Converting 'cold' to 'hot' hepatocellular carcinoma for improved immunotherapy (Review)

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Abstract. Hepatocellular carcinoma (HCC) often exhibits an immunologically 'cold' tumor microenvironment (TME) characterized by poor T cell infiltration and active immunosuppressive mechanisms, limiting the efficacy of immunotherapies such as immune checkpoint inhibitors (ICIs). Therefore, converting immunologically cold HCC tumors into 'hot', immune-reactive tumors has emerged as a critical strategy to enhance immunotherapy responsiveness. In the present review, the tumor immune landscape in HCC is summarized, and the mechanisms underlying its immunologically cold phenotype, and current strategies for reprogramming the TME toward an immune-active state are described. In addition, the roles of various immune cells, cytokines and tumor-intrinsic pathways in driving immune exclusion and tolerance are discussed. Therapeutic approaches include ICI-based combinations with anti-angiogenic agents or locoregional therapies, as well as dual checkpoint blockade. Other strategies involve targeting immunosuppressive cell populations, oncolytic virus therapy, cancer vaccines, adoptive cell therapies and epigenetic modulators. Clinical evidence supports the potential of these strategies, with several combinations demonstrating improved response rates and survival. Research aims to optimize these therapies, identify predictive biomarkers and explore novel immune targets to further improve outcomes. Overall, converting HCC from an immunologically cold-to-hot tumor represents a promising paradigm to potentiate immunotherapy efficacy, although additional studies and innovative strategies are required to achieve durable benefits for a broader population of patients with HCC.

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Abbreviations: AFP, α-fetoprotein; CAF, cancer-associated fibroblast; CAR, chimeric antigen receptor; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; CR, complete response; CSF, colony stimulating factor; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; FGFR, fibroblast growth factor receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPC3, glypican-3; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; HLA, human leukocyte antigen; ICI, immune checkpoint inhibitor; IFN-γ, interferon-γ; IL, interleukin; LAG-3, lymphocyte-activation gene 3; LSEC, liver sinusoidal endothelial cell; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; NASH, non-alcoholic steatohepatitis; NK, natural killer; OPN, osteopontin; ORR, objective response rate; OS, overall survival; OV, oncolytic virus; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; T-VEC, talimogene laherparepvec; TACE, transarterial chemoembolization; TAM, tumor-associated macrophage; TCR, T cell receptor; TGF-β, transforming growth factor-β; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell immunoglobulin and mucin-domain containing protein 3; TME, tumor microenvironment; Treg, regulator T cell; VEGF, vascular endothelial growth factor

Key words: hepatocellular carcinoma, immunotherapy, tumor microenvironment, immune checkpoint inhibitors, T cell infiltration

Contents

1. Introduction

Hepatocellular carcinoma (HCC) is a major global health burden, representing the most common primary liver cancer

and the third leading cause of cancer-related death worldwide (1). Most cases are diagnosed at an intermediate or advanced stage due to the insidious onset of the disease, and high post-treatment relapse rates further contribute to a poor overall prognosis (2,3). While conventional therapies, such as surgical resection, ablation, transarterial chemoembolization (TACE) and kinase inhibitors, remain central to HCC management, the emergence of cancer immunotherapy has introduced new therapeutic options. Immune-based treatments, particularly immune checkpoint inhibitors (ICIs) targeting programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), demonstrate durable clinical responses in several types of solid tumors, including efficacy in HCC (4,5). For example, it has been shown that in advanced HCC, single-agent ICIs can induce objective responses in 15-20% of patients (6).

However, most patients with HCC do not respond adequately to immunotherapy (7). This has been attributed, in part, to the distinct tumor immune microenvironment of HCC, which often renders the tumor immunologically 'cold' or non-inflamed, thereby diminishing immune-mediated antitumor activity (5,8). The concept of 'hot' and 'cold' tumors refers to the degree of immune cell infiltration and activity in the tumor microenvironment (TME). Hot HCC tumors are characterized by high densities of $CD8^+$ T cells infiltrating the tumor the core and margin, a strong interferon-γ (IFN-γ) gene expression signature indicative of an active T helper 1 immune response, and the upregulated expression of checkpoint molecules such as PD-L1 (9,10). Conversely, cold tumors in general exhibit sparse T-cell infiltration, diminished levels of IFN-γ-associated inflammatory cytokines, and minimal checkpoint expression, reflecting an immunologically inactive microenvironment (9,10).

Several quantitative approaches have been developed to distinguish between cold and hot tumors in HCC and other solid cancers. These include immunohistochemical analysis of intratumoral $CD8^+$T-cell density, commonly using multiplex immunohistochemistry (11), IFN-γ-associated immune gene expression profiling, for example using RNA sequencing or targeted Nanostring panels to generate a T-cell-inflamed or tumor inflammation signature (12), and spatial immune cell profiling (13). Composite immune scoring systems such as the Immunoscore, which quantifies $CD3^+$ and $CD8^+$ T cells *in situ*, are also used to stratify tumors immunologically (14).

HCC typically arise in the context of chronic inflammation, such as that caused by hepatitis B or C infection or non-alcoholic steatohepatitis (NASH), but paradoxically often develops in an immunosuppressive microenvironment (15). Chronic viral antigen exposure and liver-specific tolerogenic mechanisms contribute to T-cell exhaustion and immune evasion in HCC (8). Notably, 25-30% of HCC tumors exhibit an 'immune-excluded' phenotype, associated with Wnt/β-catenin pathway activation and characterized by a lack of T-cell infiltration, which predicts resistance to ICIs (10).

Converting an immunologically cold HCC into a hot tumor is a promising strategy to overcome immune resistance and improve patient responses. In the present review, a condensed overview of the HCC tumor immune landscape is provided and the mechanisms underlying its immunologically cold phenotype, including the roles of various immune cells, cytokines and tumor-intrinsic pathways in suppressing antitumor immunity are presented. The established and emerging therapeutic strategies aimed at reprogramming the HCC microenvironment to a more inflamed, T-cell-permissive state are also discussed.

2. Tumor immune landscape in HCC

HCC develops within a complex TME composed of tumor cells, stromal elements and a diverse array of immune cells. The baseline immune landscape of HCC is often immunosuppressive and heterogeneous, contributing to variable immune surveillance and inconsistent therapeutic responses (16). Chronic liver inflammation, resulting from viral hepatitis, alcohol or fatty liver disease, serves as the foundation for immune dysfunction even before malignant transformation occurs (17). As HCC develops, the intrinsic tolerogenic milieu of the liver, which normally prevents overactivation by persistent exposure to gut-derived antigens, is co-opted by the tumor to escape immune elimination (18). The roles of different cell types in the TME of HCC are summarized in Table I (8,16,19-27).

One hallmark of the HCC immune landscape is the abundance of immunosuppressive and tumor-promoting immune cells relative to effector cells (28). Tumor-associated macrophages (TAMs), which are often skewed toward an M2-like phenotype, are typically the most abundant immune infiltrates in HCC and are associated with a poor prognosis (29). TAMs promote tumor growth by secreting immunosuppressive cytokines, such as IL-10 and transforming growth factor-β (TGF-β), promoting angiogenesis and directly inhibiting T cell activity (30).

Similarly, tumor-associated neutrophils in HCC can suppress T cell cytotoxicity by expressing PD-L1 and arginase-1, which contribute to the exhaustion of local T cells (20). Dendritic cells (DCs) are present in the HCC microenvironment but are often functionally impaired: Conventional DCs may be reduced in number or exhibit dysfunctional antigen-presenting capacity, whereas plasmacytoid DCs tend to induce immune tolerance and are associated with the accumulation of regulatory T cells (Tregs) (8).

Crucially, cytotoxic T lymphocytes, the primary effectors targeted by immunotherapies, are often excluded from HCC tumor nests or rendered anergic or functionally exhausted (31). While a subset of HCCs exhibit an 'immune-active' phenotype characterized by substantial $CD8^+$ T-cell infiltration and IFN signaling, most HCC tumors are categorized as 'immune-exhausted' or 'immune-excluded'. These are characterized by high expression of checkpoint receptors, including PD-1 and T cell immunoglobulin and mucin-domain containing protein 3 (TIM-3), on T cells, or by the physical exclusion of T cells from the tumor parenchyma (10,32).

Cancer-associated fibroblasts (CAFs) and endothelial cells in HCC further shape the immune landscape. CAFs produce extracellular matrix proteins and desmoplastic stroma, which act as physical barriers to immune cell infiltration (33,34). They also secrete TGF-β and cytokines including IL-6, IL-8, IL-10, CXCL12, prostaglandin E2 and vascular endothelial growth factor (VEGF), which promote Treg differentiation

Table I. Properties of the tumor immune microenvironment in HCC.

Cell type	Marker	Role in HCC tumor microenvironment	(Refs.)
TAMs	CD68; M2 markers (CD163, CD206); M1 markers (CD80, CD86)	Often polarized to immunosuppressive M2 phenotype; secrete IL-10, TGF-β and other factors that suppress T cell activity. High TAM density is associated with reduced $CD8^+$ T cell infiltration and poor prognosis	(8,19)
TANs	CD15, CD66b; high PD-L1 and arginase-1 expression	Can inhibit CTL function by releasing reactive oxygen species and expressing immunosuppressive molecules, such as PD-L1. TANs blunt $CD8^+$ T cell cytotoxicity in HCC and are associated with aggressive disease	(20)
cDCs	cDC1: $CLEC9A^+$ $XCR1^+$;cDC2: $CD1c^+CD11b^+$	Primary antigen-presenting cells that prime T cells. In HCC, cDCs are often functionally impaired or present in insufficient numbers, limiting effective anti-tumor T-cell priming	(16)
pDCs	BDCA-2, CD303	Tend to accumulate in HCC and promote tolerance. pDC infiltration is associated with Treg expansion and poorer outcomes, suggesting pDCs contribute to immunosuppressive networks in HCC	(8)
NK cells and ILCs	NK: $CD56^+CD16^+$ (cytotoxic NK); ILCs: $CD127^+$ (ILC1-3)	NK cells can directly kill HCC cells, and are associated with improved survival, but often functionally suppressed in HCC. Certain ILC subsets (ILC2 and ILC3) mimic Th2/Th17 functions and secrete IL-13, IL-17, promoting tumor growth and suppressing immunity	(21)
CTLs	CD8, granzyme B, IFN-γ	Essential effectors for tumor clearance. Active, granzyme B-positive CD8 T cells indicate a hot tumor and are associated with a favorable prognosis. However, numerous HCCs exhibit low CD8 infiltration or dysfunctional CTLs due to chronic antigen stimulation and inhibitory checkpoint signaling	(22)
Exhausted T cells	$PD\text{-}1^+TIM\text{-}3^+CTLA\text{-}4^+$ CD8 T cells	High levels of exhausted T cells (expressing markers such as PD-1 and TIM-3) are characteristic of HCC. They have impaired effector function and their enrichment is associated with shorter progression-free and overall survival	(19).
TRM cells	CD69, CD103	TRM cells reside in liver tissue and provide local immune surveillance. In HCC, $CD69^+CD103^+$ TRM cells are associated with improved response to ICIs and superior outcomes, suggesting a pre-existing local immunity	(22)
Tregs	$CD4^+FOXP3^+$, often $CTLA\text{-}4^+$ high	Abundant in HCC and the surrounding liver. Tregs suppress anti-tumor T-cell responses via IL-10, TGF-β and checkpoint molecules. Elevated intratumoral Tregs are associated with CD8 dysfunction and poor survival	(23)
Th cells	Th1: IFN-γ, IL-2; Th2: IL-4, IL-5; Th17: IL-17	Th1 cells support antitumor immunity by producing IFN-γ, whereas Th2 and Th17-skewed responses can aid tumor progression. HCC with a dominant Th2/Th17 cytokine profile tends to have poor immune-mediated control	(24)
B cells	CD19, CD20 (plasma cells: CD138)	Can form TLS in HCC. TLS with B cells and follicular helper T cells are associated with an enhanced immune response and improved prognosis. Some B cells, however, may act as regulatory B cells producing IL-10	(25)
CAFs	α-SMA, collagen I, FAP	Create dense stroma and physical barriers to immune cells. CAFs secrete TGF-β and other factors that foster Treg differentiation and exclude T cells. They directly interact with immune cells to mediate immune evasion and are associated with poor immunotherapy responses	(26)
ECs	$VEGFR^+CD31^+$ (VEGF-responsive vasculature)	Abnormal tumor endothelium limits lymphocyte infiltration. VEGF from HCC drives angiogenesis and suppresses DC and T-cell function. Anti-VEGF therapy normalizes vessels and improves T-cell entry. ECs in HCC can express immune checkpoint ligands such as PD-L1, contributing to local T-cell suppression	(27)

BDCA-2, blood DC antigen 2; CAF, cancer-associated fibroblast; cDC, conventional DC; CLEC9A, C-type lectin domain family 9 member A; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; EC, endothelial cell; FAP, fibroblast activation protein; FOXP, forkhead box P; HCC, hepatocellular carcinoma; ILC, innate lymphoid cell; NK, natural killer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; pDC, plasmacytoid DC; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil; TGF-β, transforming growth factor- β; Th, T helper; TIM-3, T cell immunoglobulin and mucin-domain containing protein 3; TLS, tertiary lymphoid structure; Treg, regulatory T cell; TRM, tissue-resident memory; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; XCR1, X-C motif chemokine receptor 1; α-SMA, α-smooth muscle actin.

and inhibit effector T cell activity, thereby facilitating immune evasion (33,34). The tumor endothelium in HCC is often abnormal, expressing molecules such as PD-L1, Fas ligand and vascular cell adhesion molecule-1, which can impair lymphocyte trafficking or induce T-cell apoptosis, a phenomenon termed the endothelial checkpoint (35,36). Elevated vascular endothelial growth factor (VEGF) levels in HCC not only drive angiogenesis but also directly suppress DC maturation and T-cell responses, contributing further to immune exclusion (37).

Despite the generally immunosuppressive landscape, HCC retains a degree of immunogenicity, as evidenced by occasional spontaneous tumor regression and the observed efficacy of donor lymphocyte infusions following liver transplantation (38). It is reported that 15-25% of HCC tumors exhibit a more inflamed, hot phenotype, characterized by higher $CD8^+$ T-cell infiltration and IFN signaling. These tumors are associated with an improved prognosis and greater responsiveness to immunotherapy (5).

3. Mechanisms of immune 'coldness' in HCC

Multiple interrelated mechanisms contribute to the immunologically cold phenotype of HCC tumors, acting at the tumor cell-intrinsic level, within the broader liver microenvironment, and at the systemic level.

Tumor-intrinsic immune evasion pathways. Genetic and epigenetic alterations in HCC cells can contribute to an immune-deserted microenvironment. Notably, activation of the Wnt/β-catenin signaling pathway is observed in 30-50% of HCCs and is strongly associated with the exclusion of T cells from the tumor (10,39). β-catenin activation in tumor cells downregulates the expression of C-C motif chemokine ligand (CCL) 4, which impairs DC recruitment and thereby prevents the effective priming of anti-tumor T cells. This creates an immunologically cold tumor niche. Clinically, HCC tumors with catenin β-1 gene mutations that activate β-catenin rarely respond to ICIs, making this pathway a recognized driver of immune resistance (40).

Other oncogenic pathways frequently altered in HCC also contribute to immunosuppression; for example, MYC oncogene overexpression, which is detected in ~50% of HCCs, upregulates PD-L1 in tumor cells and modulates cell metabolism to favor an immunosuppressive milieu (39). Similarly, the loss-of-function of phosphatase and tensin homologs or activation of PI3K-AKT signaling can promote an immunoresistant environment by increasing the expression of inhibitory molecules and recruiting suppressive myeloid cells (41).

HCC cells actively upregulate immune checkpoint ligands and other immunoinhibitory molecules. HCCs frequently express PD-L1 on their surfaces of their cells, especially those with upregulated MYC expression or enriched in progenitor-like traits. PD-L1 can directly bind to PD-1 on T cells, thereby promoting T-cell exhaustion (16). In addition, some HCC cells express galectin-9, an immunosuppressive lectin that interacts with TIM-3 on T cells, further contributing to T-cell exhaustion (42).

Immunosuppressive cellular constituents. The immune infiltrate in HCC is skewed toward cell types that sustain an immunosuppressive, cold TME (28). Tregs are often enriched in the blood and tumor tissues of patients with HCC, where they suppress antitumor immunity by secreting IL-10 and TGF-β, and by consuming IL-2, which limits the proliferation of effector T cells (43,44).

TAMs of the M2 phenotype produce high levels of IL-10 and prostaglandins, and express immune checkpoint molecules such as PD-1, which suppress phagocytosis and innate immune responses (45). These TAMs also recruit Tregs via CCL22 and promote tissue remodeling, which impairing or inactivating effector T cell functions. Myeloid-derived suppressor cells (MDSCs), a heterogeneous population of immature monocytes and neutrophils with immunosuppressive activity, are also present in HCC. MDSCs release arginase and inducible nitric oxide synthase, which metabolically starve T cells of L-arginine and produce nitric oxide, thereby causing T-cell dysfunction (44).

Cytokine and metabolic milieu. The TME of HCC is enriched with immunosuppressive cytokines (40,46). TGF-β, often abundant due to cirrhosis and CAF activity, is a key driver of immune exclusion. It inhibits the proliferation and effector functions of T cells and natural killer (NK) cells, while promoting the differentiation of Tregs and M2 macrophages (46). A high-TGF-β signature in HCC is associated with an immune-excluded phenotype and resistance to immune checkpoint blockade (40).

HCC tumors are frequently hypoxic due to their rapid growth and abnormal vasculature (47). Hypoxia induces hypoxia-inducible factor-1α, which upregulates adenosine production and VEGF expression, both of which suppress antitumor immunity. Adenosine accumulates in the TME via CD39/CD73 ectonucleotidase activity in cancer and stromal cells. It potently inhibits T- and NK-cell activity through A2A adenosime receptors, while promoting the expansion and functional activation of Tregs and the polarization of macrophages to the M2 phenotype (48).

Liver-specific tolerogenic factors. The unique immune environment of the liver plays a central role in the immune evasion of HCC. Under normal conditions, the liver is constantly exposed to food antigens and microbial products from the gut via the portal circulation. Therefore, non-immunogenic (tolerogenic) mechanisms have evolved in the liver to prevent unnecessary immune activation. Kupffer cells, which are liver-resident macrophages, and liver sinusoidal endothelial cells (LSECs) constitutively express inhibitory molecules, including PD-L1 and Fas ligand, and secrete anti-inflammatory cytokines such as IL-10 and TGF-β, which induce T-cell tolerance (49-51). Naïve T cells encountering antigens in the liver often become anergic or differentiate into Tregs (52,53).

HCC exploits these tolerogenic pathways: Kupffer cells may present tumor antigens in a tolerogenic manner, while LSECs can induce the deletion of tumor-reactive T cells, thereby preventing effective antitumor immune priming (54). In addition, cirrhotic livers exhibit expanded populations of immunosuppressive myeloid and stellate cells, contributing to an immunosuppressive fibrotic microenvironment, even before malignant transformation (55).

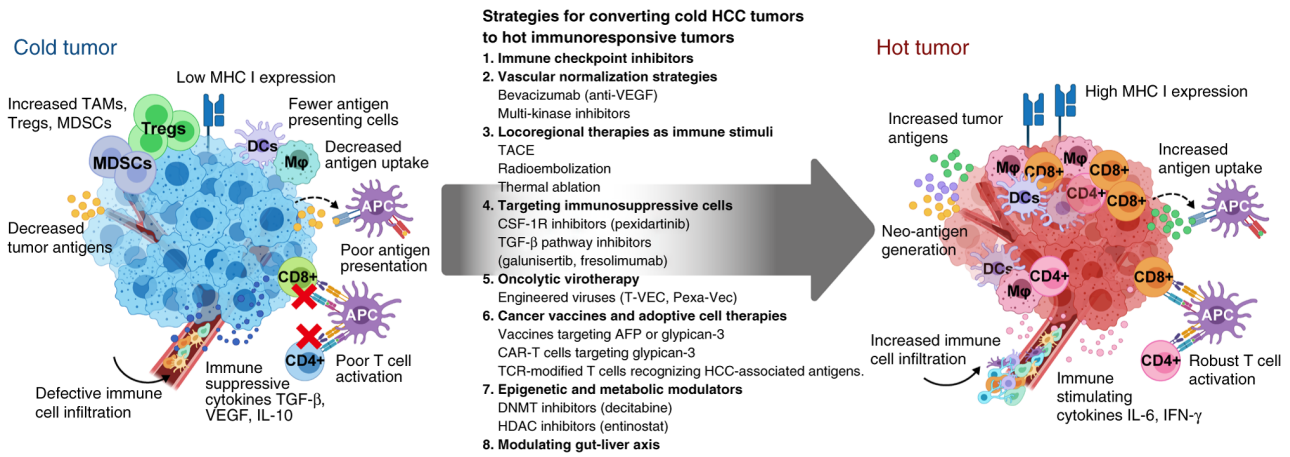

Figure 1. Transformation of immunologically cold HCC tumors into hot immunoresponsive tumors by various therapeutic strategies. The left panel depicts a cold tumor microenvironment characterized by low MHC I expression, low levels of tumor antigens, defective immune cell infiltration and immunosuppressive features, including abundant TAMs, Tregs and MDSCs. These tumors show poor antigen presentation, decreased antigen uptake by DCs, and ineffective T-cell activation due to immunosuppressive cytokines TGF-β, VEGF and IL-10. The right panel shows a successfully converted hot tumor with high MHC I expression, increased tumor antigen levels and uptake, and robust immune cell infiltration. This environment features activated $CD8^+$ and $CD4^+$ T cells, functional APCs, and immune-stimulating cytokines IL-6 and IFN-γ. The central arrow outlines eight key strategic approaches for this conversion. Each strategy includes specific therapeutic examples currently being investigated for HCC treatment. AFP, α-fetoprotein; APC, antigen-presenting cell; CAR-T, chimeric antigen receptor-T; CSF-1R, colony stimulating factor-1 receptor; DC, dendritic cell; DNMT, DNA methyltransferase; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; Mj, macrophage; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; Pexa-Vec, pexastimogene devacirepvec; T-VEC, talimogene laherparepvec; TACE, transarterial chemoembolization; TAMs, tumor-associated macrophages; TCR, T cell receptor; TGF-β, transforming growth factor-β; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor.

Immune editing and antigen loss. During HCC development, immune editing can occur, whereby the adaptive immune system initially eliminates highly immunogenic tumor cell clones, leading to the selection of tumor cells that are less detectable by T cells (56). Over time, HCC tumors may exhibit the downregulation or loss of certain tumor-associated antigens. For example, tumor variants that no longer express common antigens, such as α-fetoprotein (AFP) or glypican-3 (GPC3), may evade T-cell- or antibody-based therapies targeting these antigens (57). Similarly, the loss of major histocompatibility complex (MHC) class I molecule expression due to β2-microglobulin mutations or defects in the antigen presentation machinery can impair cytotoxic T-cell recognition, although this may potentially increase the susceptibility of the tumor to NK cells (58,59).

4. Strategies to reprogram the immune microenvironment in HCC

Given the array of mechanisms that render HCC an immunologically cold tumor, various therapeutic strategies have been developed to modulate the TME and warm it up, thereby enhancing the efficacy of immunotherapies. These strategies involve combining ICIs with other treatments, targeting specific immunosuppressive pathways or cell populations, and introducing proinflammatory stimuli into the tumor milieu. A summary of these strategies is presented in Fig. 1. In addition, a flowchart illustrating key combination therapies used to convert an immunologically cold HCC tumor into a hot tumor is shown in Fig. 2.

ICIs: Foundation for combination therapies. ICIs include anti-PD-1 antibodies, such as nivolumab and pembrolizumab, anti-PD-L1 antibodies, such as atezolizumab and durvalumab, and anti-CTLA-4 antibodies, such as ipilimumab and tremelimumab. These agents form the foundation of immunotherapy strategies in HCC, and are able to activate T-cell responses if primed T cells are already present in the TME. However, single-agent ICIs have shown limited overall response rates in HCC (~15%), indicating that numerous tumors lack preexisting active T cells (60,61). Therefore, ICIs are commonly combined with other agents that can initiate or amplify antitumor immunity. One such approach is dual checkpoint blockade, such as PD-1 plus CTLA-4 inhibition, where CTLA-4 blockade primarily acts on the lymphoid organs to expand T-cell clonal diversity and reduce Treg-mediated suppression, whereas PD-1/PD-L1 blockade reactivates exhausted T cells in the tumor (62).

In HCC, the combination of nivolumab and ipilimumab has demonstrated manageable toxicity and a notably higher response rate compared with nivolumab monotherapy , indicating that dual blockage can convert a subset of immunologically cold tumors into responsive ones (6). Similarly, a combination of tremelimumab and durvalumab was evaluated in the phase III HIMALAYA trial, using a single priming dose of tremelimumab followed by ongoing durvalumab treatment. This regimen induced an immunogenic boost in patients with unresectable HCC that improved survival compared with that of patients treated with sorafenib (63).

Anti-angiogenic and vascular normalization strategies. Abnormal blood vessels in HCC contribute to immune evasion by promoting hypoxia and serving as physical barriers to immune cell trafficking. Anti-angiogenic therapies, including the anti-VEGF antibody bevacizumab and multi-kinase inhibitors, such as sorafenib, lenvatinib, cabozantinib and regorafenib, not only inhibit tumor angiogenesis but also modulate the TME (37). VEGF blockade has immunomodulatory

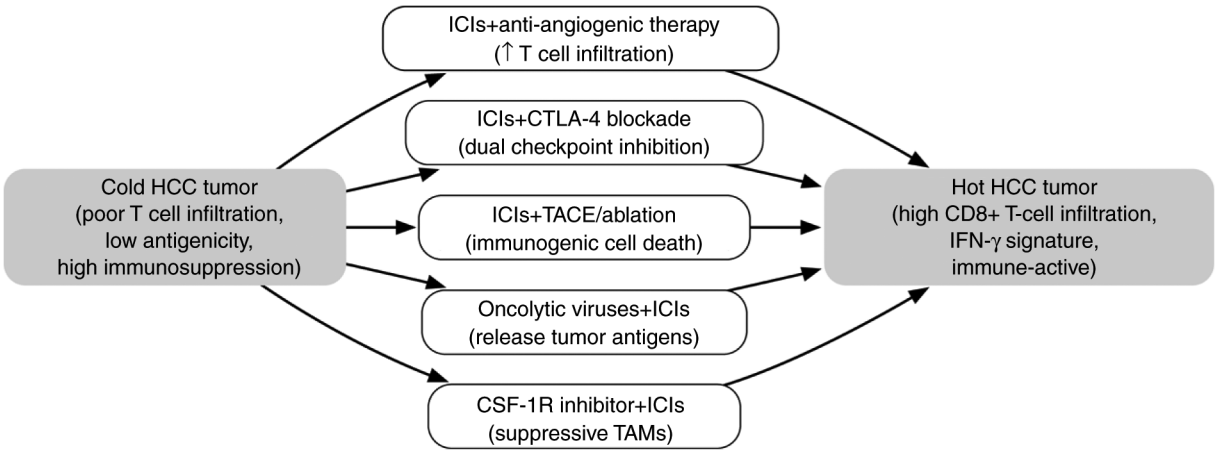

Figure 2. Flowchart illustrating key combination therapies to convert an immunologically cold HCC tumor into a hot tumor. Cold tumors exhibit low T-cell infiltration and strong immunosuppressive factors, whereas hot tumors have abundant $CD8^+$ T cells and an inflamed microenvironment. Each arrow represents a combination strategy designed to overcome a specific barrier to antitumor immunity. ICIs (e.g., PD-1/PD-L1 inhibitor) + anti-angiogenic therapy (e.g., bevacizumab): Targeting angiogenesis normalizes VEGF-driven abnormal tumor vasculature and reduces VEGF-mediated immunosuppression, thereby increasing T-cell infiltration into the tumor. ICIs (e.g., PD-1/PD-L1 inhibitor) + CTLA-4 inhibitor: Dual checkpoint blockade enhances T-cell priming and reverses T-cell exhaustion, thereby reducing Treg-mediated suppression. ICI + TACE or ablation: Locoregional treatments such as TACE or ablation induce immunogenic cell death, the release of neoantigens and antigen presentation, thereby promoting T-cell priming and infiltration. Oncolytic viruses + ICIs: Viral oncolysis triggers innate immune sensing, tumor antigen release and inflammatory signals, which are sustained and amplified by ICIs. CSF-1R inhibitor + ICIs: CSF-1R inhibitors deplete or reprogram immunosuppressive tumor-associated macrophages, relieving macrophage-induced T cell suppression. In combination with anti-PD-1/PD-L1 therapy, this increases cytotoxic T cell activity. CSF-1R, colony stimulating factor-1 receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HCC, hepatocellular carcinoma; ICIs, immune checkpoint inhibitors; IFN-γ, interferon-γ; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TACE, transarterial chemoembolization; TAMs, tumor-associated macrophages; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.

effects; it can normalize tumor vasculature, thereby enhancing lymphocyte infiltration and alleviating the VEGF-mediated suppression of DCs and T cells (37).

The combination of PD-L1 inhibitor atezolizumab with bevacizumab in the IMbrave150 phase III trial significantly improved response as well as overall survival (OS) and progression-free survival (PFS) in patients with unresectable HCC compared with those of patients treated with sorafenib (60). This exemplifies how combining immune checkpoint blockade with angiogenesis inhibition can convert a cold tumor into a more immune-active one. However, the results of the IMbrave050 trial, a follow-up study of adjuvant treatment with atezolizumab plus bevacizumab after resection or ablation in high-risk HCC showed that the benefit in recurrence-free survival was not sustained, and the effect on OS remained non-significant (64). The IMbrave150 trial succeeded because advanced HCC presents abundant neoantigens and ongoing immune activity that respond to PD-L1 and VEGF blockade, whereas the adjuvant IMbrave050 setting involved minimal residual disease and immune quiescence, limiting checkpoint and angiogenesis inhibition efficacy in preventing recurrence. It is suggested that bevacizumab enhances T-cell access to the tumor and reduces VEGF-induced immunosuppression, thereby allowing atezolizumab-activated T cells to function more effectively (65).

Locoregional therapies as immune stimuli. Traditional locoregional treatments for HCC, including TACE, radioembolization with yttrium-90 and thermal ablation, including radiofrequency or microwave ablation, can exert immunological effects that may be exploited to ignite antitumor immunity (66). These treatments cause tumor cell death and promote the release of tumor antigens and damage-associated molecular patterns into the environment, a process known as immunogenic cell death (66).

TACE induces ischemic and chemotherapeutic stress in tumor cells, potentially triggering a surge in neoantigen and proinflammatory cytokine release that attracts immune cells (67). Evidence suggests that TACE or ablation can lead to transient increases in T-cell activation and clonal expansion targeting tumor antigens (68). Radiation therapy upregulates MHC class I expression on tumor cells and increases chemokine levels for T-cell recruitment, while also inducing an abscopal effect, whereby localized radiation leads to systemic antitumor immunity (69).

Clinical trials have combined ICIs with locoregional therapies. For example, tremelimumab combined with ablation has been shown to enhance T-cell responses and induce objective tumor regression in patients with advanced HCC (67). Ongoing trials, such as EMERALD-1 and -2, are evaluating the PD-L1 inhibitor durvalumab in combination with TACE for patients with intermediate-stage HCC (70,71). Selected clinical trials and combination immunotherapy strategies for advanced HCC are summarized in Table II (6,60,67,72-77).

Targeting immunosuppressive cell populations. Another approach to convert cold tumors into more immunogenic ones is to deplete or reprogram immunosuppressive cells in the TME. Colony stimulating factor-1 receptor (CSF-1R) inhibitors, such as pexidartinib, can reduce TAM numbers or alter TAMs from an immunosuppressive M2-like phenotype toward a more proinflammatory, antitumor M1-like phenotype (78). In HCC mouse models, the inhibition of CSF-1/CSF-1R signaling,

Table II. Selected clinical trials and combination immunotherapy strategies in advanced HCC.

Therapy/trial	Regimen	Key outcomes	Implications	(Refs.)
IMbrave150, phase III	Atezolizumab (anti-PD-L1) + bevacizumab (anti-VEGF) vs. sorafenib in unresectable HCC, 1st line	Improved median OS (19.2 vs. 13.4 months), ORR (27 vs. 12%) and 1-year OS (~67 vs. 55%). Achieved first regulatory approval for an ICI combination in HCC.	VEGF inhibition + PD-L1 blockade synergistically converts HCCs to responsive tumors; new standard of care for front-line therapy. However, updated results in patients with more advanced disease showed that the initial benefit in recurrence-free survival was not sustained, and the change in OS remained non-significant	(60)
HIMALAYA, phase III	Durvalumab (anti-PD-L1) + tremelimumab (anti-CTLA-4). Single high-dose tremelimumab + durvalumab maintenance vs. sorafenib, 1st line	Improved median OS (16.4 vs. 13.8 months) with 3-year OS ~30% and ORR ~20%. Durvalumab monotherapy was non-inferior to sorafenib. FDA-approved in Oct 2022	Short CTLA-4 blockade pulse + PD-L1 inhibition can induce durable immunity; offers an alternative 1st-line regimen, particularly for patients unsuitable for bevacizumab	(72)
CheckMate-040, phase II	Nivolumab + ipilimumab in advanced HCC post-sorafenib; 3 dosing arms	Best arm (nivolumab 1 mg/kg + ipilimumab 3 mg/kg at 3 weekly intervals, 4 doses in total) had an ORR of 32%, CR rate of 8% and median OS of 22 months. Other arms with a lower ipilimumab dose had an ORR of 27%, and OS of 12 months. Accelerated FDA approval (second-line setting)	Dual checkpoint blockade yields high response rates and some cures, at cost of increased immune toxicity. CTLA-4 dose intensity appears critical for maximal efficacy	(6)
KEYNOTE-240, phase III	Pembrolizumab (anti-PD-1) vs. placebo in advanced HCC post-sorafenib, 2nd line	Did not meet P<0.0175, despite a trend of increased OS (13.9 vs. 10.6 months; P=0.023) and improved PFS. ORR was 18 vs. 4% for placebo. Generally well tolerated	Single-agent PD-1 blockade shows activity but under-powered survival benefit; indicates requirement for combination in most patients. Led to exploration of pembro-lizumab in combinations, e.g., with lenvatinib	(60)
LEAP-002, phase III	Pembrolizumab + lenvatinib vs. lenvatinib alone, 1st line	Median OS increase (21.2 vs. 19.0 months; HR, 0.84; P=0.0227), did not meet required P<0.017. ORR (26.1 vs. 17.5%) and PFS (8.2 vs. 8.1 months; HR, 0.87). No new safety signals observed	No significant improvement in OS, likely due to the efficacy of lenvatinib monotherapy. However, higher response rate with the combination suggests certain patients derived added benefit; highlights the challenge of improving upon strong TKI performance	(73)
RESCUE, phase III	Camrelizumab (anti-PD-1) + apatinib (VEGFR2 TKI) vs. sorafenib, 1st line	Median OS (22.1 vs. 15.2 months; HR, ~0.62), ORR (25 vs. 5%) and PFS (5.6 vs. 3.7 months). Manageable toxicities, including hand-foot skin reaction and hypertension. Approved in China	Reinforces class effect of PD-1 + anti-angiogenic synergy in HCC. Achieved one of the longest OS reported, albeit in a selected population	(74)

Table II. Continued.

Therapy/trial	Regimen	Key outcomes	Implications	(Refs.)
Locoregional + ICI, phase Ib/II	Tremelimumab (single dose) + computed tomography-guided tumor ablation (30% tumor volume) in advanced HCC	ORR 26% (unirradiated lesions) with disease control 89%. Post-treatment, increased $CD8^+$ T cells and PD-1^+ T cells in blood; 20% had >30% tumor necrosis in non-ablated lesions (abscopal effect).	Feasible and promising immunogenic synergy between ablation and CTLA-4 blockade, priming T cells that attack distant tumors. Supports larger trials of ICI + RFA/TACE	(67)
KEYNOTE-524, phase Ib	Pembrolizumab + lenvatinib in advanced unresectable HCC with no prior systemic treatment	ORR 36% according to modified RECIST, including 1 CR. Median PFS 9.7 months and OS 22 months. No additive toxicity beyond known profiles	Early sign that PD-1 + multi-kinase inhibitor yields a high response rate, justifying phase III (LEAP-002). PFS/OS were encouraging in the single-arm setting	(75)
Oncolytic virotherapy + ICI, phase Ib	Intratumoral T-VEC (modified HSV-1 GM-CSF virus) + pembrolizumab in advanced liver tumors, including HCC	Among patients with HCC, responses were observed in injected and non-injected lesions. Overall response rate ~23% across the cohort. Treatment well tolerated, with no grade 4 or 5 events	Provides clinical evidence that an oncolytic virus can inflame the TME and improve responses to PD-1 blockade. Injection approach is viable in liver tumors	(76)
TIL therapy, pilot	Autologous TIL infusion + IL-2 after partial hepatectomy, adjuvant setting	Feasibility demonstrated: TILs were expanded from resected tumor and infused. 1-year recurrence rate appeared lower than the historical value (~33 vs. 50%), but the sample was very small	Suggests TIL therapy is feasible for HCC and may help eliminate microscopic disease. Larger studies are necessary, and combining TILs with ICIs may enhance the persistence of infused TILs	(77)

CR, complete response; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FDA, Food and Drug Administration; GM-CSF, granulocyte-macrophage colony-stimulating factor; HCC, hepatocellular carcinoma; HSV-1, herpes simplex virus type 1; ICI, immune checkpoint inhibitor; IL-2, interleukin-2; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TIL, tumor-infiltrating lymphocyte; TKI, tyrosine kinase inhibitor; TME, tumor microenvironment; T-VEC, talimogene laherparepvec; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2

which is critical for monocyte-to-macrophage differentiation and survival, decreases TAM infiltration and promotes a shift toward a pro-inflammatory environment, thereby sensitizing tumors to anti-PD-1 therapy (79).

C-C motif chemokine receptor 2 (CCR2) antagonists also show promise by preventing the recruitment of monocytes with high expression of lymphocyte antigen 6 complex, locus C, a key surface glycoprotein marker used to distinguish functional subsets of monocytes, that are precursors for MDSCs and TAMs (80). The inhibition of CCL2-CCR2 signaling reduces the accumulation of intratumoral macrophages and leads to T-cell-dependent tumor regression in preclinical HCC models (81).

Tregs are more challenging to target selectively, but low-dose cyclophosphamide or anti-CD25 antibodies have been used in solid tumors such as ovarian, breast cancer, prostate cancer, renal cell carcinoma, and melanoma to transiently deplete Treg populations (82).

Finally, TGF-β pathway inhibitors are another class of compounds used to remove a major suppressive influence. Inhibitors such as galunisertib, a TGF-β receptor kinase inhibitor, or fresolimumab, an anti-TGF-β antibody, have been investigated in patients with HCC (83,84). Although these agents have not yet been tested in phase III trials in combination with ICIs, preclinical analysis suggests that blocking TGF-β signaling enhances the penetration of T cells into tumors and is a promising mechanism for use in combinations that aim to convert cold tumors into hot ones (85).

Oncolytic virotherapy. Oncolytic viruses (OVs) are engineered or naturally occurring viruses that selectively replicate in and lyse cancer cells while stimulating an antitumor immune response (86). This lytic process releases tumor antigens and viral pathogen signals, effectively transforming the tumor into a vaccine depot. Several OVs have been evaluated for the treatment of HCC.

Talimogene laherparepvec (T-VEC), a modified herpes simplex virus type-1 that encodes granulocyte-macrophage (GM)-CSF, has been approved for melanoma treatment and tested via intratumoral injection in liver tumors (87). Early trials combining T-VEC with ICIs have shown increased local immune cell infiltration in HCC and demonstrated the feasibility of generating a localized autitumor immune response (88).

Pexastimogene devacirepvec (Pexa-Vec; JX-594), an oncolytic vaccinia virus expressing GM-CSF, has also been evaluated in HCC. A phase II trial showed signs of improved survival in a subset of patients (89), In addition, a phase III trial (PHOCUS), evaluating Pexa-Vec combined with sorafenib, was conducted in advanced HCC (86). The PHOCUS phase III trial showed that adding Pexa-Vec to sorafenib does not improve overall survival in advanced HCC, leading to early study termination, though Pexa-Vec was well tolerated with manageable safety (90).

Cancer vaccines and adoptive cell therapies. Active immunization strategies aim to stimulate the immune system to recognize HCC-specific antigens, thereby enhancing T-cell priming against the tumor. Although past vaccine trials in HCC targeting antigens such as AFP (91), GPC3 (92) or multi-peptide mixtures (93) have shown only modest success, they all have demonstrated immunogenicity, indicating that T cells targeting these antigens can be expanded in patients. A summary of emerging immunotherapeutic approaches for HCC is provided in Table III (21,23,35,67,79,89,94-101). Certain approaches-particularly cancer vaccines, oncolytic viruses, and certain adoptive cell therapies-have shown evidence of inducing tumor-specific immune responses in clinical or preclinical studies, whereas others (metabolic, microbiome, and TAM-targeting strategies) are still at the investigational or early-trial stage and remain to be validated for immunogenic effects.

Combining cancer vaccines with ICIs or other TME modulators is a rational strategy. For example, DC vaccines pulsed with HCC tumor antigens can generate a wave of tumor-specific T cells, and concurrent PD-1 blockade can prevent their exhaustion, allowing them to penetrate and exert cytotoxic activity within tumors (102).

Adoptive cell therapy supplies effector cells directly to patients. In HCC, efforts have focused on engineering T cells to target tumor antigens. Chimeric antigen receptor (CAR) T cells specific for GPC3, an oncofetal antigen expressed in HCC cells, have been tested in early-phase trials (102). A phase I study of CAR-GPC3 T cells in advanced HCC showed some partial responses; however, it also revealed that the immunosuppressive TME limited CAR-T persistence and function (100).

A preclinical study used murine and xenograft HCC models to show that CAR-T cells engineered to secrete IL-12 upon antigen engagement could remodel the TME and enhance systemic antitumor activity (103). Researchers isolated and characterized high-affinity TCRs specific for AFP peptides presented by HLA-A*02:01. These engineered TCR-T cells recognize AFP-expressing HCC cells and exhibited potent, antigen-specific cytotoxicity *in vitro* and in mouse xenograft models. Follow-up studies using the same AFP TCR construct led to a first-in-human phase I trial (NCT03971747) in HCC patients (104). These TCR-T cells can recognize intracellular antigens presented on MHC molecules, potentially broadening targetable proteins beyond surface markers.

Epigenetic and metabolic modulators. An emerging strategy to enhance antitumor immunity involves modulating epigenetic or metabolic pathways to render tumor and immune cells more immunogenic. Epigenetic drugs, including DNA methyltransferase inhibitors such as decitabine or histone deacetylase (HDAC) inhibitors such as entinostat, can restore the expression of silenced tumor-associated antigens and increase MHC molecule presentation on cancer cells, thereby enhancing their recognition by T cells (105).

In addition to their effects on tumor cells, epigenetic modulators can also alter the differentiation of immune cells. For example, HDAC inhibition has been shown to promote a pro-inflammatory macrophage phenotype and reduce the accumulation of MDSCs, effectively boosting immune responses (106). Furthermore, in HCC models, combining HDAC inhibitors with PD-1 blockade increased intratumoral T-cell infiltration and led to tumor regression (106).

Modulating the gut-liver axis. The gut microbiome has a major influence on systemic immunity and has been shown to affect the response to ICIs in melanoma, non-small cell lung cancer and renal cell carcinoma. Therefore, researchers are interested in modulating the gut microbiota to shift the TME in HCC to a hotter immune state (95). Certain bacterial metabolites in the gut can enhance antitumor immunity. Fecal microbiota transplantation from ICI-responsive patients has been shown to improve therapeutic responses in melanoma and may hold promise for application in HCC (95).

5. Clinical and preclinical evidence

ICI combinations in advanced HCC. The first wave of phase III trials of single-agent ICIs for advanced HCC has yielded mixed results. In the CheckMate-459 trial, nivolumab showed durable responses in a subset of patients when used as a first-line treatment compared with sorafenib, but it did not significantly improve OS in the primary analysis (107). Similarly, in the second-line setting of the KEYNOTE-240 trial, pembrolizumab yielded a modest improvement in survival compared with placebo in the second-line setting, however this was not significant (108).

Atezolizumab + bevacizumab (anti-PD-L1 + anti-VEGF). The IMbrave150 trial demonstrated that combining atezolizumab with bevacizumab significantly improved OS and the objective response rate (ORR) in treatment-naïve patients with advanced HCC. The median OS was 19.2 months in the combination arm compared with 13.4 months in patients treated with sorafenib, while the ORR was ~ 27% compared with ~12%, respectively (60). Numerous responders showed substantial tumor shrinkage, and some achieved durable remission (60).

Durvalumab + tremelimumab (anti-PD-L1 + anti-CTLA-4). In the phase III HIMALAYA trial, a single high dose of tremelimumab (300 mg) combined with chronic dosing of durvalumab achieved a superior OS compared with

Table III. Emerging immunotherapeutic approaches in HCC.

Strategy	Description	Evidence	Status	(Refs.)
TAM targeting	CSF-1R inhibitors, including pexidartinib and cabiralizumab	CSF-1R blockade decreases M2 TAMs and synergized with PD-L1 inhibition, increasing T cell activity	Phase I trials ongoing with CSF-1R mAb + nivolumab; biomarkers required for TAM-high tumors	(79)
Treg targeting	CTLA-4 inhibitors; anti-CD25 antibodies	Tremelimumab reduced Tregs and increased effector T cells; improved CD8:Treg ratios are associated with tumor control	CTLA-4 inhibitors are included in approved combinations; cyclophosphamideanti-CD25 trials underway; however, autoimmunity is a risk factor	(23,67)
Neutrophil/ MDSC targeting	CXCR2 inhibitors; ARG1 inhibitors; IL-8 blockers	CXCR2 antagonist + PD-1 blockade reduced TANs, and improved the T-cell response; high neutrophils predict poor ICI outcomes	CXCR2 inhibitor + durvalumab/bevacizumab trial ongoing; IL-8 antibodies in ongoing clinical trials	(60,94)
OVs	JX-594 (vaccinia-GM-CSF); T-VEC (HSV-GM-CSF); AFP-targeting HDV	JX-594 increased immune infiltration in tumors. OVs upregulate IFN genes and enhance antigen presentation	Pexastimogene devacirepvec + sorafenib, phase III, negative; OV + ICI combinations in phase I/II; T-VEC + pembro-lizumab, ~20% response	(89,101)
CAR T/NK cells	Engineered cells targeting GPC3, AFP and EpCAM admini-stered intravenously or via the hepatic artery	GPC3-CAR T induced tumor necrosis, IFN-γ secretion; PD-1 knockout CAR-Ts overcame exhaustion	Early trials, GPC3-CAR T/ NK and AFP-TCR T cells; next-generation, IL-15 secretion and TGF-β resistance	(35,100)
Cancer vaccines	Peptide/neoantigen vaccines; DC vaccines; oncolytic vaccines	DC vaccine improved recurrence-free survival post-surgery; neoantigen vaccines induced tumor-infiltrating T cells	OVAX + nivolumab, GPC3 peptide + ICI trials ongoing; optimum effect for minimal disease or with checkpoints	(92,99)
Epigenetic modulators + ICIs	DNMT inhibitors; HDAC inhibitors + ICIs	DNMT inhibition increased MHC-I and antigen presentation; HDAC blockade reprogrammed macrophages and enhanced ICI efficacy	Durvalumab + tremelimumab + guadecitabine and nivolumab + entinostat trials ongoing to 'unmask' tumors	(98)
Metabolic modulation	A2A antagonists; IDO inhibitors; lactate modulators	A2A blockade restored T cell function in hypoxia; IDO inhibition + PD-1 blockade showed synergy in preclinical models	A2A antagonist trial (CPI-444) in phase I; metformin showing promise in NASH-HCC	(21,97)
Microbiome modulation	Probiotics; FMT; antibiotic management	*Akkermansia* and *Bifidobacterium* are associated with improved outcomes; FMT from responders improved response in resistant models	Early FMT trials promising; HCC-specific probiotic interventions in development	(95,96)

A2A, adenosine A2A receptor; AFP, α-fetoprotein; ARG1, arginase 1; CAR, chimeric antigen receptor; CSF-1R, colony stimulating factor 1 receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CXCR2, C-X-C motif chemokine receptor 2; DC, dendritic cell; DNMT, DNA methyltransferase; EpCAM, epithelial cell adhesion molecule; FMT, fecal microbiota transplantation; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPC3, glypican-3; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; HSV, herpes simplex virus; ICI, immune checkpoint inhibitor; IDO, indoleamine 2,3-dioxygenase; MDSC, myeloid-derived suppressor cell; MHC-I, major histocompatibility complex class I; NASH, non-alcoholic steatohepatitis; NK, natural killer; OV, oncolytic virus; OVAX, recombinant oncolytic vaccinia virus; PD-1, programmed cell death protein 1; ; PD-L1, programmed death-ligand 1; T-VEC, talimogene laherparepvec; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil; TCR, T cell receptor; TGF-β, transforming growth factor- β; Treg, regulatory T cell.

that of sorafenib in front-line advanced HCC (63). This Single Tremelimumab Regular Interval Durvalumab (STRIDE) regimen capitalized on an initial wave of broad T-cell activation from CTLA-4 blockade, followed by sustained PD-L1 blockade. The median OS was 16.4 months compared with 13.8 months in patients treated with sorafenib. A subset of patients treated with the STRIDE regimen achieved long-term survival >2 years (63).

Nivolumab + ipilimumab (anti-PD-1 + anti-CTLA-4). In the CheckMate-040 cohort 4 phase II study, three different regimens combining nivolumab with ipilimumab were evaluated in patients who had progressed on sorafenib. An ORR of 32% and median OS of 22 months was obtained in the arm with higher ipilimumab dosage (6). Approximately 8% of patients achieved a CR (6).

Locoregional and multimodal therapy evidence. A pilot trial combining the CTLA-4 inhibitor tremelimumab with subtotal radiofrequency ablation in patients with advanced HCC whose tumors were suitable for partial ablation reported that 26% of patients had a partial response in non-ablated lesions, and disease control was achieved in 89% of patients (67). Notably, an increase in intratumoral $CD8^+$ T cells and a reduction in viral load in hepatitis B virus (HBV)-infected patients were observed after treatment, indicating a systemic immune effect (67). These findings support the concept that local tumor destruction can synergize with checkpoint blockade.

Retrospective analyses also suggest that patients receiving radiotherapy or TACE shortly before or during nivolumab therapy have improved response rates compared with those receiving nivolumab alone (109). For example, one study found an abscopal response in ~20% of patients who received radiotherapy for a single lesion while receiving nivolumab treatment compared with <10% in those treated with nivolumab alone (110).

Adoptive cell therapy and vaccines

CAR T cells. A phase I trial of CAR T cells targeting GPC3 in patients with advanced HCC reported a partial response in 2 of 13 patients, with disease stabilization observed in seven patients (100). Although the clinical response was modest, tumor biopsies following CAR T infusion demonstrated the accumulation of CAR T cells at tumor sites, and some patients exhibited substantial tumor necrosis. These findings indicate that CAR T cells are able to traffic to and attack HCC lesions (111). However, the expansion and persistence of CAR T cells *in vivo* is limited, possibly due to immunosuppressive checkpoints and the dense TME.

TCR-engineered T cells. A first-in-human study of T cells engineered with a TCR against an AFP peptide presented by HLA-A2 in patients with AFP-positive HCC showed that the treatment was safe and resulted in transient reductions in serum AFP levels and tumor shrinkage in certain patients (112). Although no objective responses were observed according to RECIST criteria, one patient exhibited a measurable decrease in tumor burden that does not meet the threshold for a partial response (PR) , and others achieved stable disease (112).

DC vaccines. A randomized phase II trial of patients with early-stage HCC compared a tumor lysate-activated autologous DC vaccine with a control group of patients treated with best supportive care following curative resection or ablation (113). The vaccinated group experienced a significantly longer median time to recurrence than the control group (36 vs. 25 months, respectively) and a higher recurrence-free survival at 1 year (75.6 vs. 61.1%, respectively) (113).

Preclinical insights. Several mouse model studies have directly demonstrated the benefits of reprogramming the HCC microenvironment:

NASH-HCC model. A study of mice with diet-induced NASH revealed that PD-1 blockade did not protect against liver cancer and instead promoted the incidence and progression of HCC due to activated, but dysfunctional, $CD8^+$ T cells producing TNF-α. However, when those $CD8^+$ T cells were depleted or TNF-α was neutralized, checkpoint blockade efficacy improved (15).

Osteopontin (OPN)/CSF-1R axis. In a chemically-induced HCC mouse model, it was revealed that tumor-derived OPN recruits TAMs through the CSF-1 pathway. The blockade of CSF-1R not only reduced TAM infiltration but also led to increased $CD8^+$ T-cell activity and responsiveness to anti-PD-L1 therapy (79).

Fibroblast growth factor receptor 4 (FGFR4) and Tregs. A study of lenvatinib demonstrated that the inhibition of FGFR4, a protein that is overexpressed in some HCC tissues, not only affected tumor cell growth but also reduced intratumoral Treg accumulation in mouse xenografts and PD-L1 expression in tumor cells. This dual effect was associated with improved anti-PD-1 antibody efficacy and tumor rejection in mouse models (114).

CAR T cells with checkpoint knockout. In an orthotopic HCC model in mice, PD-1 gene-deleted CAR T cells targeting GPC3 outperformed conventional CAR T cells, as they were more persistent and infiltrated the tumors more deeply. In addition, these modified CAR T cells resulted in complete tumor eradication in a higher proportion of the mice (35).

Other therapeutical targets. Preclinical findings have revealed new targets, including CSF-1R (78), CXCR2 (115) and adenosine (116), and confirmed mechanisms such as Wnt-mediated exclusion, that can be therapeutically targeted (116).

6. Future directions

The field of HCC immunotherapy is rapidly evolving, and the paradigm of converting cold tumors into hot ones will continue to guide future research and clinical practice. Several key directions are anticipated.

Personalized immunotherapy and biomarker-driven treatment. As our understanding of HCC immune biology deepens, there is growing recognition that not all patients should receive the same immunotherapy regimen. Future treatment algorithms will likely incorporate biomarkers to stratify patients. For example, tumors with Wnt/β-catenin mutations or an immune-excluded phenotype may be prioritized for trials evaluating Wnt pathway inhibitors or other T-cell-recruiting strategies (39).

A robust immune score for HCC could be developed, analogous to the Immunoscore used in colorectal cancer (117). This could help to determine the required intensity of immunotherapy. Liquid biopsies, including circulating tumor DNA and immune cell profiling assays, may enable the early assessment of whether a tumor is becoming hotter or remains immunologically cold, allowing for adaptive treatment adjustments.

Next-generation checkpoint targets. While PD-1/PD-L1 and CTLA-4 are the current targets, several other inhibitory pathways are being explored in clinical trials and could be integrated into HCC treatment (5). Antibodies targeting lymphocyte-activation gene 3 (LAG-3), known as relatlimab, were recently approved by the US Food and Drug Administration for the treatment of melanoma and are of interest in HCC, as LAG-3 is upregulated in exhausted T cells in the liver (118).

T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is another checkpoint protein expressed on T and NK cells; anti-TIGIT antibodies, such as tiragolumab, combined with anti-PD-1 therapy are in trials for HCC following promising results in other cancer types (119). These emerging checkpoint inhibitors may help to revive exhausted T cells in HCC when PD-1 blockade alone is inadequate.

Triplet therapies and beyond. Building on the success of doublet regimens, such as atezolizumab + bevacizumab and durvalumab + tremelimumab, the next wave of studies is testing triplet combinations to further convert cold tumors into a hot state. For example, the COSMIC-312 trial has evaluated a triplet of PD-1 + CTLA-4 + tyrosine kinase inhibitors, namely nivolumab + ipilimumab + cabozantinib, to simultaneously target T cells, Tregs and tumor angiogenesis (120). The COSMIC-312 phase III trial reported that cabozantinib + atezolizumab significantly improved progression-free survival vs. sorafenib (6.8 vs 4.2 months; HR 0.63) but did not improve overall survival (121). However, the nivolumab + ipilimumab + cabozantinib triplet remains under investigation, with no published efficacy results yet for this regimen.

However, as toxicity and cost increase with each added agent, future research must identify which patient subsets truly require multilayered therapy. Sequential approaches, such as an induction phase followed by a maintenance phase, rather than concurrent triple therapy, may also mitigate toxicity.

Immunotherapy in earlier-stage HCC. Although most immunotherapy trials have focused on advanced HCC, there is a strong rationale for applying these strategies in earlier stages of the disease, when immune function is less impaired and the tumor burden is lower. Neoadjuvant immunotherapy prior to surgery is being investigated, and small studies administering ICIs or combinations prior to resection have shown that some patients achieve significant tumor necrosis and robust pathologic responses, which might translate to a lower recurrence risk (40).

In the adjuvant setting, after curative resection or ablation, the goal is to eradicate residual disease and prevent recurrence. The CheckMate-9DX phase III trial of adjuvant nivolumab did not meet the primary endpoint of recurrence-free survival (122), possibly due to trial design considerations or insufficient treatment duration. However, other adjuvant trials are ongoing, including the KEYNOTE-937 trial of pembrolizumab and the EMERALD-2 trial of durvalumab ± bevacizumab (123,124).

Addressing etiology-specific immune contexts. As already noted, NASH-related HCC has a suppressive immune milieu that may require tailored therapeutic strategies (15). One future approach is to treat underlying liver conditions in parallel with the cancer itself. In NASH, this could involve therapies that reduce hepatic inflammation, such as farnesoid X receptor agonists, acetyl-CoA carboxylase inhibitors or anti-IL-1β agents, administered in conjunction with immunotherapy, to prevent the non-productive activation of T cells. For HBV-associated HCC, continuing antiviral therapy is crucial, and additional strategies, such as therapeutic HBV vaccines or TCR-based approaches targeting HBV antigens, may boost antitumor immunity, given that some HCC tumor cells express HBV-derived proteins.

Novel delivery systems. Accessibility of the liver via the hepatic artery allows for innovative strategies for the delivery of immunotherapies directly to the tumor or liver, thereby maximizing local immune activation effects while minimizing systemic toxicity. Ongoing trials are currently examining the locoregional delivery of IL-12 plasmids (125), toll-like receptor 9 agonists (126), and CAR T cells through hepatic artery infusion (127). Nanotechnology-based approaches may enable nanoparticles carrying immune stimulants, such as stimulators of IFN gene agonists, or small interfering RNAs targeting immune checkpoints, to be delivered selectively to liver tumors (96). Such approaches can convert the tumor site into an immune-reactive zone without exposing the whole body to high cytokine or drug levels, thereby potentially reducing systemic side effects.

Monitoring and managing immune-related toxicity. As immunotherapy strategies are intensified to convert cold tumors into hot ones, the risk of collateral damage to normal liver tissue increases. Future protocols will require robust monitoring procedures for immune-related adverse events and potentially prophylactic measures. For example, patients with cirrhosis are at risk of decompensation if immunotherapy triggers autoimmune hepatitis (128,129). Gut microbiome manipulation is being considered as a means of improving efficacy and also mitigating toxicity, since certain microbiome profiles are associated with the risk of colitis (130,131). Researchers are also exploring selective immunosuppressants capable of controlling toxicity without completely compromising antitumor immunity (132,133).

Integration of artificial intelligence and systems biology. As data from genomic, transcriptomic, imaging and clinical sources accumulate, artificial intelligence and machine learning will likely play a role in the identification of features that define cold and hot tumors and in guiding treatment selection. For example, deep learning applied to histological images can quantify immune infiltrates and their spatial distribution, thereby potentially predicting outcomes (115). Multiomics analysis may identify new therapeutic targets, for example, by identifying that a certain chemokine is the key factor

limiting T-cell infiltration in a subset of patients, which could be targeted by drugs. Systems biology approaches may be used to model complex interactions within the HCC immune ecosystem, simulate the performance of a given combination and guide the rational design of treatment regimens.

7. Conclusions

HCC has long been considered an immunologically cold tumor, posing a formidable challenge for immunotherapy. In the present review, numerous factors, including immunosuppressive cell populations, inhibitory cytokines, tumor-intrinsic pathways and liver-induced tolerance are described that are able to blunt antitumor immunity in HCC. Crucially, it is emphasized that these barriers are not insurmountable. Through various strategies, researchers and clinicians have demonstrated that the active transformation of cold HCC tumors into hot, immune-reactive tumors is possible.

Combination therapies combining ICIs with agents such as anti-angiogenic drugs or CTLA-4 blockers have already yielded significantly improved clinical outcomes, validating the principle that immune silencing in HCC can be reversed. Emerging modalities, including locoregional therapy combined with immunotherapy, OVs, CAR T/NK-cell approaches, and metabolic or epigenetic modulators, have further expanded the therapeutic options for reprogramming the TME.

As the immune landscape of HCC is progressively understood and manipulated, it is anticipated that what was once considered a non-immunogenic cancer may become routinely manageable, and potentially even curable, by harnessing the immune system of the patient. The journey from cold to hot HCC (Fig. 1) exemplifies a paradigm shift in oncology: Rather than treating the tumor alone, the TME and the host immune response are also targeted, creating a situation in which the immune system contributes to HCC eradication.

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Competing interests

The authors declare that they have no competing interests.

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