment have a high recurrence rate, typically manifesting as recurrence in liver remnants [11]. The recurrence rate in early HCC patients remains high at 5 years post curable excision[11-13]. Most HCCs (> 70%) are diagnosed at an advanced stage [14]. Radical treatment of recurrent HCC includes repeated hepatic resection and liver transplantation; these radical treatments are complex owing to the shortage of donors, small residual areas of liver after hepatectomy, hepatic dysfunction, and multiple metastases. Due to the particularity of advanced and recurrent HCC, radiotherapy alone is not recommended. Systematic chemotherapy is also rarely recommended due to resistance to multiple cytotoxic drugs and abnormal liver function [15]. Therefore, local interventional therapies have been developed to treat recurrence, including transcatheter arterial chemoembolization (TACE) [16] and hepatic artery infusion chemotherapy (HAIC) [17]. Locoregional therapy is, for the most part, not a radical

treatment, with recurrence and local disease progression being typical. For patients undergoing these, there is an urgent need to explore new therapies to treat recurrent HCC.

Sorafenib, which was been recommended as a first-line treatment for liver cancer with Child-Pugh type A liver function and BCLC-C in 2007, is a multi-tyrosine kinase inhibitor (TKI) that can extend median overall survival (mOS) and the time to radiologic progression by three months [18]. Lenvatinib, which is an alternative first-line treatment for advanced HCC [19], was not inferior to sorafenib. However, lenvatinib was associated with significant improvements compared with sorafenib in terms of higher objective response rate (ORR), prolonged progression-free survival (PFS), and prolonged time to progression (TTP) [20, 21]. Regorafenib [22], cabozantinib [23], and ramucirumab [24, 25] were recommended as second-line treatment of advanced HCC [26]. These licensed systemic multi-TKIs may be poorly tolerated owing to their significant side effects, drug resistance, and modest benefits in mOS [21, 27-29]. Since nivolumab was approved as a second-line treatment for advanced HCC in 2017, immunotherapy for recurrent or advanced HCC has witnessed rapid development. Nivolumab, pembrolizumab, atezolizumab, durvalumab, ipilimumab, tremelimumab, tislelizumab, sintilimab, and camrelizumab and their combinations have been approved in succession for HCC treatment[8]. The advent of cancer immunotherapy has completely changed the traditional treatment concept for HCC by stimulating the immune system of individuals to kill tumor cells selectively. Other immunotherapy strategies, such as chimeric antigen receptor-engineered T cells (CAR-Ts) and therapeutic cancer vaccines, have matured to the stage of clinical trials, offering new hope for HCC patients[30-32]. This article reviews approved immunotherapies and those in clinical development for HCC treatment.

LIVER AND HCC IMMUNITY

The liver, which receives arterial and venous blood is exposed to pathogens in the systemic circulation (mainly from the gut). Liver immunosurveillance is one of the most critical lines of defense. The liver contains a variety of immune cells, some of which are

innate immune cells, including neutrophils, macrophages, natural killer cells (NKs), natural killer T cells (NKTs), dendritic cells (DCs), and Kupffer cells, all of which are essential immune sentinels and antigen-presenting cells [33-37]. Kupffer cells can capture antigens under flowing conditions, while NKs and NKTs can be activated upon detection of antigens and directly release granulosin and perforase to act on target cells or release large amounts of cytokines (such as IFN-γ) to direct the immune response [38-40]. DCs are the most potent antigen presenting cells (APCs), which can effectively take up, process, and present antigens. As important immune cells, DCs can participate in the development and activation of T and B cells. DCs can also secrete a variety of cytokines (interleukin, interferon, and tumor necrosis factor) and chemokines to participate in the immune function regulation and mediate the chemotaxis of other immune cells [33, 41, 42]. Neutrophils promote the progression of HCC by interacting with macrophages and regulatory T cells. Large numbers of neutrophils predict poor tumor status[43, 44]. Conversely, adaptive immune cells include B cells, plasma cells, and effector T cells. A normal liver provides a tolerant microenvironment that inhibits innate and adaptive immunity in homeostasis and prevents inflammation or tissue damage in the liver [45, 46].

The immune system of the liver plays a vital role in controlling the occurrence and development of HCC. The interaction between innate and adaptive immunity can lead to effective anti-tumor immunosurveillance [47]. Tumor cells, regulatory T cells (Tregs), inhibitory B lymphocytes and other inhibitory cells mediate the tumor microenvironment by regulating negative costimulatory molecules to achieve immune escape [48]. In addition, myeloid suppressor cells (MDSC) or M2-polarized tumor-associated macrophages (TAM) generate an inflammatory microenvironment, which can also serve as a medium for tumor initiation, angiogenesis and metastasis [49]. Transforming growth factor beta (TGF-β) is the primary mediator for this activity [50] and plays a central role in inflammation, fibrogenesis, and immunomodulation in the HCC microenvironment [51, 52]. Therefore, controlling the synthesis and activation of TGF-β during tumor progression is important.

PRINCIPLES OF HEPATOCELLULAR IMMUNOTHERAPY AND ICI

Tumor cells inhibit immune checkpoint overactivation and express corresponding ligands to achieve an immune escape [53]. We have previously studied various immunosuppressive receptors, including programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), lymphocyte-activation gene 3 (LAG3), T cell immunoglobulin and mucin domain containing-3 (TIM3), and B- and T-lymphocyte attenuator (BTLA) [54, 55]. For example, the inhibitory receptor on T cells, PD-1, can be expressed in various immune cell types and binds to programmed death ligand-1 (PD-L1) of the corresponding target cells to inhibit the effects of T cells. CTLA-4 is expressed on the surface of activated T cells by competing with CD28 and binding to CD80 and CD86 to reduce the co-inhibitory signal of CD28 and induce T cell apoptosis. Meanwhile, CTLA-4, an essential gene in Treg differentiation, development, and maintenance of cell functions is highly expressed in Tregs [56]. The concept of blocking inhibitory immune receptors and activating the anti-tumor function of reinvigorated immune cells has been experimentally demonstrated and translated into the clinical treatment of many types of tumors [57]. Inhibitors of PD-1, PD-L1, and CTLA-4, known as ICIs, are an essential part of immunotherapy for many tumors, including melanoma, non-small cell lung cancer, and colorectal cancer [58]. ICIs, which can block the influence of negative immune costimulatory molecules, can exhibit anti-tumor activity and kill tumor cells by promoting and upregulating the activation of T cells, thereby restoring normal physiological functions of the human body [59]. ICIs have shown that effective immune response can exterminate tumor cells. Some approved ICIs and those in clinical research and their related targets of HCC are summarized in this review (Figure 1).

SINGLE IMMUNE AGENT THERAPY

Nivolumab

Nivolumab was approved in 2017 for patients with recurrent HCC who showed no respond to sorafenib treatment [60]. Nivolumab showed noble safety and tolerability in the phase of escalation (0.1 – 10 mg/Kg) in the CheckMate 040 study. Only 12 of 48 patients (25%) experienced grade 3 or 4 adverse reactions, and no deaths linked to nivolumab treatment were confirmed. In the phase of dose expansion (3 mg/Kg), ORR, disease control rate (DCR), and mPFS were 20%, 40%, and four months, respectively. Compared with the phase of escalation, the indices of the dose-expansion phase were significantly improved[61]. In the CheckMate 040 study (NCT01658878) (Table 1), a single nivolumab showed an enduring response, controlled safety, and satisfactory survival in patients with advanced HCC. As the CheckMate 040 study lacked a randomized control, the CheckMate 459 randomized trial (NCT02576509) (Table 1) was conducted to evaluate the efficacy of nivolumab *vs* sorafenib in a first-line setting. Although nivolumab did not significantly improve mOS (16.4 vs. 14.7 mo; hazard ratio [HR] =0.85; P = 0.075) compared with sorafenib, a lower proportion of grade 3 or 4 treatment-related adverse events, persistent response frequency, and clinical activity make nivolumab a broader treatment prospect [62].

Pembrolizumab

Pembrolizumab, an anti-PD-1 mAb, has demonstrated promising antineoplastic effects and safety in a variety of malignant tumors [63]. KEYNOTE-224 study (NCT02702414) (Table1) was conducted to evaluate the efficacy and safety of pembrolizumab in patients with recurrent HCC with no response to sorafenib. Results included ORR of 17%, DCR of 62%, mPFS of 4.9 mo, mOS of 12.9 mo, and grade 3 or 4 adverse reactions that occurred in 25% of the clinical trial participants. Therefore, the FDA approved pembrolizumab for treating unresectable intermediate and advanced HCC in November 2018 [64]. Pembrolizumab showed good efficacy and a controllable safety profile in patients with advanced HCC who had previously received sorafenib; therefore a worldwide phase III study of pembrolizumab (KEYNOTE-240) (NCT02702401) (Table1) was conducted. In the second-line treatment of advanced HCC,

mOS of Pembrolizumab and placebo were 13.9 vs. 10.6 mo (HR, 0.77), mPFS was 3.3 vs. 2.8 mo (HR, 0.70), and OS and PFS did not meet the specified criteria for statistical significance. Improvements in ORR, DCR, PFS, and OS with pembrolizumab treatment were consistent with the results of the single-cohort KEYNOTE-224 study (Table 1). The difference in ORR (18.4 vs. 4.4%) favored pembrolizumab[65]. Accelerated FDA approval was acquired for pembrolizumab use for treating advanced HCC in patients who failed to respond to prior sorafenib therapy.

Camrelizumab

Camrelizumab, a IgG4 anti-PD-1 mAb, is used to treat several cancers, including lymphoma, lung cancer, esophageal cancer, and HCC [66, 67]. Camrelizumab showed significant anti-tumor efficacy and tolerance in patients with advanced solid tumors in phase I trials [68-70]. To continue evaluating the activity and safety of camrelizumab as a second-line or higher treatment for advanced or recurrent HCC, a randomized phase II trial (NCT02989922) (Table 1) was conducted. A total of 217 patients with advanced HCC were randomly assigned in a 1:1 ratio to two groups, including two weeks of camrelizumab (3 mg/kg) (n = 109) treatment and three weeks of camrelizumab (3 mg/kg) (n = 108) treatment. At the end of data cutoff, survival metrics from the 2- or 3-week group, including mOS (14.2 mo vs. 13.2 mo), mPFS (2.3 vs. 2 mo), DCR (47.7 vs. 44 %) and ORR (11.9 vs. 17.6%) showed good anti-tumor activity. In terms of safety, grade 3 or 4 adverse events occurred in 47 patients (22%) [71].

Compared with other PD-1 inhibitors, camrelizumab experienced a significantly lower DCR (44.2 vs. 55% with nivolumab [62] in sorafenib-patients and 47.7 vs. 62% with pembrolizumab [64] in the second-line setting after sorafenib use) and shorter mPFS (2.1 vs. 4.9 mo with pembrolizumab and 3.7 mo with nivolumab). Overall, camrelizumab demonstrated potential anti-tumor efficacy and safety. However, the efficacy of single camrelizumab was limited; hence, a combination with targeted agents and other ICIs are needed to improve the efficacy.

In March 2020, camrelizumab was approved by the Chinese Food and Drug Administration for treating patients with advanced HCC who had received sorafenib or chemotherapy with oxaliplatin. Camrelizumab is also the first PD-1 inhibitor with HCC indications approved in China, which is a breakthrough in immunotherapy in China.

Tremelimumab

Tremelimumab is a human IgG2 mAb that blocks the binding of CTLA-4 [72]. ORR was 17.6% with a DCR of 76.4% in a clinical trial of tremelimumab in patients with HCC and chronic HCV. Surprisingly, tremelimumab showed satisfactory anti-tumor activity, antiviral activity, and safety in patients with advanced HCC developed from HCV-induced liver cirrhosis. However, the first trial of tremelimumab for HCC included only 20 patients and therefore could not account for chance results caused by multiple clinical covariates [73]. In a phase II clinical trial of tremelimumab in combination with durvalumab for HCC (NCT02519348) (Tables 1 and 2), 326 patients were assigned to 4 cohorts, namely the tremelimumab monotherapy arm (750 mg once every 4 wk [seven doses] and then once every 12 wk), durvalumab monotherapy arm and T300+D arm (tremelimumab 300 mg plus durvalumab 1,500 mg [one dose each during the first cycle] followed by durvalumab 1,500 mg once every 4 wk), and T75+D arm (750 mg once every 4 wk [seven doses] and then once every 12 wk). The tremelimumab monotherapy arm represented the first large cohort of HCC patients receiving anti-CTLA-4 monotherapy. The ORR was 7.2%, DCR was 49.3%, mOS was 15.1 mo, and mPFS was 2.69 mo. Although the ORR of this cohort was the lowest (7.2%), the mOS was the second longest, and the median duration of response (mDOR) was prolonged (23.95 mo). However, the grade 1-4 adverse events of T300+D were highest (82.4%), while that of grade ≥ 3 adverse events of tremelimumab monotherapy was the highest (43.5%). Among the four arms, the tremelimumab monotherapy received the highest dose of tremelimumab; therefore, serious adverse reactions were considered to be dose-related to tremelimumab. Compared with tremelimumab monotherapy, the combination of T300+D significantly enhanced anti-tumor efficacy [74].

Durvalumab

In the phase II clinical trial of tremelimumab in combination with durvalumab for HCC (NCT02519348) (Tables 1 and 2) mentioned before, 104 patients with HCC who had progressed on, were intolerant to, or refused sorafenib were randomly assigned to receive durvalumab monotherapy; ORR was 10.6%, DCR was 37.5%, mOS was 13.6 mo, and mPFS was 2.07 mo [74]. Meanwhile, in a phase III (NCT03298451) (Table 1) of tremelimumab in combination with durvalumab for HCC, the durvalumab monotherapy arm was non-inferior to sorafenib in ORR (17 VS. 5.1%) and mOS (16.56 VS. 13.77 mo). Compared with durvalumab monotherapy in the phase II study, durvalumab in this phase III study had significantly increased activity with ORR of 17%, DCR of 54.8%, mPFS of 3.65 mo, and mOS of 16.56 mo [82].

Tislelizumab

Tislelizumab (BGB-A317) is a humanized IgG4 mAb with high affinity and binding specificity for PD-1. Unlike nivolumab and pembrolizumab, tislelizumab evades the efficacy mediated by FcγR1 and minimizes the binding of macrophages to FcγR; this, may mitigate potential adverse interactions with other immune cells, including macrophages and MDSC[75-77]. Tislelizumab has demonstrated satisfactory tolerability and significant anti-tumor activity in patients with advanced HCC. Fifty advanced HCC patients who had previously received other anti-tumor therapies were reported in the HCC cohort, with an ORR of 12.2% (95%CI, 4.6 – 24.8), a DCR of 51% (95%CI, 36.3 – 65.6), and an average DOR of 15.7 mo. Preliminary safety and anti-tumor activity support the continued exploration and development of tislelizumab in patients with advanced HCC[78, 79]. Therefore, the phase II open-label clinical trial of tislelizumab (NCT03419897) further explored the efficacy and safety of Tislelizumab in the second-line treatment of advanced HCC and a phase III randomized controlled trial (NCT03412773) is currently evaluating the efficacy and safety of tislelizumab and

sorafenib as a first-line treatment for unresectable HCC. These results will provide more options for treating advanced and recurrent HCC.

COMBINATION OF DOUBLE IMMUNE AGENTS THERAPIES

Nivolumab and pembrolizumab have demonstrated anti-tumor properties in treating advanced HCC. PD-1/PD-L1 inhibitors and CTLA-4 inhibitors influence T cell response through a complementary mechanism to enhance anti-tumor efficacy [80]. These positive results inspired the study of the combination of PD-1/PD-L1 inhibitors with CTLA-4 inhibitors with an aim of longer survival and higher response rates. Several combinations of ICIs have been tested to prove their efficacy (Table 2), whereas some remain in the experimental research and development stage (Table 4). According to the preliminary results of the nivolumab and ipilimumab combination compared with nivolumab monotherapy, the ORR (34%) and mOS (22.8 mo) significantly increased with the combination of PD-1 and CTLA-4[81] (NCT01658878) (Table 2). The rate of adverse events was significantly higher with the combination of nivolumab and ipilimumab than with nivolumab monotherapy. More than 50% of patients in the Checkmate 040 study required corticosteroids, and the discontinuation rate was 22%, due to tolerably high immunotoxicity [61]. Similar results that showed the anti-tumor activity of dual immunoblockers being superior to that of single drug were also observed in tremelimumab and durvalumab for patients with unresectable HCC (NCT02519348). Compared with tremelimumab or durvalumab monotherapy, T300+D showed the most encouraging benefit-risk profile[74], which promotes T300+D to enter into phase III clinical trial (NCT03298451). For the 393 patients, the ORR was 20.1%, DCR was 60.1%, mOS was 16.4 mo and mPFS was 3.7 mo (Table 2). Durvalumab was not inferior to tremelimumab; however, the combination of T300+D showed superior efficacy and a favorable benefit-risk profile compared with durvalumab and tremelimumab monotherapy. Compared with the combination of nivolumab and ipilimumab, the incidence of immunotoxicity requiring systemic corticosteroids in the T300 + D regimen was 24.3%. The discontinuation rate was only 10.8%, due to adverse

reactions [82]. Overall, the results of these two studies demonstrated that PD-1/PD-L1 and CTLA-4 had different and complementary anti-tumor mechanisms.

COMBINATION OF IMMUNE AGENTS AND ANTIANGIOGENNIC DRUG THERAPIES

Combination of atezolizumab and bevacizumab

The overexpression of vascular endothelial growth factor (VEGFR) is important in the occurrence and development of HCC. Anti-VEGFR drugs (sorafenib and lenvatinib) effectively reduce VEGFR-mediated immune suppression and promote T cell activity in the tumor environment[83, 84]. Sorafenib was the first anti-VEGFR drug used to treat advanced HCC in the past decade. Since then, until the emergence of atezolizumab in combination with bevacizumab, no treatment has surpassed the first-line efficacy of sorafenib[18, 85]. In a phase Ib randomized cohort trial comprising 119 patients, atezolizumab in combination with bevacizumab resulted in significantly higher mPFS (7 mo) and ORR (36%) than atezolizumab monotherapy[86]. In the IMbrave150 clinical trial (NCT03434379) (Table 3), compared with sorafenib, the combination of atezolizumab (PD-L1) and bevacizumab (a vascular epidermal growth factor inhibitor) reduced the risk of death by 42% and extended mPFS and mOS by 2.5 and 5.8 mo (median follow-up 15.6 mo) respectively. The results showed an ORR of 27.3%, DCR of 74%, mPFS of 6.9 mo, and mOS of 19.2 mo. Notably, the ORR of this combination even reached more than twice that of sorafenib [85]. With long-term follow-up, to the best of our knowledge, this combination had the longest mOS observed in a phase III trial for HCC until now. In terms of safety, the grade ≥ 3 adverse events of the combination occurred in 160 patients (49%), which were consistent with the known adverse events of each drug [87]. The combination of atezolizumab and bevacizumab was approved by the FDA for treating patients with advanced or recurrent HCC who had not previously received systemic treatment [88].

Combination of pembrolizumab and lenvatinib

Lenvatinib was not statistically inferior to sorafenib in a phase III trial comparing lenvatinib with sorafenib as the first-line treatment for unresectable HCC. Compared with sorafenib, lenvatinib showed significant and clinically significant improvements in ORR, PFS, and TTP [21]. However, pembrolizumab also exhibited substantial anti-tumor activity and safety. Lenvatinib, in combination with pembrolizumab, has received accelerated approval for the treatment of advanced tumors that do not have high microsatellite instability or mismatch repair defects[89]. The encouraging preliminary trial data has led to a phase Ib study for the combinations of lenvatinib and pembrolizumab to treat unresectable HCC (NCT03006926) (Table 3). Surprisingly, the combination achieved an ORR of 46.0% (95%CI, 36.0–56.3%, mRECIST standard) and a DCR of >85% (regardless of the RECIST category). mPFS and mOS were 9.3 and 22 mo, respectively. The combination of lenvatinib and pembrolizumab showed no new adverse events [90]. Based on the interim data from this study, the FDA granted lenvatinib in combination with pembrolizumab as first-line therapy for advanced HCC. The combination is being studied in a randomized phase III trial (NCT03713593) and compared with the first-line treatment of unresectable or metastatic HCC using lenvatinib (Table 4).

Other combination of ICIs and antiangiogenic drugs therapy

ICIs combined with anti-angiogenic agents open a new avenue for treating HCC. In contrast, FDA-approved first-line combination therapies for HCC are only available in a few regions worldwide. Therefore, alternative therapies need to be developed and approved. Currently, PD-1/PD-L1 checkpoint inhibitors, CTLA-4 checkpoint inhibitors, Tyrosine kinase inhibitors (TKI), along with other anti-tumor agents are undergoing randomized phase I–III trials as monotherapy or combination therapy (Table 4). Cabozantinib, approved in 2019 by the FDA as a second-line treatment of sorafenib, has shown promising antitumor activity. COSMIC312 (NCT03755791) evaluated the combination of cabozantinib and atezolizumab *vs* sorafenib as first-line systemic therapy for HCC. Compared with sorafenib, the combination arm significantly

improved PFS (HR, 0.63; 99% CI, 0.44–0.91; *P* = 0.0012; mPFS 6.8 mo vs. 4.2 mo). However, OS was not improved[91] (HR, 0.90; 96% CI, 0.69-1.18; *P* = 0.438). At the end of 2020, the ORIENT-32 trial, which enrolled 571 HCC patients without systemic therapy, reported that combination of sintilizumab (PD-1) and bevacizumab biosimilar (IBI305) was significantly superior to sorafenib in terms of OS and PFS, as shown in Table 3. After a median follow-up of 10 mo, the mOS was not achieved in the combination line (sintilizumab and IBI305), while it was 10.4 mo in the sorafenib group (HR, 0.57; 95%CI, 0.43–0.75; P < 0.0001); mPFS (4.6 mo 95%CI, 4.1–5.7) was significantly prolonged (HR, 0.56, 95%CI, 0.46-0.70; P < 0.0001) [92]. In early 2021, camrelizumab (PD-1) in combination with apatinib (a selective vegFR-2 tyrosine kinase inhibitor) was assessed in phase II (NCT03463876) as the first- and second-line treatment for advanced HCC. Significant anti-tumor activity was achieved in ORR, DOR, and OS for both first- and second-line treatments [93] (Table 3). Encouraging anti-tumor properties continue to emerge in the new combination therapies with ICIs and TKIs, which will provide options for recurrent HCC treatment.

COMBINATION OF IMMUNE AGENTS AND LOCOREGIONAL THERAPIES

Some locoregional therapies for HCC, including radiotherapy, RFA, TACE and HAIC, can release or produce alertin substances from cancer cells to stimulate the aggregation of DCs into tumor tissues. This can upregulate the expression and antigenicity of tumor-associated antigens (TAAs) and trigger injury-related molecular patterns to induce "immunogenic cell death" [94-96]. Locoregional therapies can induce the release of proinflammatory cytokines to activate and expand innate and adaptive immune cells (NK and cytotoxic T cells) and reduce the activity of immunosuppressive cells (Tregs and MDSCs) [97-100]. Meanwhile, immunotherapy can not only improve the hypoxic microenvironment in tumors and enhance the effect of radiotherapy by inducing vascular normalization through a T cell-dependent pathway, but also enhance the immune induction effect of radiotherapy to slow the growth of distant tumors (abscopal effect). Radiotherapy and immunotherapy synergize to exert more potent local effects in

the irradiated tumors [101-102]. The IMMUTACE trial initially evaluated the efficacy of nivolumab plus TACE in 49 patients with mid-stage HCC; the ORR was 71.4% (95%CI, 56.8%-83.4%), including 16.3% complete responses (CRs) and 55.1% partial responses (PRs). Despite the small number of patients in each group, subgroup analyses did not reveal differences in treatment responses [103]. In the CA 209-678 study (NCT03033446) of Y90-radioembolisation followed by nivolumab in 36 patients with advanced HCC, the ORR of 30.6% compared favorably with an ORR of approximately 20% noted with Y90-radioembolisation. Notably, 81% of patients showed regression of radiation-field target lesions. This combination is safe and tolerable with grade 3–4 treatment related adverse events or serious adverse events noted in 14% of patients [104]. Many clinical trials of locoregional therapies combined with ICIs are being conducted successively (Table 5). This combination is expected to become the mainstream treatment for HCC in the future.

CAR-T CELL THERAPY FOR HCC

CAR-T therapy is a developing immunotherapy approach for treating malignant tumors. Owing to the great success of CAR-T therapy in the treatment of CD19-positive hematological malignancies, such as a complete response rate of up to 90% with anti-CD19 CAR-T cells in B-cell acute lymphoblastic leukemia (B-ALL)[105-108], two CAR-T cell therapies, Kymriah® and Yescarta®, were approved by the FDA for lymphoma studies in 2018 and 2017, respectively. Because of this lymphoma breakthrough, CAR-T's application in treating solid tumors, such as HCC, has also been explored. Glypican-3 (GPC-3), a member of the Glypican family, is a 70-kDa heparan sulfate proteoglycan overexpressed in HCC and associated with poor diagnosis and prognosis[109-112]. Several clinical trials have evaluated the safety and efficacy of GPC-3 CAR-T cells. Shanghai Renji Hospital combined lymphodepleting chemotherapy (LDC) with GPC-3 CAR-T cells in 13 patients with GPC3-positive HCC and confirmed the anti-tumor efficacy and safety of GPC3 CAR-T cells (NCT02395250) [113]. GPC-3 CAR-T cells combined with sorafenib may be a promising option for treating of HCC [114].Chongqing Xinqiao

Hospital has attempted to combine TACE with CAR-T to treat GPC3-positive advanced HCC (NCT03084380). Other clinical trials are recruiting patients to improve the efficacy of intratumoral or intravenous administration of GPC3-CART cells (NCT03130712, NCT02715362, NCT04951141, NCT03198546, and NCT05155189). In conclusion, GPC-3 is a promising target for future therapeutic strategies in HCC. Mucin1 glycoprotein (MUC-1) [115, 116] and epithelial cell adhesion molecule (EpCAM) [117] are two transmembrane glycoproteins that can be overexpressed during the occurrence and development of HCC and can be used as biomarkers and therapeutic targets for HCC. One clinical trial of MUC-1 CAR-T cells (NCT02587689) and two clinical trials of EpCAM CAR-T cells (NCT03013712 and NCT02729493) are ongoing. AFP, which is overexpressed in HCC, is another potential therapeutic target being explored. However, AFP is a glycoprotein of the cellular endocrine system and expression and is therefore considered inappropriate for the chimeric antigen receptor (CAR). Some researchers designed a highly specific antibody of the (AFP)-MHC complex to be expressed as the CAR and found that CAR-T cells of this antibody had an apparent inhibitory effect on HCC; this provided a promising new approach for HCC immunotherapy [118].

TUMOR VACCINE

Tumor vaccines are active immunotherapies that require the injection of tumor antigens, including viruses, DNA, peptides, and tumor cell-expressed genes, into patients to trigger tumor-associated antigen-specific immune responses and mediate powerful anti-tumor effects [119]. Therapeutic tumor vaccines include peptides, DCs, whole-cell vaccines, oncolytic viruses, and DNA reagents.

Peptides

Several peptide-based cancer vaccines have been assessed for HCC treatment. As a biomarker of HCC, AFP was constructed as a peptide vaccine, used in two patients with AFP-expressing tumors and showed high levels of AFP-specific CD8+ T cell expression and apparent safety (NCT00093548). GPC-3 is highly expressed in most malignant

tumors and is rarely in normal tissues; therefore, GPC-3 is considered an ideal tumor-associated antigen for developing cancer vaccines [111, 120]. The GPC-3 vaccine is well-tolerated and safe [121, 122]. Similarly, multidrug resistance-associated protein 3(MRP3), a vector-type transporter, highly expressed and associated with various cancers [123], is a great potential candidate for tumor vaccine development. In a phase I trial, the MRP3-derived peptides (MRP3765) showed promising safety and antitumor properties in 12 HLA-A24-positive HCC patients. MRP3-specific T-cell responses were induced in eight patients (72.7%) and the mOS was 14 mo (95%CI, 9.6-18.5) [124]. Other TAAs, including SSX-2, NY-ESO-1, hTERT and MAGE-A, can also be valuable targets for HCC immunotherapy, but no clinical trials have verified the clinical response to these antigens in HCC [125]. Although peptide vaccines have achieved some success in terms of safety, tolerability, and mOS improvement, they have fewer clinical benefits and more stringent screening conditions than ICIs.

Dendritic Cell Vaccines

DCs as antigen-presenting cells can stimulate T cells and increase the anti-tumor effect [126]. Peripheral monocytes were isolated *in vitro* and the DC population was expanded by adding cofactors (GM-CSF or IL-4). Mature DCs are activated with autologous tumor lysates or specific TAAs. Finally, these cells are reinfused into the patient to stimulate the adaptive cells to mount an antitumor immune response [36, 127, 128]. Currently, several clinical trials have confirmed the immunogenicity and safety of DCs. In a phase I trial of 17 patients with HCC treated with immunoprimers (ilixadencel), 73% had an increased frequency of tumor-specific CD8+-T cells in their peripheral blood [129]. Meanwhile, a phase I clinical trial in Japan injected DCs pulsed with tumor lysates (TLs) into 10 patients with unresectable HCC. All patients had an excellent immune tolerance; one patient experienced significant tumor shrinkage, while two experienced considerable tumor marker decrease [130]. In another phase II study, the intravenous administration of mature DCs pulsed with tumor lysate (HepG2) showed promising antitumor properties and safety in 35 patients with HCC [131]. When DCs were combined with TACE, tumor-

specific immune responses were enhanced more effectively than when TACE was used alone [132]. Multiple clinical trials on DCs are in progress (NCT01821482, NCT02638857, NCT02882659, NCT03674073, and NCT03203005). A growing body of evidence suggests that DC vaccines have general safety and anti-tumor properties as primary therapy and adjunct to other established therapies. DC vaccines are promising mainstream immunotherapy for HCC.

Oncolytic Viruses

An oncolytic virus (OV) is a specially modified intracellular pathogen that can achieve an anti-tumor response by massive replication in tumor cells, leading to direct lysis of tumor cells to produce soluble TAAs [133, 134]. OVs have been shown to improve ORR and mOS in advanced melanoma (NCT00769704) [135]. Currently, adenovirus and vesicular stomatitis virus are the main oncolytic viruses used to treat HCC, which can preferentially infect HCC tumor cells, followed by the herpes simplex virus and vaccinia virus [136]. In a recent randomized phase II trial (NCT00554372), JX-549 (Pexa-Vec) was injected into the tumors of 30 HCC patients, and mOS was significantly longer in the high-dose group than in the low-dose group (14.1 vs. 6.7 mo) (HR, 0.39; $P = 0.020$) [137]. Unfortunately, the phase IIb trial (NCT01387555), which compared Pexa-Vec to placebo as second-line therapy in patients with advanced HCC with no response to sorafenib therapy, did not achieve its OS [138]. A phase III trial (NCT02562755) is currently underway, which compares the safety and efficacy of sorafenib with Pexa-Vec against sorafenib alone in HCC. Currently, two clinical trials are underway to evaluate the efficacy of the combination of OVs and ICIs in HCC (NCT03647163 and NCT03071094) [139].

CONCLUSION

The rapid development of immunotherapy has changed the traditional treatment modalities for recurrent HCC. Immunotherapy can play a unique role in the comprehensive treatment of HCC, including prolonging and improving quality of life

and even curing HCC. Several clinical trials have attempted to evaluate the anti-tumor properties and safety of ICIs and their combinations in recurrent HCC, and have reported encouraging results. Although ICIs are the leading immunotherapy for recurrent HCC, other immunotherapy modalities, including CAR-T cells, DC vaccines, and OVs, are rapidly evolving. Among the multiple treatment options for recurrent HCC, achieving satisfactory results with single immunotherapy has become challenging.The development of synergistic immunotherapy may be a promising direction for HCC treatment in the future. In addition, immunosuppression of HCC remains a significant obstacle for immunotherapy drugs in which they must exert their anti-tumor properties. Another priority is to actively exploring the mechanisms of immunotherapy resistance or overcoming immune drug resistance through multiple anti-tumor drugs. Immunotherapy can lead to future breakthroughs and progress in treating recurrent HCC.

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