

Drug co-administration in the tumor immune microenvironment of Hepatocellular carcinoma

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Abstract

The etiology and exact molecular mechanisms of primary hepatocellular carcinoma (HCC) remain unclear, and its incidence has continued to increase in recent years. Despite tremendous advances in systemic therapies such as molecularly targeted drugs, HCC has some of the worst prognoses owing to drug resistance, frequent recurrence, and metastasis. Hepatocellular carcinoma is a widespread disease and its progression is regulated by the immune system. Traditional Chinese medicine (TCM) has been gradually theorized and systematized to have a holistic regulatory role for use in the prevention and treatment of tumors. Although half of the patients with HCC receive systemic therapy, traditionally sorafenib or lenvatinib are used as first-line treatment modalities. TCM is also widely used in the treatment of HCC, and the same immune checkpoint inhibitors (ICIs) such as PD-L1 have also received much focus in the field of continuously changing cancer treatment. Owing to the high probability of resistance to specific drugs and unsatisfactory efficacy due to administration of chemotherapy in single doses, the combination of drugs is the newest therapeutic option for patients with tumors and has become increasingly prominent for treatment. In this article, the research progress on combination therapy in the immunology of HCC is reviewed and the unique advantages of synergistic anti-tumor therapy with combination drugs are highlighted to provide new solutions for the clinical treatment of tumors.

Keywords: Combination drug; Hepatocellular carcinoma; Immune checkpoint inhibitors; Traditional Chinese medicine; Tumor microenvironment

Graphical abstract: <http://links.lww.com/AHM/A65>

Introduction

Liver cancer ranks fourth worldwide in cancer-related deaths and is the sixth most common cancer in humans, with hepatocellular carcinoma (HCC) accounting for 90% of all liver cancers. Hepatocellular carcinoma continues to be an exceptionally devastating disease, despite significant advancements in knowledge brought about by substantial baseline and clinical research over the previous 10 years^[1]. To date, HCC research and treatment have not yielded any effective treatments for HCC, even though sorafenib initially

treated kidney cancer, and immune checkpoint inhibitors (ICIs) against other solid tumor types initially showed efficacy^[2–3].

Cancer treatment has been revolutionized by immunotherapies such as IC blockades (ICBs) and adoptive cellular immunotherapy (ACT). Cancer immunotherapy involves recognizing the immune system hijacked by cancer cells and suppress the IC of anti-cancer immunity^[4], especially in patients whose disease is considered incurable. While most chemotherapy drugs are exploited for their direct cytotoxic effects without consideration for their effects on the immune system, several studies have already proven the immunological contribution of cytotoxic chemotherapy in the fight against cancer. Traditional Chinese medicine (TCM) can adjust the balance of the body, improve poor physique, support positive energy, improve immunity, control pre-cancerous lesions, prevent and treat tumor occurrence, and improve the survival rate of patients with tumors.

Therefore, extensive exploration of combination therapies is underway, and the use of several chemotherapeutic agents, natural compounds, or ICIs in combination may not only improve the effectiveness of cancer treatment, and boost anti-cancer potency but also reduce the toxicity and side effects of chemotherapeutic agents^[5], as is presented in this overview.

In this review, the most recent advancements in combination therapy for HCC are investigated and the implications of combination therapy for the treatment of HCC are discussed based on CNKI and PubMed. What remains to be seen is an overview of the foremost fields of HCC exploration, including new immune scenarios

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and clinical trials that are expected to help in the future clinical management of HCC.

Effectiveness of treatment for HCC

Hepatocellular carcinoma is challenging to treat because, in addition to the fact that more than 70% of patients are mid-stage to late-stage at the time of diagnosis and have lost the opportunity for surgical resection, HCC is usually resistant to chemotherapy. In contrast, the treatment options for early surgical resection, liver transplantation, and local ablation are limited^[6]. Hepatocellular carcinoma has significant molecular heterogeneity with a concordance rate of only 13% for different loci and alleles within the same tumor. In 2007, the approved usage of sorafenib allowed patients with advanced HCC to receive effective systemic therapy drugs for the first time^[7]. However, drug application was minimal, with only a limited number of drugs, such as sorafenib and lenvatinib^[8].

According to the theory of TCM, HCC results from a systemic disease with pathogenic cumulative toxicity, *qi* deficiency, and *qi* stagnation, leading to an imbalance between *yin* and *yang*. Therefore, the strategy of TCM in treating HCC is to apply multiple methods according to different evidence while treating the patient as a whole to restore the balance of *yin* and *yang* in the body and maintain the smooth flow of *qi* and blood. Chinese medicine helps reduce the toxicity of radiotherapy and chemotherapy and can be used as an important adjunct to HCC treatment to improve cancer-related symptoms and quality of life. Through traditional diagnosis and treatment, tonics and modern Chinese medicine preparations, including anti-cancer pills Kanglaite and Huachansu, have been widely used for the treatment of HCC and have

accumulated many practical examples in clinical practice, with certain efficacy and respective characteristics, and better patient compliance, safety, and tolerability.

Immune cycle of cancer

As a series of steps are initiated by the body, they progress and expand iteratively such that the anti-cancer immune response effectively kills cancer cells, which we refer to as the cancer immune cycle^[9]. Developing immunity against cancer is a self-propagating process involving the accumulation of immunostimulatory factors, which, in principle, amplify the T-cell response; however, features of the cycle also include feedback mechanisms that inhibit factors leading to the regulation of immunity, thus preventing or limiting its development. This cycle can be categorized into seven major steps: cancer antigen release, cancer antigen presentation, initiation and activation, T-cell trafficking to the tumor, T-cell infiltration into the tumor, and T-cell recognition by cancer cells (Figure 1). T-cell clearance in cancer is merely one step in the tumor immune cycle, which strikes a delicate balance between prophylactic autoimmunity and identifying the non-self. The cancer immune cycle plays a vital role not only for T cells but also for many other immune cells.

Cancer immunotherapy aims to induce or restart self-sustaining immunity against cancer so that it can magnify and propagate, albeit as far as possible without producing those unrestricted autoinflammatory responses^[10–11]. Every step has built-in checkpoints and inhibitors to oppose continuous amplification and can inhibit or block anti-tumor immune responses to achieve better therapeutic results. The IC, a regulator of the immune system, can be “hijacked” by tumors and

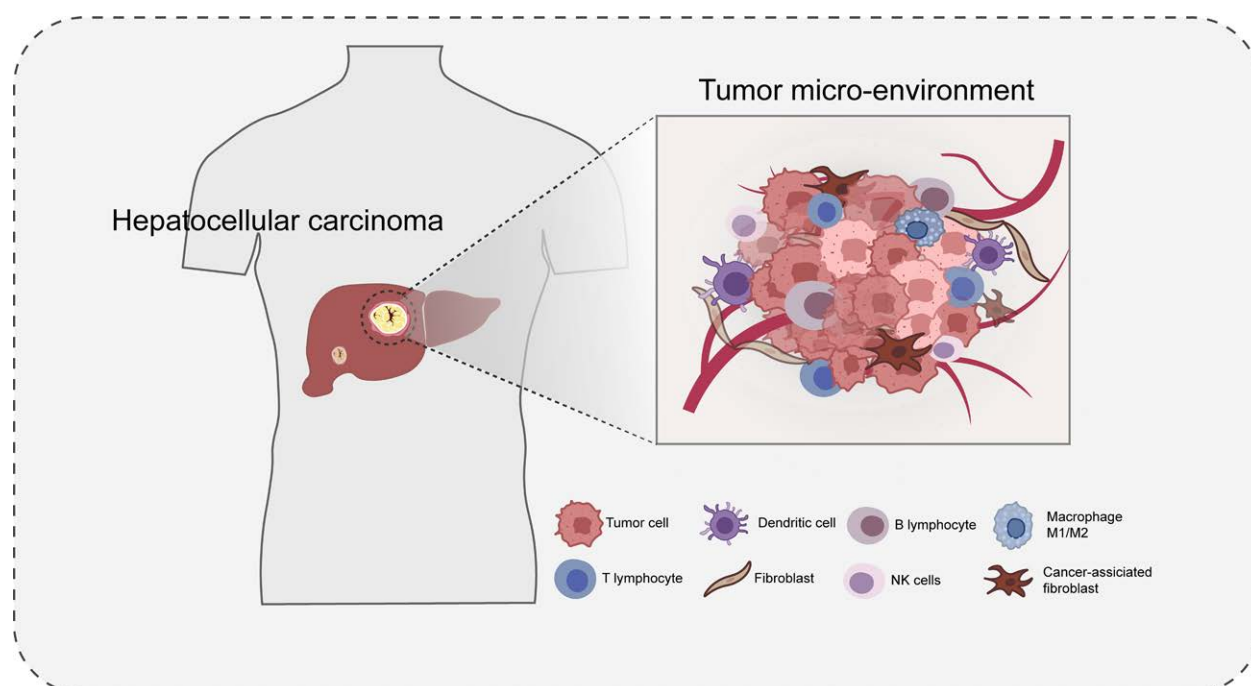


Figure 1. Immune cycle in HCC. The immune cycle refers to a series of progressive events that the body initiates and allows to proceed and iteratively expand as the anti-cancer immune response effectively kills cancer cells. Cancer immunity is a cyclical process that amplifies the T-cell response and inhibits tumor progression, as well as the feedback mechanisms of immune regulation that prevent or limit the development of immunity. HCC: Hepatocellular carcinoma.

continuously activated to suppress anti-tumor immunity and promote tumorigenesis. Current Food and Drug Administration (FDA)-approved checkpoint inhibitors block Lymphocyte Activation Gene-3 (Lag-3), Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), and programmed cell death (PD-1)/programmed cell death 1 ligand 1 (PD-L1); however, only the latter two have been used in HCC^[12].

HCC tumor microenvironment

The liver is an organ composed mainly of hepatic parenchymal cells and is the most extensive reticuloendothelial cell phagocytic system, which plays an important role in systemic immune regulation. Its immune functions are mainly reflected in immunomodulatory and phagocytic effects. As a major metabolic organ that receives blood from the hepatic artery and intestine through the portal vein, the liver plays a central role in immune regulation. Within the tumor microenvironment (TME) of HCC, tumor and immune cells secrete various immunosuppressive cytokines to induce immune tolerance (Figure 2). Cellular factors within the TME affect the migration and recruitment of immune cells not only to the tumor site but also regulate immune cell activity^[13]. Numerous mechanisms have been identified that cause anti-cancer immune resistance once immune cells enter the TME, including intrinsic cancer cell factors, immunosuppressive environments, and immune cells.

Enriched in the liver with natural killer cells (NK cells), NKT cells, and TCR $\gamma\delta$ T cells, the proportion

of NK cells is exceptionally high in human liver lymphocytes, up to 50%, and has a more substantial cytotoxic effect on tumor cells compared to NK cells in other organs, whereas the ratio of CD8⁺ to CD4⁺ T cells is also higher in the liver relative to peripheral blood. Immunomodulation, on the other hand, is the filtration, clearance, and induction of immune tolerance to antigens by the liver, which is a defense mechanism. Phagocytosis is the role of E phagocytes in the liver and is employed to remove and filter heterogeneous substances and regulate immune responses^[14]. The liver is also the largest digestive gland in the digestive system, filtering out toxic substances and excreting them from the body, thus playing a detoxifying role.

Current status of cancer immunotherapy

As the innate immune system is the first line of defense against foreign infections and substances, it must induce an adaptive immune response but also effectively remove foreign substances, including killing T-cell-independent tumor cells. NK cells^[15-16] and phagocytes, including dendritic cells (DCs)^[17] and macrophages^[18], are typically used in cancer immune therapies. These innate anti-tumor capabilities are activated in a coordinated manner leading to the removal of “high-risk” transformation units in the pre-cancerous stage, thus preventing the development of HCC^[19]. Meanwhile, the design of effective hepatocellular immunotherapies must address two critical issues; the strength of tumor cell immunogenicity in the liver and specific mechanisms of immune evasion that promote

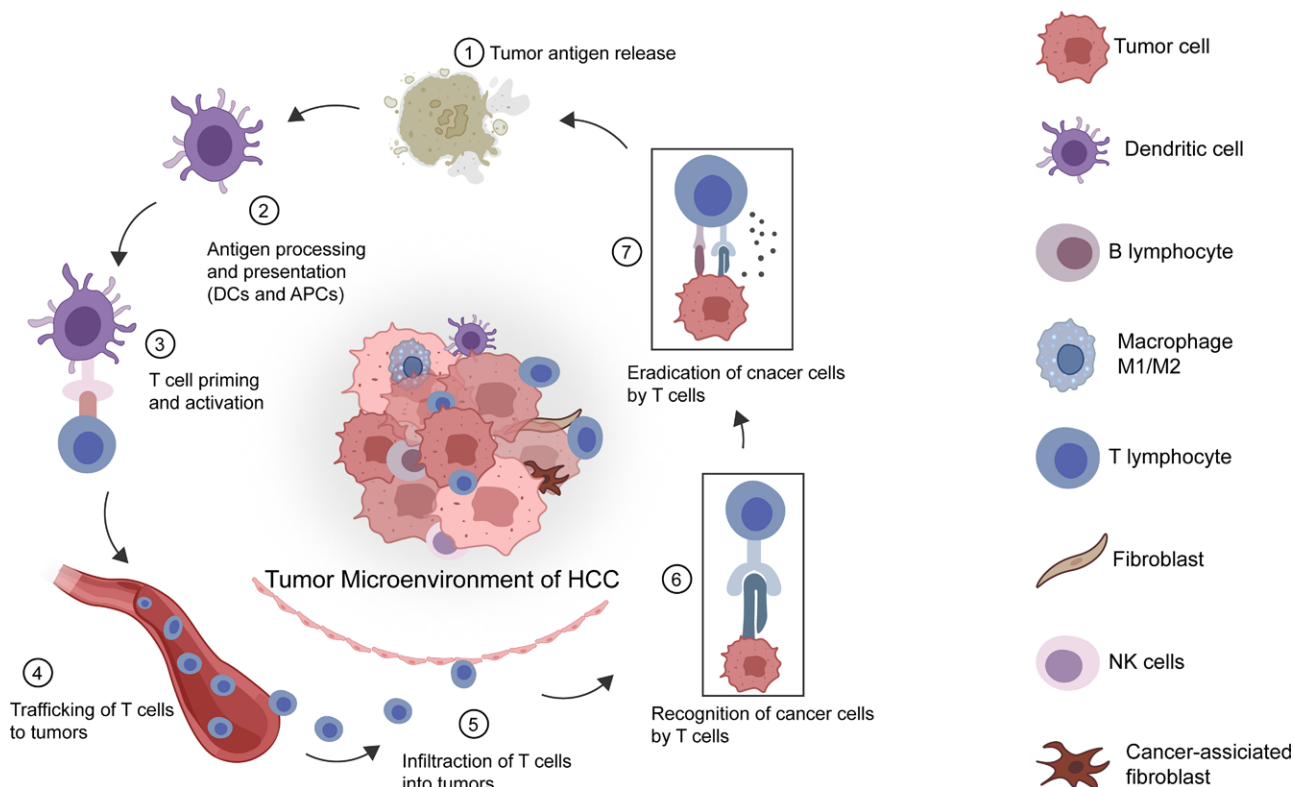


Figure 2. Tumor microenvironment in HCC. As an organ mainly composed of hepatic parenchymal cells, the liver is the largest reticuloendothelial cell phagocytic system and its immune functions are mainly reflected in its immunomodulatory effects and phagocytosis. Because the innate immune system is the first line of defense against infections and foreign substances, there are a variety of immune cells in the lymphocyte and tumor microenvironments of the human liver that perform different immune functions. HCC: Hepatocellular carcinoma.

HCC progression. A truly patient-oriented, personalized approach awaits future cancer immunotherapy.

A new class of agents, known as ICIs, was rapidly introduced into clinical studies on HCC in global multicenter trials after being pioneered in the treatment of other solid tumors^[20]. In 2013, a study reported a pilot clinical trial of the CTLA-4 antibody, tremelimumab, was conducted in patients with advanced HCC developing on cirrhosis^[21]. In 2017, an additional publication on the use of navilizumab (anti-PD-1 Ab) in patients with advanced HCC (CheckMate 040) was led by El-Khoveiry and many collaborators worldwide^[22]. Together, these results emphasize the necessity of refining strategies to incorporate biomarkers into the ICI treatment of HCC. Numerous additional global ICI clinical trials for HCC are currently underway, including two phase III trials (KEYNOTE-240 and KEYNOTE-394) involving patients with second-line advanced HCC^[23].

Small molecules from natural products

Drug therapy, which can overcome the limitations of surgery and RT, and has significant efficacy for tumors that have spread and metastasized; it is one of the primary means of treating tumors; however, chemotherapy is accompanied by adverse effects such as pain, recurrence, and destruction of immunity, and does not improve patients' suffering.

Combination therapy with tyrosine kinase inhibitors

Quercetin, a flavanol found in plants, has good anti-cancer and anti-inflammatory properties^[24], and in this study, quercetin was used as a therapeutic agent alone or in combination with sorafenib chemotherapeutic agents to improve the conventional treatment of HCC with sorafenib by increasing the efficacy and reducing the toxicity of sorafenib. It was found to be effective in combination therapy, which can be attributed to the significant effects of quercetin in regulating cell cycle blockade, oxidative stress and inflammation, and modulating immunity.

Combining saffron with sorafenib improved several parameters, including inflammation, histopathology, and serum inflammatory markers (C-reactive protein). The regimen of crocin and sorafenib reduced hepatotoxicity, inhibited the development of HCC, and improved liver function while affecting interleukin-6 (IL-6) expression. Patients with HCC had significantly increased levels of lactate dehydrogenase (LDH) and oxidative stress markers, and crocin and sorafenib showed synergistic anti-tumor effects on HepG2 cells^[25].

Artemisinin (ARS) is a commonly used antimalarial drug in clinical practice^[26], and several ARS derivatives, such as artesunate (ART), dihydroartemisinin (DHA), and artemether, have been developed successively. Recent studies have revealed that the role of ARS derivatives is not limited to the treatment of malaria, but may also have therapeutic implications for several other diseases, including cancer^[27–28]. ART and DHA are the most active derivatives of ARS, they have a significant inhibitory effect on human HCC cells, can improve the efficacy of sorafenib, and sensitize and reverse drug resistance through a combination^[29].

Bufalin, the active ingredient extracted from the TCM Toadstool, is the main ingredient of Huazhuxin tablets, and combined with sorafenib, it can significantly inhibit the proliferation of human HCC HepG2 cells, which induced apoptosis and blocked the cells in the S phase. The combination produced a synergistic effect with significantly enhanced anti-tumor effects. Further studies confirmed that the mechanism of action was related to the inhibition of phosphorylation of the Akt/NF- κ B signaling pathway.

Combination of drugs with ICIs

Paclitaxel (PTX) increased ATAD3A expression, which disrupts the stability of PD-L1 by inhibiting PINK1-dependent mitosis. The results of preclinical studies further demonstrated that targeting ATAD3A could reset a favorable anti-tumor immune microenvironment and improve the efficacy of ICI combination therapy, increasing the effectiveness of ICI plus PTX combination therapy^[30].

Curcumin prevents HCC progression by decreasing methemoglobin levels in the liver tissue, increasing the number of immune cells (eg, NK cells), inhibiting EMT by regulating IL-6/JAK/STAT3 and IL-1 β /NF- κ B pathways, inhibiting anaerobic glycolysis by inhibiting LDH and HIF-1 α , and reducing lipid synthesis by downregulating FASN and upregulating serum HDL-C and mRNA levels of ApoA1 in Sorafenib-treated mice^[31]. Meanwhile, curcumin and anti-PD-1 have the synergistic effects of slowing down Hep3B cell proliferation, activating lymphocytes, inhibiting immune evasion, and downregulating TGF- β 1 expression. *In vivo*, the combination of curcumin and anti-PD-1 reduced the HCC growth rate and improved the tumor microenvironment.

Antibiotic (ATB) combined with anti-PD-1 had a significant inhibitory effect on tumor growth, upregulated the proportion of CD3⁺ and CD8⁺ T cells in T lymphocytes, the secretion levels of IL-2, IL-10, and IFN γ cytokines, and regulated T-cell immune function by upregulating the expression levels of CD3⁺ and CD8⁺ T cells in tumor tissues in order to continuously activate the immune system and suppress tumors.

Small-molecule targeted inhibitors

Tyrosine kinase inhibitors (TKIs) offer the possibility of improved overall survival (OS) in patients with advanced disease, although sorafenib has little clinical benefit^[7]. The complicated molecular pathogenesis of HCC facilitates the study of TKI in combination with other molecularly targeted agents. After the development and launch of sorafenib, four other TKIs were launched, including first-line lenvatinib^[32], second- and third-line regorafenib^[33], and cabozantinib^[34].

Sorafenib

Sorafenib is currently used in combination with anti-angiogenic drugs and Mitogen-activated Extracellular signal-regulated Kinase/Extracellular Regulated protein Kinases, mammalian Target of Rapamycin, and Epidermal Growth Factor/Epidermal Growth Factor

Receptor pathway inhibitors. The combination of sorafenib and doxorubicin significantly inhibited tumor growth *in vivo*. Doxorubicin increased PD-L1 expression *in vitro* and *in vivo*, whereas sorafenib inhibited doxorubicin-induced PD-L1 upregulation. The proportion of CD8⁺ T cells capable of secreting interferon-gamma was significantly increased in tumor tissues when sorafenib was combined with doxorubicin, whereas the proportions of CD4⁺ T cells, CD8⁺ T cells, and Tregs⁺ T cells did not change significantly^[35].

Sorafenib, in concert with radiation therapy (RT) and immunosurveillance, inhibited PLC5 cell viability *in vitro*^[36], and RT was able to induce IFN- α and IL-6 expression and activate STAT1 and STAT3 phosphorylation through autocrine and paracrine effects, leading to HCC cells becoming radioresistant and PD-L1 overexpression. Sorafenib inhibits PLC5 cells, suppresses PD-L1 expression in HCC cells, activating RT-suppressed CD8⁺ T-cell killing. Previous anti-PD-1 antibody therapy amplified the response of HCC to sorafenib therapy and increased survival, while anti-PD-1 therapy with subsequent sorafenib therapy demonstrated vascular protection in HCC and enhanced the combination in a CD8⁺ T-cell-dependent manner^[37].

A combination strategy combining sorafenib with STAT3 inhibition^[38], which directly and effectively interferes with the tumor characteristics of HCC cells, improves the tumor microenvironment and induces anti-HCC immune responses through the cGAS-STING-I-type IFNs axis of DC cells, which may be a potential therapeutic strategy for HCC. TROY is an HCC-cancer stem cell (CSC) marker that is regulated by TGF-1 secreted by CAF and interacts with HCC stemness to facilitate the spread of cancer^[39], while sorafenib's tumor stemness is effectively inhibited by the PI3K inhibitor wortmannin. Patients with HCC may benefit from the combination of chemotherapy or targeted agents with wortmannin to treat TROY-positive CSC.

Sorafenib treatment increased PD-L1 expression in HCC cells, suggesting that it may recruit T cells to the tumor and induce PD-L1 expression^[40], which may be linked to its ability to induce cell death and inhibit certain tyrosine kinases such as those related to vascular endothelial growth factor receptors.

Lenvatinib

Lenvatinib can activate the endogenous mTOR pathway in depleted CD8⁺ T cells^[41]; therefore, the addition of mTOR inhibitors promotes the anti-tumor effects of lenvatinib and the activation of CD8⁺ T cells. Tolerant of anti-PD-1 by HCC-derived CSF1 can drive immune escape and immune escape, and the protein kinase inhibitor Gö6976 and multi-kinase inhibitor lenvatinib can reset the tumor microenvironment and restore anti-PD-1 sensitivity by blocking the PKC α /ZFP1/CSF64 axis^[42]. Lenvatinib improves anti-PD-1 efficacy by blocking FGFR1 to reduce PD-L1 levels in tumors and differentiation of Treg T cells, while FGFR4 expression and Treg T-cell infiltration levels in tumors can be used as biomarkers for screening patients with HCC for lenvatinib plus anti-PD-1 combination therapy^[43].

Regorafenib

Antigen-specific T cells that metastasize in the presence of regorafenib have enhanced anti-tumor efficacy^[44], and multiple immune-related pathways in the tumor microenvironment are involved in regorafenib and anti-PD-1's synergistic anti-tumor effects. By modulating macrophage polarization, regorafenib may enhance anti-tumor immunity independent of its anti-angiogenic activity. Optimizing the regorafenib dose also allows for a more rational design of combination therapies to improve clinical indices. When regorafenib is combined with anti-PD-1 therapy, tumor growth is inhibited. Survival is improved by normalizing the tumor vascular system and increasing tumor CXCR8⁺/CD10⁺ T-cell infiltration by enhancing CXCL3 expression in HCC cells^[45].

Cabozantinib

Combining cabozantinib with the pan-mTOR inhibitor MLN0128 resulted in tumor regression in c-Met/ β -catenin mice. By blocking c-MET and ERK activity in HCC cells, cabozantinib reduces PKM2 expression and increases p21 expression in pathways involving c-Met/CDK8 and Akt/c-Met^[46]. When combined with anti-PD-1, cabozantinib enhances anti-tumor immunity by combining neutrophil-driven innate and adaptive immune responses, significantly reducing the infiltration of regulatory T cells (Tregs) and increasing the ratio of total T cells and memory/effector T cells, while decreasing the percentage of neutrophils to lymphocytes^[47].

Other

Small-molecule inhibitors targeting TTK (CFI-402257)^[48] effectively inhibited HCC growth and induced DNA damage and micronucleus formation in highly aneuploid HCC cells. Patients with HCC might benefit from combination therapy with ICIs, such as CFI-402257 which was also found to increase survival in mice treated with anti-PD-1, both as a single agent and in combination therapy.

Radiotherapy-stimulated CD8⁺ T-cell infiltration and activation were further enhanced by AZD6738, and the immunosuppressive effect of radiation on xenograft tumor Tregs was restored. As a result of AZD6738 and radioimmunotherapy activating the cyclic GMP-AMP synthase/interferon gene stimulator (cGAS/STING) signaling pathway, synergistic anti-tumor effects occurred^[49].

DNA replication stress is induced by CDC7 inhibitors in HCC cells, regardless of whether ATR or CHK1 is inhibited^[50]; thus, CDC7 inhibition has a striking synergy with ATR-CHK1 inhibition. Abnormal mitotic processes may explain the synergistic effect of ATR-CHK1 and CDC7 inhibition.

Chinese herbal compounds

The anti-tumor effect of TCM is mainly divided into two types: one is to have a direct inhibitory effect on the growth of tumor cells; the other is to have an indirect anti-tumor effect by improving the pathological

state of the body, enhancing immunity, and normalizing the damaged immune function to activate the immune response of tumor cells. The active ingredients of Chinese medicine can not only enhance the immune function of the body, improve the tumor microenvironment, and induce apoptosis of tumor cells but also increases the effectiveness and reduces toxicity through a combination with chemotherapy drugs and reversing multidrug resistance.

Chinese medicine takes a holistic view of the treatment concept, considering the five viscera and six internal organs as a whole, and elucidates the nature of the disease by regulating the balance of multiple internal organs to treat the disease. The combination of Chinese medicine with sorafenib was superior to the sorafenib alone in improving liver function indexes of total bilirubin, direct bilirubin, alanine aminotransferase, albumin, and globulin, and the combination improved liver function, reducing its deterioration.

Immunomodulatory function of Han Fang Compound Bitter Ginseng Injection (CKI) and its anti-tumor efficacy in combination with low-dose sorafenib^[51], CKI-activated pro-inflammatory responses and attenuated immunosuppression of tumor-associated macrophages in the HCC microenvironment by triggering the tumor necrosis factor receptor superfamily member 1 (TNFR1)-mediated NF- κ B and p38MAPK signaling cascade. CKI-activated macrophages significantly promoted the proliferation and cytotoxic capacity of CD8⁺ T cells and reduced their depletion, leading to HCC cell apoptosis.

Chinese medicine combined with targeted drugs provides a new pathway for the treatment of primary HCC. However, despite being proven as clinically compelling, current studies are still limited and lack perspectives, large samples, and multicenter clinical data. Primary HCC treatment should emphasize evidence-based treatment, which indirectly affects the results of evidence-based therapy owing to significant individual differences among patients. Foundational research on Chinese medicine is relatively limited, and the characteristics of drug composition, compounding characteristics, mechanism of action, pharmacology, and toxicology need to be further studied and explored.

Chemotherapeutic agents

Currently, the tumor drug therapy market in China is dominated by chemotherapeutic drugs. Chemotherapeutic drugs such as nimostatin, doxorubicin, and vincristine are the main categories of domestic chemotherapeutic drug therapy for tumors. Traditional chemotherapy drugs can be divided into alkylating agents, anti-metabolites, anti-cancer antibiotics, botanicals, hormones, and platinum drugs, most of which act directly or indirectly on DNA synthesis and replication of tumor cells to inhibit the proliferation of tumor cells. Chemotherapeutic drugs are the most traditional tumor treatment drugs, but the apparent disadvantage of chemotherapy is its high toxicity. Therefore, combining chemotherapeutic drugs with Chinese medicines, targeted drugs, or immune drugs can reduce toxicity and enhance the therapeutic effect, which is the focus of tumor treatment.

Mechanisms of action

Studies have shown that cytotoxic chemotherapy induces immunogenic cell death (ICD), a form of regulated cell death that activates adaptive immune responses in immunocompetent hosts and enhances immunotherapy^[52]. Cytotoxic chemotherapy induces ICD and enhances immunotherapy, leading to cancer cell damage, thus promoting the release and repositioning of DAMPs^[53–54]. The main benefit of cytotoxic chemotherapy is tumor reduction, with tumor cells being the main contributors to the immunosuppressive TME. Simultaneously, ICDs increase cancer cell antigenicity, and cytotoxic drugs upregulate antigen presentation mechanisms^[55–56], and many commonly used cytotoxic drugs, such as anthracyclines, cyclophosphamides, platinum, and PTX, target cell cycle processes in proliferating cells and induce apoptosis. When tumor cells die, immune cells are presented with tumor neoantigens by antigen-presenting cells that phagocytose dying tumor cells.

Therapy depletes immunosuppressive cells and several subpopulations of immune cells are known to suppress anti-cancer immunity^[56–60], such as MDSC, macrophages, and Treg cells, whereas chemotherapy alters the TME, favoring the differentiation of immune cells that support anti-cancer immunity and the differentiation of macrophages into the M1 type^[61]. Enhanced and restored sensitivity to chemotherapy, immunotherapy, and cytotoxic chemotherapy are mutually reinforcing, and some patients with chemoresistant tumors respond to reinitiation of chemotherapy following anti-PD-1 treatment for disease progression^[62]. However, the double-sided nature of chemotherapy in immunotherapy, which may cause lymphocyte depletion after chemotherapy treatment^[63], may also contribute to immunosuppression and cause tertiary lymphoid structures (TLS), the presence and high density of which in tumors are positively correlated with the prognosis of several cancer types^[64–66]. In contrast, chemotherapy-induced lymphocyte depletion can also influence TLS through the immediate cytotoxic effects of chemotherapeutic agents or related treatments like corticosteroids^[67].

Numerous chemimmunotherapy combinations have been clinically tested in almost all major cancers and have received multiple FDA approvals, primarily in lung, breast, head, and neck cancers^[68–70], and more in-depth studies are needed on HCC. Immunotherapy serves as a maintenance therapy after chemotherapy is completed; however, chemoimmunotherapy combinations are not a panacea for all solid tumors, and more research is needed to determine the optimal combination, sequence, drug selection, and underlying mechanisms of the different responses is needed^[71–72].

Combination of chemotherapy drugs

Oxaliplatin in combination with 5-fluorouracil can be used in the systemic treatment of HCC^[73] and can be applied in combination with anti-PD-1 drugs as a new combination therapy regimen. However, its effectiveness needs to be further validated by clinical results. In the clinical treatment of primary HCC, the application of oxaliplatin, followed by the use of Doxorubicin can

achieve optimal results, reduce tumor markers, prolong survival time, and have a high safety profile with fewer adverse effects.

Doxorubicin is another chemotherapeutic agent that induces ICD and is commonly used for the chemoembolization of HCC^[74–75]. A clinical study to validate the combination of chemoembolization and doxorubicin drug elution with nivolumab is currently underway. In addition, investigators have suggested that doxorubicin has the potential to stimulate APCs exposed to tumor antigens, which in turn initiate anti-tumor T cells. In conclusion, the combination of chemotherapeutic agents with systemic immunotherapy should be validated in further clinical trials.

5-FU induces intra-tumor MDSC accumulation and counteracts the infiltration of T lymphocytes and natural killer cells, thus neutralizing the anti-tumor efficacy of PD-L1 blockade, which is crucial to combine it with anti-PD-L1 antibodies to rationally design of a combination immunotherapy strategy for patients with HCC^[76].

Supplementing capecitabine with cyclophosphamide for advanced HCC^[77] improved the physical status of patients, resulting in the normalization of alpha-fetoprotein (AFP) and recovery of Child-Pugh A. Meanwhile, the immunomodulatory effects of the combination deserve to be investigated.

Antibody drugs

In 2020, the first- and second-line treatments for advanced HCC ushered in a significant change with the selection of several new therapies, including atezolizumab combined with bevacizumab, IC monoclonal antibody monotherapy, and combination regimens. Immunotherapy for HCC spans from second-to first-line therapy and begins to enter the adjuvant and neoadjuvant phases of treatment, reshaping the competitive landscape. To date, ICBs approved by the US FDA include antibodies against PD-1^[77], PD-L1^[78], LAG-3^[79], and CTLA-4^[80]. Anti-CTLA-4 and anti-PD-1/PD-L1 combination therapies have slightly higher response rates but at the cost of higher immune-mediated toxicity. Combination therapies to improve the clinical efficacy of ICI have been approved by the FDA.

Antibody-drug conjugates (ADCs) combine the targeting properties of antibodies with the anti-tumor effects of small-molecule drugs, ADCs. These immunocouples are composed of antibodies or antibody fragments linked to “warhead” drugs by linkage chains^[81], the substances that can be used as “warheads” are radionuclides, chemotherapeutic drugs and toxins, and these “warheads” are linked to antibodies to form radioimmunocouples, chemoimmunocouples, and immunotoxins, respectively. The current generation of anti-tumor drugs is divided into two categories according to their mechanism of action: disruption of the DNA chemical structure and interference with microtubule protein synthesis. In short, it is a precision radar for chemotherapeutic drugs that builds a bridge for chemotherapeutic medications to precisely destroy cancer cells.

Currently, the US FDA approves the PD-1 monoclonal antibodies nivolumab and pembrolizumab for the treatment of HCC, both of which directly inhibit PD-1 on the T-cell surface to overcome tumor immune escape. The human immunoglobulin G1 monoclonal antibody MEDI4736 (durvalumab) is well-tolerated against PD-L1; however, studies have shown that no patient has responded significantly to this treatment. In preclinical studies, investigators found that a combination of anti-PD-L1 and anti-CTLA-4 monoclonal antibodies achieved stronger anti-tumor activity than monotherapy. In a phase I/II clinical study (NCT02519348), durvalumab in combination with tremelimumab controlled 57.5% of the patients with HCC who could not undergo resection.

Targeting Gasdermin-D (GSDMD) promotes interferon expression and downregulates PD-L1 expression by inactivating the cGAS pathway^[82]. Therefore, the combination of anti-PD-1 and GSDMD inhibitors may be an effective therapeutic option for patients with GSDMD-overexpressing HCC. PARG can act as an oncogene in HCC by regulating PARG/DDB1/c-Myc signaling, and PARG inhibitors work synergistically with anti-PD-1 antibodies in an *in situ* mouse model. PARG inhibition increases the efficacy of anti-PD-1 treatment (immunotherapy) efficacy in HCC^[83]. The combination of CXCR4-targeted p53 mRNA nanoparticles with anti-PD-1 therapy was also found to be effective in inducing global reprogramming of the cellular and molecular components of the immune TME, reversing immunosuppression in HCC when p53 mRNA nanodrugs were used in combination with ICB, supporting the implementation of this strategy in cancer therapy^[84].

In 2020, results from the global randomized phase 3 trial IMBrave150 showed that the combination of atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF) improved overall and progression-free survival compared to sorafenib in the treatment of non-resectable HCC^[85]. A renewed analysis indicated a clinically meaningful improvement in the reported quality of life, disease symptoms, and function with atezolizumab plus bevacizumab compared with sorafenib alone, with the survival benefit of the combination maintained^[86] (Table 1). As the first successful therapy for HCC, other than sorafenib, it has great potential as a combination immunotherapy.

Anti-PD-1/CTLA-4 combined with sorafenib and cabozantinib can be used as first-line treatment for CheckMate-9DW (NCT04039607)^[87]. Duvalizumab (anti-PD-L1) in combination with tremelimumab (anti-CTLA-4) was an ICI-like combination that was found to exhibit remarkable clinical activity after Sorafenib as well as tolerance to non-resectable HCC in a phase I/II study. A Himalayan phase III trial (NCT03298451) compared the efficacy of Durvalumab (anti-PD-L1) in combination with or without Tremelimumab (anti-CTLA-4) and Sorafenib as a first-line treatment for advanced HCC. Preliminary results showed that durvalumab plus tremelimumab significantly outperformed sorafenib in terms of OS. Immunotherapy combinations have also demonstrated a favorable safety profile and may be alternative first-line agents.

Table 1**Currently investigation of small molecule targeted inhibitors in combination with immunotherapy combinations**

Name	Combinations	Test model	Function
Sorafenib	Doxorubicin	<i>In vitro</i> / mouse	Sorafenib inhibits doxorubicin-induced PD-L1 upregulation and increases the proportion of CD8 ⁺ T cells
	Radiation therapy	<i>In vitro</i>	Killing of HCC cells by activated RT-suppressed CD8 ⁺ T cells
	Anti-PD-1	Mouse	Anti-PD-1 antibody combined with sorafenib enhances treatment efficacy and survival in HCC in a CD8 ⁺ T-cell-dependent manner
	STAT3 Knockdown	<i>In vitro</i>	Improving tumor microenvironment and inducing anti-HCC immune response through cGAS-STING-I type IFNs axis of DC cells
Lenvatinib	Wortmannin	Mouse	Wortmannin, a PI3K inhibitor, effectively inhibits tumor stemness in combination with sorafenib targeting TROY-positive CSC for HCC
	mTORs inhibitors	<i>In vitro</i>	Inhibitors of mTOR promote the anti-tumor effects of Lenvatinib and activate CD8 ⁺ T cells
	Gö6976	<i>In vitro</i> / mouse	Combination administration resets the tumor microenvironment to restore anti-PD-1 sensitivity by blocking the PKCα/ZFP1/CSF64 axis
	anti-PD-1	Mouse	Combination therapy improves anti-PD-1 efficacy by blocking FGFR1 to reduce PD-L1 levels in tumors and differentiation of Treg T cells
Regorafenib	Anti-PD-1	Mouse	Combination administration synergizes anti-tumor efficacy through multiple immune pathways in the tumor microenvironment
	Anti-PD-1	Mouse	Combination therapy increases the infiltration of CXCR8 ⁺ /CD10 ⁺ T cells in the tumor by enhancing CXCL3 expression in HCC cells, thereby inhibiting tumor growth and improving survival
Cabozantinib	MLN0128	Mouse	Combination causes hepatocellular carcinoma tumor regression in c-Met/β-catenin mice
	Anti-PD-1	Mouse	Combination of pooled innate neutrophil-driven and adaptive immune responses augmented anti-tumor immunity and Treg T-cell infiltration
CFI-402257	Anti-PD-1	Mouse	CFI-402257 improved the survival of mice with HCC treated with anti-PD-1
AZD6738	Radioimmunotherapy	Mouse	Synergistic anti-tumor of AZD6738 with radioimmunotherapy through activation of the cGAS/STING signaling pathway
ATR or CHK1 inhibitors	CDC7 inhibitors	<i>In vitro</i>	Synergy between ATR-CHK1 inhibition and CDC7 inhibition may be due to mitotic abnormalities

The complex molecular pathogenesis of HCC has facilitated research into the combination of TKI with other molecularly targeted drugs. ART: Artesunate; cGAS/STING: Cyclic GMP-AMP synthase/interferon gene stimulator; HCC: Hepatocellular carcinoma; PD1: Programmed cell death; PD-L1: Programmed cell death 1 ligand 1; RT: Radiation therapy; TKI: Tyrosine kinase inhibitor.

Nivolumab in combination with ipilimumab (CTLA-4) had the best median OS at 22.8 months with 54% disease control compared to the other groups from a set of CheckMate-040 assessments^[88]. The combination of nivolumab and ipilimumab was approved by the FDA for HCC patients after sorafenib treatment in 2020 (Table 2).

Pembrolizumab received accelerated FDA approval for treatment-resistant HCC patients treated with sorafenib and improved patient survival cycles^[89]. Efficacy of pembrolizumab as a second-line agent in the treatment of patients with HCC (NCT02702401). The combination of pembrolizumab and lenvatinib showed an overall remission rate of 46% and has been shown to have favorable outcomes when targeted therapy is associated with anti-angiogenic agents^[90]. A phase Ib trial evaluating the combination of lenvatinib and pembrolizumab showed a 9.5-month improvement in progression-free survival and a 22-month improvement in OS. A phase III study comparing lenvatinib plus pembrolizumab with lenvatinib placebo is ongoing (NCT03713593). In a phase Ib/II clinical trial, camrelizumab was investigated in combination with the chemotherapy regimen FOLFOX4 as a first-line treatment for advanced HCC^[91].

Other ICI/anti-angiogenic combinations, such as apatinib and camrelizumab (NCT03764293), durvalumab and tivozanib (NCT03970616), and regorafenib plus atezolizumab (NCT04183088), are currently being investigated in clinical trials (Table 3).

Discussion

Cancer immunotherapy has emerged as an essential aspect of cancer treatment history^[92]. Durable responses to monotherapy continue to be reported, and a wide range of human cancer treatments using several different agents are being investigated. For certain patients, monotherapy may not meet the therapeutic needs to achieve the therapeutic goals, making the need for combination therapy more urgent. Immunotherapy using ICI has transformed cancer treatments. However, although several clinical trials are underway worldwide, it is unclear how this paradigm shift strategy will benefit patients with HCC. Although low response rates can be improved by selecting patients based on reliable biomarkers, another approach is to convert low or non-responders into ICI responders using herbs or other drugs to increase treatment efficacy through combination therapy.

Table 2**Currently approved immunotherapy combinations in HCC**

Combinations	Indications	Approval dates
Atezolizumab + bevacizumab	HCC first-line	May 29, 2020
Nivolumab + ipilimumab	HCC after sorafenib	March 11, 2020

To date, the Food and Drug Administration has approved several combination therapies for different cancer types; however, only two have been approved for HCC. HCC: Hepatocellular carcinoma.

Table 3
Ongoing clinical trials of combination therapies in HCC

Phase	Clinical trial	Intervention	Study population	Estimated completion date
3	NCT03755791	Atezolizumab + cabozantinib	Patients with aHCC (first line)	2023
3	NCT03298451	Durvalumab + tremelimumab	Patients with aHCC (first line)	2024
½	NCT04170556	Nivolumab + regorafenib	Patients with aHCC progressing after first-line therapy	2023
2	NCT04310709	Nivolumab + regorafenib	Patients with aHCC naive to systemic therapy	2023
3	NCT04039607	Nivolumab + ipilimumab	Patients with aHCC (first line)	2025
2	NCT04050462	Nivolumab + cabrializumab	Patients with aHCC	2024
3	NCT03713593	Pembrolizumab + lenvatinib	Patients with aHCC (first line)	2023
3	NCT04246177	Pembrolizumab + lenvatinib + TACE	Patients with aHCC (first line)	2029
2	NCT03519997	Pembrolizumab + bavituximab	Patients with aHCC	2023
2	NCT03316872	Pembrolizumab + SBRT	Patients with aHCC	2023
2	NCT04696055	Pembrolizumab + regorafenib	Patients with aHCC previously treated with PD-1/PD-L1 ICI	2024

Ongoing clinical trials of combination therapies in patients with HCC are summarized. aHCC: advanced hepatocellular carcinoma; HCC: Hepatocellular carcinoma; ICI: Immune checkpoint inhibitor.

Chinese medicine has emerged as a new source of anti-cancer drugs owing to its advantages in ameliorating the adverse effects of chemotherapy, and its pharmacological mechanisms in the treatment of HCC have been explored in numerous studies. The combination of Chinese and Western medicines is becoming a new trend in the prevention and treatment of HCC in modern society. Although there are still some challenges facing the combination, further well-designed clinical trials and experimental studies will provide hope to understand the mechanism of action of the combination of Chinese and Western medicines and promote the modernization of the combination of Chinese and Western medicine for the treatment and prevention of HCC. The limited activity observed with previous immune-based therapies can be explained by the complex tumor microenvironment and the presence of inhibitory factors; therefore, drug combinations may be more effective treatments for these patients.

Conflict of interest statement

The authors declare no conflict of interest.

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Ethical approval of studies and informed consent

Not applicable.

Author contributions

Conceptualization: Yingying Shao, Ranran Su, and Haiyang Yu; Picture drawing and discussion: Yingying Shao, Yu Wang, and Haiyang Yu; writing—original draft preparation: Yingying Shao, Weiling Pu, and Sangho Koo; writing-review and editing: Yingying Shao, Shuangshuang Yin and Haiyang Yu. All the authors have read and agreed to the published version of the manuscript.

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Data availability

Not applicable.

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