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Recent advance in immune checkpoint inhibitors-based therapy of advanced hepatocellular carcinoma

Running title: hepatocellular carcinoma and immune checkpoint inhibitor

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and is associated with a high mortality rate. Its occult origin often results in the loss of the optimal timeframe for liver transplantation and resection. During the past few decades, tremendous advances in the treatment of HCC have been achieved, and immunotherapy has become an attractive approach with promising results in clinical trials. In the present work, we will review immune checkpoint inhibitors (ICIs) for their function and role in treating cancers, particularly advanced HCC, summarize recent therapeutic progress with various ICIs or their combinations with other options/therapeutic agents, and discuss works related to the development of biomarkers that predict therapeutic response as well as the limitations of ICIs. Future directions for immune checkpoint therapy (ICP) have also been addressed.

Keywords: immune checkpoint inhibitor; immunotherapy; cancer; prognostic biomarker; therapeutic response; surgical resection

Introduction

Liver cancer ranks the sixth in malignant tumors and is the fourth leading cause of cancer-related death (1, 2). The overall 5-year survival rate of patients with advanced hepatocellular carcinoma (HCC) is approximately 18%, and its incidence has been increasing in recent years. For example, the incidence rate was approximately 18.3 per/million people, with a mortality rate of approximately 17.1/100000 in China (3). The causes of HCC include hepatitis B and hepatitis C infection, genetic factors and other internal and external factors such as alcohol, tobacco, obesity, and diabetes (4, 5) (Figure 1). Recently, long noncoding RNA is implicated in HCC as an oncogenic factor (6). Orthotopic liver transplantation (OLT) is considered the best therapeutic

option for end-stage liver disease, including HCC. However, due to the insidious onset, most patients with HCC are already at the late stage once diagnosed, and less than 20% of them are able to receive OLT or other surgical treatment, while the remaining patients can only be treated palliatively (7, 8). Before 2007, transcatheter arterial chemoembolization (TACE) was the first choice for treating patients with unresectable HCC (9). Although TACE is currently one of the major HCC treatment options, no global guidelines have been established regarding the dosage, choice, or combination of cytotoxic drugs used for TACE (9). Furthermore, the response rate to TACE remains relatively low (approximately 30%) (10). In 2007, a multi-target tyrosine kinase inhibitor (TKI), sorafenib, was shown to increase the overall survival (OS) of patients participating in the Sorafenib HCC Assessment Randomized Protocol and Asia-Pacific trials. Since then, this drug has become the standard treatment for advanced HCC (11-13). Lenvatinib**,** also a TKI that displays promising therapeutic effects against various solid tumors, was found to be comparable to sorafenib with regard to OS in advanced HCC (14) and had more favorable outcomes for advanced HCC when used with Vp3/4 (15). In addition, regorafenib and cabozantinib (both TKIs) and ramucirumab (a vascular endothelial growth factor (VEGF) receptor inhibitor) have been approved by the Food and Drug Administration (FDA) as second-line systemic therapeutics for patients who are not responsive to sorafenib (16, 17). However, for the majority of patients, monotherapy has limited clinical outcomes. The survival rate after single TKI treatment was only 3 months in patients with unresectable HCC (14, 18) and acquired resistance to TKI may develop due to EGFR mutations, leading the treatment failure (19).

Recently, the mechanisms underlying tumor cell immune escape have been intensively studied, leading to the development of various immunotherapy drugs that can suppress the development and progression of malignant tumor (20-22). The programmed cell death 1 (PD-1) receptor and

its ligands PD-L1 and PD-L2, and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) are known to play crucial roles in tumor cell immune escape mechanisms (23). Immune checkpoint inhibitors (ICI), such as antibodies against PD1, PD-L1, and CTLA-4, can activate T cells and block immunosuppression in the tumor microenvironment (24, 25). The blockade of CTLA-4 and PD-1/PD-L1 signaling with antibodies against PD-1/PD-L1 and CTLA-4, such as nivolumab, avelumab, and ipilimumab, significantly prolonged recurrence-free survival, OS, and distant metastasis-free survival as compared to placebo in a stage III trial of melanoma therapy (26-28), although the incidence rate of adverse events was still high, particularly in patients with endocrinopathies (29, 30). Nevertheless, ICP has brought new hope to patients with advanced HCC (31). Since ICP is a very important approach for cancer treatment, it has attracted numerous researchers. As a result, a number of reviews have been published (32, 33). To further enhace our understanding of current reseach in this area, we searched the literature published in PubMed and MEDLINE between 2005 and 2023 using hepatocellular carcinoma, immunotherapy, immune checkpoint inhibitor, biomarker, therapeutic response as top search terms. In this review, we add new information regarding immune resistance, image-based biomarker and locoregional treatments as well as the current understanding of the mechanisms of tumor cell immune escape, the role and effect of ICIs in treating cancers, particularly advanced HCC. We also summarize recent therapeutic progress with various ICIs alone or jointly with other methods, describe emerging biomarkers that help predict therapeutic response following ICP, and address future directions for ICP.

Immune escape mechanisms and immunotherapy in HCC

The liver contains blood from the portal veins and hepatic arteries and has both autoantigens and endogenous antigens. When the blood containing the two types of antigens circulates through the

liver, it develops autoimmune tolerance that prevents liver cells from being injured as a result of autoimmunity (34, 35). Owing to this immune tolerance, liver tumor cells can avoid being recognized and cleared by the immune system. When tumor cells grow, they release antigens that are recognized by T lymphocytes through antigen-presenting cells (APC), resulting in the specific killing of tumor cells. Immune checkpoints are inhibitory or stimulatory protein molecules synthesized on the cytoplasmic membrane of different immune cells, such as natural killer cells, dendritic cells, macrophages, monocytes, B and T cells, and tumor cells, or other cell types that regulate immune system activation and maintain immune homeostasis (36). Immune checkpoint PD-1 is present mainly in lymphocytes. The levels of PD-L1 are also abundantly expressed in innate cells such as macrophages (specifically Kupffer cells). In the normal physiological state, PD-1 binds to PD-L1 / PD-L2 to release inhibitory signals to inhibit the proliferation and activation of T lymphocytes via various pathways, resulting in the inhibition of autoimmune reactions, which confers immune resistance to tissues and cells (Figure 2). When the levels of PD-1 and PD-L1 / PD-L2 are elevated, T lymphocytes are activated, resulting in reduced proliferation and increased escape of tumor cells from immunity (37). A study using mouse models revealed that inhibition of PD-1 enhances the T lymphocyte-mediated immune response (38). PD-L1 / PD-L2 synthesized in human autoimmune cells also plays an important role in tumor cell immune escape (39). The immune checkpoint, CTLA-4, is produced by regulatory T lymphocytes. It releases signals that inhibit T cell proliferation, leading to the immune escape of tumor cells (40). Therefore, blocking these mechanisms with ICIs can result in the early recognition and killing of tumor cells.

Anti-tumor mechanism of ICI

The anti-tumor immune response can be reactivated by ICIs by suppressing inhibitory receptor signaling in T-cells(41). This process also involves various other immune cells. First, APCs (such as dendritic cells) are motivated to recognize tumor-related antigenic peptides displayed on major histocompatibility complex (MHC) I/II, and then these antigens are processed and presented to T cells to produce $CD8^+$ T cells that can recognize tumor cells. The tumor-specific $CD8^+$ T cells are then differentiated into effector T cells, which are cloned and proliferated in the tumor microenvironment, and finally eliminate the tumor cells by releasing cytolytic effectors, such as granzyme A/B and perforin (42). Finally, with the assistance of $CD4^+$ helper T cells and dendritic cells, some effector T cells differentiate into effector memory T cells for a rapid response to antigen re-attack. This is also the reason why some patients receiving immunotherapy achieve long-term remission.

To avoid injuring non-cancer cells due to excessive immune response, immunoregulatory proteins such as PD-1 on the T cell surface transmit immunologically suppressive signals to suppress the proliferation of T cells after binding to its ligand PD-L1. If cancer cells master this mechanism, they can generate PD-L1 on their own surface to avoid being recognized by T cells, thus escaping from the siege of T cells. PD-1/PD-L1 and CTLA-4 inhibitors are antibodies designed to block the recognition process of PD-1/PD-L1 and CTLA-4 on T cells (Figure 3). This blockade partially restores the capacity of T cells to kill tumor cells (43).

PD-1/PD-L1 inhibitors

HCC often occurs on the background of inflamed livers, where PD-1 levels are high in lymphocytes, and PD-L1 and PD-L2 are highly expressed in Kupffer cells, sinusoidal endothelial cells (LSECs) that form the wall of the hepatic sinusoids, and white blood cells as a result of exposure to proinflammatory cytokines (44). PD-1 inhibitors, such as pembrolizumab, nivolumab, and cemiplimab, suppress the binding of PD-1 to PD-L1 and PD-L2, leading to enhanced recognition and clearance of cancer cells by the immune system. These monoclonal antibodies (mAbs) have been demonstrated to be effective for treating melanoma, gastric cancers, non-small cell lung cancer, bladder cancer, and head and neck squamous cell carcinoma (HNSCC) (45). Pembrolizumab (a monoclonal antibody against PD-1) was approved by the FDA in 2019 to treat patients with recurrent or metastatic HNSCC (46). Since then, several clinical trials for ICIs have been completed, and the outcomes are encouraging. For instance, nivolumab (monoclonal antibody against PD-1) and pertuzumab (humanized antibody against extracellular domain II of human epidermal growth factor receptor 2 (HER2)) are common PD-1 inhibitors for breast and lung cancers (47, 48). In phase I/II of the CheckMate 040 trial, nivolumab was administered to 262 patients with later-stage unresectable HCC. The results showed that the objective response rate (ORR) in the dose escalation and expansion groups was 20%, and the median progression-free survival (PFS) was 4.0 (2.9-5.4) months. For patients who did not receive sorafenib, the median PFS was 28.6 Â months, and for those who received sorafenib, the median PFS was 15.0 (5.0-28.1) months. In the dose-escalation group, the median OS was 15.6 (13.2-18.9) months. In these two groups, 18% and 23% of patients with and without sorafenib had 3-4 grade treatment-related adverse reactions, including fatigue and diarrhea, without new signs of cancer progression, indicating that nivolumab has a manageable safety profile in patients with later-stage HCC (49).

In a study on pembrolizumab for the treatment of advanced HCC, 104 patients were included. These patients were diagnosed as intolerant to sorafenib and were treated with 200 mg pembrolizumab every three weeks for approximately two years. After treatment, the median PFS was 4.9 months, the median OS was 12.9 months, the ORR was 17%. One patient (1%) had complete remission and 17 (16%) had partial responses. In 46 (44%) patients, the disease stabilized, and in 34 (33%) patients, the disease continued to progress. The disease control rate (DCR) was 62% in 16% of patients with grade 3 treatment-related adverse reactions, including fatigue and high levels of aspartate and alanine aminotransferases (49), indicating that pembrolizumab is clinically effective and well-tolerated in patients with advanced HCC who had previously been treated with sorafenib.

CTLA-4 mAb

CTLA-4 is an antigen expressed by T cells that is involved in the differentiation of white blood cells. CTLA-4 competes with CD28 to bind to APC surface ligands CD80/CD86 to activate inhibitory signals that limit the activation and proliferation of T cells (50). CTLA-4 mAbs block the binding of CTLA-4 to its ligand to stimulate the activation and proliferation of T-cells. As a result of the blockade, the induction and anti-tumor immune responses were enhanced. The CTLA-4 mAb was one of the earliest ICIs clinically used for cancer treatment. In 2011, the FDA approved the first humanized mAb, ipilimumab, targeting advanced melanoma (51). Another CTLA-4 mAb, tremelimumab, was tested for advanced melanoma, liver cancer, and colorectal cancer and was granted by the FDA as an orphan drug for the treatment of HCC. In a Phase II clinical trial (NCT01008358), 17 progressive cases were treated after sorafenib treatment and hepatitis C virus-related HCC. The ORR and stable disease rate were 17.6% and 76.4%,

respectively. The median time for disease progression was 6.48 months, and the incidence rate of adverse reactions (grade 3/4) was 45% (52).

A dramatic reduction in viral load was achieved, and the predominant variants present before therapy were replaced by new variants of the hypervariable region 1 of HCV. This antiviral activity is likely related to an enhanced specific anti-HCV immune response (14). A randomized, multicenter phase III study investigated the therapeutic outcomes such as OS of nivolumab plus ipilimumab vs. standard of care (SOC) (sorafenib or lenvatinib) in participants with advanced HCC who had not received prior systemic therapy. The results showed that the dual immunotherapy combination provided durable responses and long-term survival benefit (53). However, this type of immunotherapy has not yet been approved for use as a monotherapy in HCC. Tremelimumab appears to have excellent therapeutic potential; however, further exploration is needed to develop and use biomarkers to predict the immune response to this drug. Potential combination therapy strategies should be explored to overcome the high incidence rate of adverse reactions and improve the current low response rate (10–20%).

Combination immune therapy

Data from preclinical studies (54, 55) indicate that PD-1/PD-L1 mAbs have synergistic anti-tumor activity with CTLA-4 mAb. The synergistic therapeutic activity of navulizumab and epizumab was demonstrated in a Phase III clinical trial of advanced melanoma (56). The combination of duvalizumab and trimetazumab is currently being used to treat advanced HCC in Phase I/II clinical trials (NCT02519348) (57). Based on preliminary results from 40 patients, the ORR was 15%, with a stable disease rate of 57.5%. A report released at the 2019 annual meeting of the American Society of Clinical Oncology (ASCO) (58) indicated that combined treatment with navulizumab and ipiximab had an ORR of 31%, with a 5% complete response rate in 148

patients with advanced HCC, demonstrating the superiority of dual immunotherapy. Based on this result, navulizumab + ibizumab combination therapy was expeditiously approved by the FDA as the first combination treatment in second-line therapy for HCC.

Tumor growth can accelerate angiogenesis, leading to vascular leakage, hypoxia, and the stimulation of multiple immunosuppressive pathways in the tumor microenvironment. Vascular endothelial growth factor (VEGF) promotes angiogenesis in tumor and is an important promoting factor in angiogenesis, which can be inhibited by anti-angiogenic inhibitors (59). Preclinical studies have indicated that combination therapy can promote the maturation of APCs and the activation and infiltration of $CD8^+$ cytotoxic T lymphocytes (CTL), reducing myeloid-derived inhibitory cells in tumor tissue and infiltration of regulatory T cells, synergistically promoting the clearance of tumors (60). Bevacizumab is a humanized anti-VEGF antibody. In the NCT02715531 trial, atezumab monotherapy and dual therapy with bevacizumab were compared for efficacy and safety in patients with advanced HCC (61). Combination therapy significantly improved OS and response rates. The latest Phase III Clinical Trials IMbrave150 (NCT03434379) (62) used atezumab in combination with bevacizumab to treat 501 metastatic or unresectable patients with advanced HCC and found that the risk of patient death was reduced by 42%, and the 12month survival rate was improved to 67.2% compared to sorafenib.

This type of combination therapy plan breaks the bottleneck of unresectable HCC and has been approved by the FDA as a first-line immunotherapy option for patients who do not receive systemic treatment and have unresectable HCC. In a Phase Ib clinical trial (NCT04072679)

A total of 50 patients with advanced HCC were included to receive low-dose and high-dose PD-1 mAb xindilizumab and bevacizumab analog IBI305 (63). The results showed that after high-

dose treatment, the ORR and stable disease rate were as high as 33.3% and 83.3%, respectively, further validating the efficacy and safety of the combination therapy.

ICIs plus TKIs

TKIs (such as sorafenib and lenvatinib) have multiple drug targets and can inhibit multiple tyrosine kinase-mediated signaling pathways, tumor cell proliferation, and block neovascularization. These drugs also have immune regulatory effects (such as reducing myeloid-derived inhibitory cells and regulatory T cells, enhancing tumor infiltration, and activating NK and T cells.

In the 2019 ASCO annual meeting, the clinical outcome of atezumab and acetinib combination for the treatment of advanced HCC was released, with an ORR of 13.6% and a median progression-free survival time of 5.5 months. However, dual therapy had higher toxicity than monotherapy, resulting in a 3/4 grade treatment-related adverse reaction rate of 72.7% (64), suggesting that although the combination treatment plan has a significant therapeutic advantage, more studies are needed to optimize the dosage and cycle. In addition, in other clinical trials (65), atezumab combined with cabozantinib therapy (NCT03755791) and pabolizumab combined with lenvatinib (NCT03713593) were evaluated for efficacy and safety. Based on the REFLECT test results, lenvatinib is recommended as category 1 drug for first-line treatment in the NCCN guidelines (14), and in CSCO guidelines for liver cancer treatment, pabolizumab, calilizumab in combination with apatinib/oxaliplatin are recommended for use in systemic chemotherapy.

ICI plus other therapies

Tumor cells release tumor antigens once they are killed by chemotherapy, radiation therapy, or interventional therapy. For example, oxaliplatin-based chemotherapy FOLFOX4 and GEMOX regimens can induce immunogenic cell death (66). When combined with ICIs, these therapies can further maintain or enhance the activation of T cells by APCs, leading to increased tumor-specific immune responses. A comparative phase II clinical trial was conducted to evaluate the therapeutic efficacy of the carrelizumab/ FOLFOX4 combination and sorafenib as first-line therapy for advanced HCC (67). Based on the results from 34 patients, ORR and stable disease rates were 26.5% and 79.8%, respectively, the median tumor-free survival time was up to 5.5 months, and the incidence of ≥ 3 grade ICP-related adverse reactions was only 5.9%, indicating that the combination has excellent therapeutic effect and safety. In the CSCO guide 2, patients who had used sorafenib in the past were considered eligible for the ruilizumab and FOLFOX4 regimen as second-line treatment. In a retrospective clinical cohort analysis, five patients with advanced HCC received stereotactic radiotherapy combined with navolizumab. Two patients had complete remission and three had partial remission, with a median progression-free survival time of up to 14.9 months, achieving local control and survival within 1 year (68).

In another study, selective internal radiotherapy was reported to enhance the activation and recruitment of immune cells, especially PD-1-expressing immune cells, in patients with HCC (69). Radiotherapy was conducted during nivolumab treatment in 76 patients, and PFS and OS were found to be significantly higher in patients receiving radiotherapy- nivolumab combination therapy than in those receiving nivolumab alone (70). With stereotactic body radiotherapy, no classic radiation-induced liver disease (RILD), also known as radiation hepatitis, a serious side effect of radiotherapy for HCC has been observed (68). These studies suggest therapeutic

synergy between radiotherapy and ICIs therapy. However, large-scale prospective clinical studies are required to validate these conclusions.

TACE, pulsed radiofrequency ablation (RFA), or cooled RFA has also been attempted in combination with trametazumab to treat advanced HCC. The median disease progression time was 7.4 months and the median overall survival time was 12.3 months, demonstrating the feasibility of this combination therapy. However, further studies are required to confirm the efficacy and safety of this treatment. A clinical trial (NCT03397654) is currently underway to evaluate the effectiveness and safety of pembrolizumab combined with TACE to treat late-stage HCC (71). It is generally agreed that ICP combined with locoregional therapy, defined as imaging-guided liver tumour-directed procedures (72), is an important direction for precise personalized treatment of HCC as described above. The locoregional therapies have gained consideration attention in HCC treatments, including RFA, microwave and high-intensity focused ultrasound ablations (73), selective internal radiation therapy (74), stereotactic body radiotherapy (75). These percutaneous ablation, transarterial chemoembolization, and transarterial radioembolization locoregional therapies are being explored to increase overall survival while preserving liver function with promising outcomes (76).

Insert Table 2 here

The timing of ICP

ICIs and related combination therapies have been demonstrated to be effective neoadjuvant therapies for HCC, which can improve clinical outcomes and benefit patients in various aspects and stages. Refining the timing of the ICP is important to maximize these benefits. A randomized, open-label, perioperative phase II study (NCT0322076) compared 27 patients with resectable HCC who received navulizumab in combination or without combination with

ipilimumab administered during the perioperative period. The results showed that ICP before surgery resulted in complete pathological remission in five (24%) patients and partial pathological remission in three (16%) patients, suggesting that ICP may be applied to patients with early stage HCC as neoadjuvant or adjuvant therapies (77). Studies have also shown that after therapeutically reducing unresectable HCC to resectable HCC through chemotherapy, the 5-year survival rate after the second resection reached 25% to 57% with reduced tumor recurrence and improved prognosis, which is significantly higher than that without surgery (6% to 8%) (78). Therefore, ICP provides a new pathway for transforming advanced HCC into resectable HCC for subsequent surgical resection. At the 2020 ASCO annual meeting, the combined use of TKIs (apatinib or lenvatinib) and PD-1 mAb was reported to treat 60 patients with advanced and unresectable HCC (79). Among them, 11 cases (18.3%) were converted into resectable HCC, suggesting that ICIs are not only effective in significantly prolonging the survival period and improving the quality of life of patients with advanced HCC, but also valuable in early neoadjuvant therapy and the transformation of advanced HCC to resectable HCC.

Real-world studies of ICP

Randomized controlled trials (RCTs) provide a standardized approach for evaluating the safety and efficacy of new drugs. However, the inclusion and exclusion criteria used in RCTs are often too restrictive to accommodate diverse patient populations, and the outcomes from RCTs may not fully conform to real-world clinical environments and conditions. Real-world studies are thus able to provide reliable data regarding patients' responses to drugs in real diagnosis and treatment environments, which may be a better alternative and supplementary source of safety and efficacy data for new drug development. For ICIs, several real-world studies have been performed to analyze the therapeutic response in large cohorts (Table 1) (80-84). For instance, in

a real-world retrospective study, 55 patients with advanced HCC were administered PD-1 inhibitors (36 navuzumab, 13 pemuzumab and six AK105), with a median OS of 15 months, PFS of 10 months, PR of 22%, and ORR of 22%. 47 patient (67%) showed stable disease, and six (11%) had progressive disease (PD) at the first radiological evaluation. The DCR was 89%, total incidence of adverse reactions was 61.8%, and incidence of major adverse reactions was 89%. Most adverse reactions were alleviated after treatment. This study demonstrated that PD-1 inhibitors are safe and effective for advanced primary HCC (80). An international multicenter real-world cohort study with 65 patients with advanced HCC (34 treated with trastuzumab and 31 treated with pemuzumab) was conducted. The results showed that both inhibitors have encouraging efficacy and safety (81).

Biomarker development for ICP response prediction

Current data indicate that the ORR after treatment with ICIs is about 20% in advanced HCC, meaning that a considerable proportion of patients receiving ICP are neither responsive nor respond poorly. Therefore, it is particularly important to select appropriate patients for ICI treatment to achieve better ORR. An important approach is to use cellular and molecular cues to predict and stratify patients who respond to ICPs and benefit from these therapies. Although a number of prognostic biomarkers for ICIs have been identified and tested in various cancers, there are few studies on biomarkers predicting the response of patients with HCC to ICIs or ICPs because ICP is still in its infancy in HCC. Zheng et al. treated eight cases of liver cancer with PD-1 inhibitors and analyzed the characteristics of the dynamics and composition of the gut microbiome during anti-PD-1 immunotherapy in HCC using metagenomic sequencing data from the fecal samples of three responders and five non-responders. They found that the fecal samples from responders showed higher taxa richness and more gene counts than those of non-

responders. The responders were found to have more microbial species, including *Akkermansia muciniphila* and *Ruminococcaceae*. Their work demonstrated that the dynamics of intestinal bacterial flora may be an early indicator of the outcomes of immunotherapy in HCC, and can be used for disease monitoring and decision-making in treatment planning (86). Juneja et al. found that PD-L1 expression levels in immune cells might be a potential biomarker of suppressed antitumor immunity and might play a critical role in immunosuppression (87). Radiological methods have also been applied to measure the response to immunotherapy in advanced HCC. For example, magnetic resonance elastography (MRE) has been used to assess the therapeutic effect of ICP in advanced HCC. The results showed that early stiffness changes in MRE tumors were associated with therapeutic response in advanced HCC (88). With advancements in research, an increasing number of biomarkers have been proposed (Table 3). Male sex (89), old age (over the age of 60) (90) and low baseline transforming growth factor-β (TGF-β) (91) have been shown to be more responsive to immunotherapy, and tumor-infiltrating CD8(+) T cells and intratumoral CD4/CD8 T-cell ratio are also promising biomarkers of therapeutic response (92, 93). Recently, exhausted, unconventionally activated $CD8^+$ $PD1^+$ T cells have been found to progressively accumulate in non-alcoholic steatohepatitis (NASH); however, they do not lead to NASH-induced HCC regression after PD1-targeted immunotherapy. Patients with NASH-driven HCC have shorter OS than patients with other etiologies after anti-PD1 or anti-PDL1 antibody treatment, suggesting that etiology could also be an important determinant of ICI therapy (94). Magnetic resonance (MR) imaging-based techniques including chemical shift imaging, frequency-selective imaging, and MR spectroscopy can be used to quantify fat-water admixtures (95) and intratumor steatosis was associated with treatment outcomes of ICI in patients with late-

stage HCC (96), suggesting that non-invasive MR techniques may be used to predict the therapeutic outcome of ICI.

Cancer progression may occur at an accelerated and unexpectedly high rate during ICP. This is one of the key reasons for the dramatic reduction in survival time. This condition is referred to as hyperprogressive disease (HPD) and has been observed in the treatment of various tumors, including HCC (97, 98). Several studies have been conducted to identify the clinical or molecular factors that predict HPD (Table 2), although many of these biomarkers need independent validation in HCC.

Resistance mechanism of TKI

Acquired resistance to TKI remains a challenge in ICP and targeted therapy (121). Several mechanisms are related to TKI resistance. The first recognized acquired resistance was the T790M mutation in EGFR. This missense mutation affects the formation of hydrogen bonds between tyrosine kinases and TKI, disabling TKI from binding tyrosine kinases (122, 123) and is highly frequent in NSCLC patients resistant to gefitinib or erlotinib (124). Several irreversible EGFR inhibitors, such as afatinib and osimertinib, have been developed to overcome acquired resistance due to T790M mutations (125, 126). Amplification of the c-MET gene is an important mechanism of resistance. c-MET can activate the RTK system to induce cell proliferation, differentiation, migration, and angiogenesis (127), and amplification of the c-MET gene is partially responsible for TKI resistance in NSCLC patients (128). Understanding this mechanism allows the exploration of combined TKI treatments to overcome EGFR-TKI resistance (129, 130). For example, a single-arm antibody MetMAb was developed to block the binding of HGF to the MET receptor, resulting in the restoration of sensitivity to erlotinib in NSCLC patients (131). Deficiency of PTEN, a tumor suppressor, is also found in TKI-resistant cells and in

patients treated with gefitinib (132). High expression levels of JNK were found in non-responders among sorafenib-treated HCC patients. As JNK activity is associated with the level of CD133 (133), this may increase the subpopulation of multipotent cells that have higher proliferative and self-renewal abilities than those identified as cancer stem cells and proven to trigger the onset and growth of HCC (134). In addition, the Jun proto-oncogene (c-Jun) in the mitogen-activated protein kinase (MAPK) signaling pathway is upregulated in hepatoma cell lines after treatment with sorafenib, leading to reduced sorafenib-induced apoptosis, demonstrating that c-Jun is a key player in the development of sorafenib resistance in hepatoma cells (135). In human hepatoma cells, the expression of some genes in the Toll-like receptor (TLR)signaling pathway was altered after treatment with regorafenib and lenvatinib. Therefore, it may be possible to improve the treatment of patients with HCC via modulation of the TLR signaling pathway (136). Other possible resistance mechanisms include mutations in BRAF, which is located downstream of the EGFR signaling pathway and promotes cell proliferation and differentiation through interaction with RAS (137, 138), mTOR pathway suppression (139) and increased VEGF levels (140)

Conclusions and prospectives

ICP, represented by ICIs, has made significant breakthroughs in the treatment of HCC. ICIs improve OS and increase DCR to a certain extent in patients with advanced HCC who are intolerant or unresponsive to sorafenib. ICIs have fewer adverse events and are not metabolized in the liver, thus avoiding severe adverse reactions and liver injury. Combination treatments with two or more ICIs, radiotherapy, and interventional therapy may further improve anticancer efficiency. The development of more effective and safe immune combination therapy is a future direction for

treating advanced HCC. There are still urgent problems to be solved when applying ICI for HCC treatment, such as organ-specific immunity and highly immunosuppressive microcirculations. ICI-based ICP heavily relies on driving endogenous immune cells, such as existing CTLs, but the response to ICI could be reduced due to the elimination of immune cells through various inhibitory pathways and immune escape in HCC. Therefore, more research is needed to explore specific biomarkers to measure patient response to ICI/ICP, develop new drugs on known targets and combination treatment, and have a better understanding of the mechanism underlying ICP-related adverse reactions. Identification of new immune checkpoints, development of new ICP based on new immune checkpoints, and overcoming the obstacles associated with the tumor microenvironment will provide more treatment options for patients with HCC in the future. In addition, real-world studies with large sample sizes are needed to further validate the therapeutic outcomes of ICIs/ICPs for advanced HCC.

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771 Figure legend

772 Figure 1. Major causes of hepatocellular carcinoma.

Figure 2. Immune resistance mechanisms of hepatocellular carcinoma via $CD8^+$ cell activation (CTLA-4, cytotoxic T-lymphocyte antigen-4; Tregs, regulatory T cell; HSC, hematopoietic stem cell, HC, hepatocellular carcinoma; KC, Kupffer cells; LSEC, liver sinusoidal endothelial cell; PDL-1, programmed cell death ligand 1; PDL-2, programmed cell death ligand 2; PD, programmed cell death protein)

Figure 3. Modes of action of immune checkpoint inhibitors in hepatocellular carcinoma (APC, antigen-presenting cells; MHC, major histocompatibility complex; T cell, T lymphocytes; TCR, T-cell receptor; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD, programmed cell death protein; PDL-1, programmed cell death ligand)

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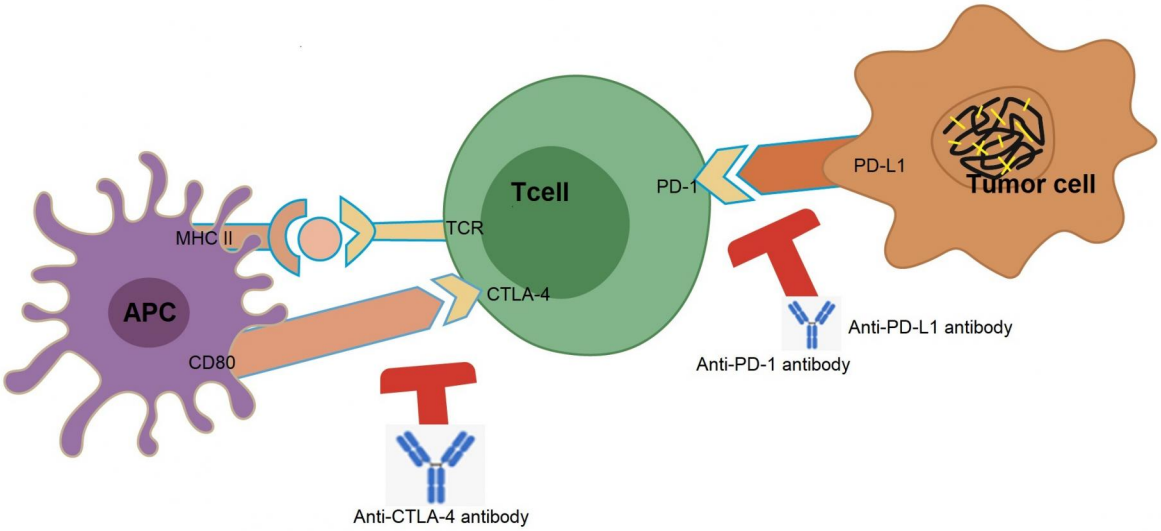
Tcell
PD-1
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Tumor cell
MHC II
TCR
APC
CTLA-4
CD80
Anti-PD-L1 antibody
Anti-PD-1 antibody
Anti-CTLA-4 antibody

Table 2. Potential biomarkers predicting therapeutic response in hyperprogressive disease (HPD) in cancers after therapy with immune checkpoint inhibitors.

Biomarkers	Prognostic significance
Circulating tumor DNA (ctDNA) (85)	High concentration of cfDNA relates to high risk for HPD and poor progress-free survival in NSCLC
Chemoattractant protein 1 (86)	Low serum monocyte chemoattractant protein is associated with HPD
Haemoglobin (87)	Serum haemoglobin level is associated with HPD
Neutrophil-lymphocyte ratio (NLR) (88)	High NLR is associated with poor overall survival and NLR increases rapidly in patients developing HPD
MDM2 (89, 90)	Amplification of MDM2 leads to poor prognosis
EGFR (91)	Overexpression of EGFR lows the response rates to ICI therapy
BRCA2 (92)	Enriched mutations in the DNA repair gene BRCA2 improves anti-PD-1 response in cancer
MMR (93, 94)	Deficiency of MMR predicts better prognosis in cancer
Regulatory T (Treg) cells (95)	Activation of Treg promotes hyperprogression of cancer

T cells (96)	Increased T_{PEX} cell frequencies are linked to increased patient survival
myeloid-derived suppressor cells (*MDSCs)* (97)	Patients with low *MDSC* count are more likely responding to ipilimumab treatment
IFN-γ (98)	IFN-γ mediates inhibition of lung cancer via upregulating expression of PD-L1, leading to better prognosis
CRP (99)	High CRP predicts HPD in gastric cancer

Table 1. Outcomes of real-world studies of immune checkpoint inhibitors in hepatocellular carcinoma

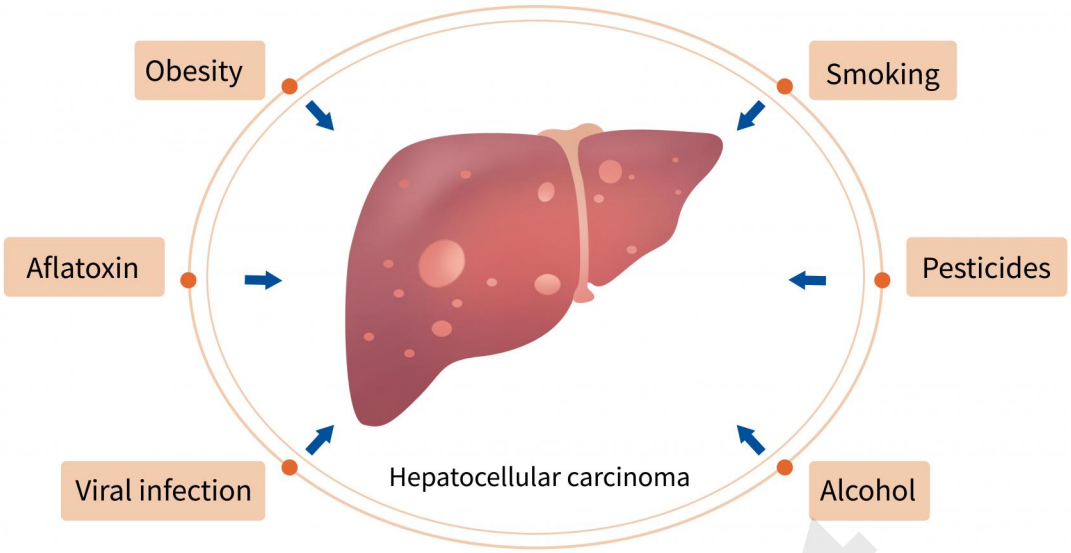
Drugs	Trial name	stage	N	OS (m)	PFS (m)	ORR (%)	DCR (%)
Anti-PD-1 antibody (80)			55	15	10	22	89
Nivolumab/pembrolizumab (81)			34/31	11.0	4.6	12/49	
Nivolumab (82)			155	10.25	3.06	23.9	
PD-(L)1 inhibitors (85)			5257	9.3	3.2		
Nivolumab/pembrolizumab (83)			1344	8.0			
Atezolizumab (84)			152	12.8			

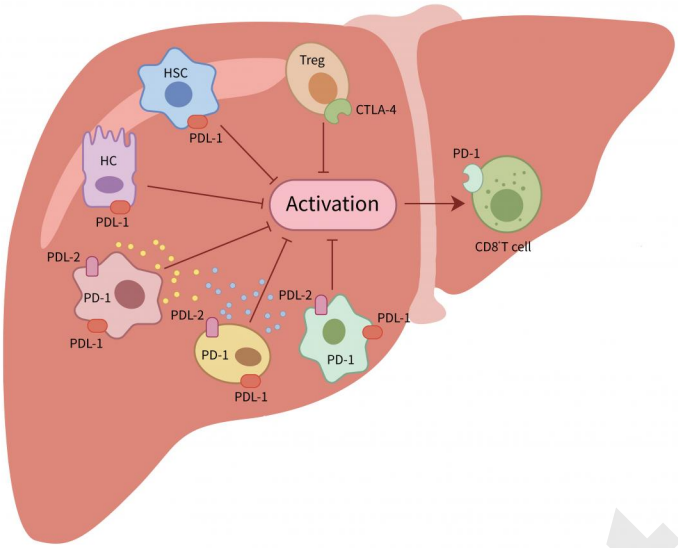
Abbreviations: Ab, antibody; CTLA-4, cytotoxic T lymphocyte antigen-4; ORR, objective response rate; OS, overall survival; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; ORR, objective remission rate; DCR, disease control rate; irAER, incidence of grade 3 immune-related adverse events.

Table 2. Potential biomarkers predicting therapeutic response in hyperprogressive disease (HPD) in cancers after therapy with immune checkpoint inhibitors.

Biomarkers	Prognostic significance
Circulating tumor DNA (ctDNA) (99)	High concentration of cfDNA relates to high risk for HPD and poor progress-free survival in NSCLC
Chemoattractant protein 1 (100)	Low serum monocyte chemoattractant protein is associated with HPD
Haemoglobin (101)	Serum haemoglobin level is associated with HPD
Neutrophil-lymphocyte ratio (NLR) (102)	High NLR is associated with poor overall survival and NLR increases rapidly in patients developing HPD
MDM2 (103, 104)	Amplification of MDM2 leads to poor prognosis
EGFR (105)	Overexpression of EGFR lows the response rates to ICI therapy
BRCA2 (106)	Enriched mutations in the DNA repair gene BRCA2 improves anti-PD-1 response in cancer
MMR (107, 108)	Deficiency of MMR predicts better prognosis in cancer
Regulatory T (Treg) cells (109)	Activation of Treg promotes hyperprogression of cancer
T cells (110)	Increased T_{PEX} cell frequencies are linked to increased patient survival

myeloid-derived suppressor cells (*MDSCs)* (111)	Patients with low *MDSC* count are more likely responding to ipilimumab treatment
IFN-γ (112)	IFN-γ mediates inhibition of lung cancer via upregulating expression of PD-L1, leading to better prognosis
CRP (113)	High CRP predicts HPD in gastric cancer
Tumor infiltrating lymphocytes (TILs) (114)	High TIL predicts good ICI treatment outcomes
Immune cells within the tumor microenvironment (115)	Number and type of immune cells affect the treatment response
Gene expression profiling (116, 117)	Helps to identify novel biomarkers for ICI responses and effectiveness in cancer
Immune milieu (118)	Can be modulated by bacteriophage and genetically-engineered microbes to increase and predict response to ICI
Neoantigen (119)	Neoantigen load is a promising biomarker for predicting the efficacy of ICIs
Tumor-specific antigens (120)	Associated with patient response to immunotherapies, including ICI, adoptive cell transfer, and dendritic cell-based vaccines and may be used to development of effective second-generation therapeutic cancer vaccines.


Obesity
Smoking
Aflatoxin
Pesticides
Viral infection
Hepatocellular carcinoma
Alcohol


HSC
PDL-1
Treg
CTLA-4
HC
PDL-1
Activation
PD-1
CD8+T cell
PDL-2
PD-1
PDL-1
PDL-2
PDL-2
PD-1
PDL-1
PDL-1
PD-1