

Tumor Microenvironment Composition and Related Therapy in Hepatocellular Carcinoma

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Abstract: Globally, primary liver cancer is the third leading cause of cancer death, and hepatocellular carcinoma (HCC) accounts for 75%–95%. The tumor microenvironment (TME), composed of the extracellular matrix, helper cells, immune cells, cytokines, chemokines, and growth factors, promotes the immune escape, invasion, and metastasis of HCC. Tumor metastasis and postoperative recurrence are the main threats to the long-term prognosis of HCC. TME-related therapies are increasingly recognized as effective treatments. Molecular-targeted therapy, immunotherapy, and their combined therapy are the main approaches. Immunotherapy, represented by immune checkpoint inhibitors (ICIs), and targeted therapy, highlighted by tyrosine kinase inhibitors (TKIs), have greatly improved the prognosis of HCC. This review focuses on the TME compositions and emerging therapeutic approaches to TME in HCC.

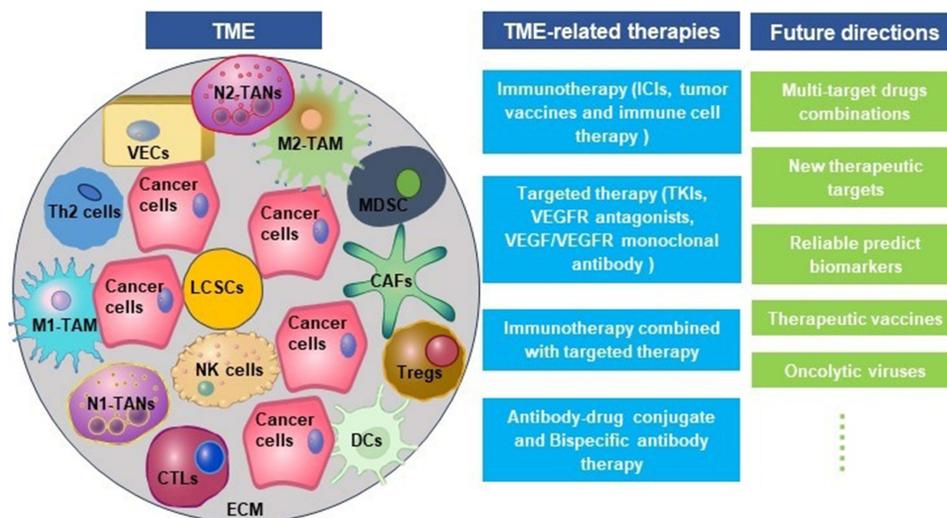
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Introduction

Primary liver cancer (PLC) was the sixth most common cancer and the third leading cause of cancer death globally in 2020, with approximately 906,000 new cases and 830,000 deaths.¹ In China, PLC is the second leading cause of cancer death, and hepatocellular carcinoma (HCC) accounted for 93.0% of PLC, intrahepatic cholangiocarcinoma (ICC) accounted for 4.3%, and combined hepatocellular-cholangiocarcinoma (CHC) accounted for 1.6%; 84.4% of HCC is seropositive for hepatitis B surface antigen.² Unlike other malignant tumors, HCC is highly malignant and has a higher mortality rate in individuals aged 40–65 than in people older than 65 years in China.³ Therapeutic interventions, including surgical measures (either resection or liver transplantation) paired with non-surgical strategies like transcatheter arterial chemoembolization, radiofrequency ablation, chemotherapy, radiotherapy, biologic treatments, and traditional Chinese medicine, remain the cornerstone of HCC management. Unfortunately, most patients do not get survival benefits, and patients with survival benefits also have the problem of rapid recurrence and metastasis.^{4,5}

The tumor microenvironment (TME) plays a crucial role in the development of HCC, including cell proliferation, migration, invasion, epithelial–mesenchymal transition (EMT), immune escape, neovascularization, and treatment resistance.⁶ Characteristics of the TME include hypoxia,⁶ abnormal vascular proliferation,⁷ acidification,⁸ inflammation,⁹ and immunosuppression.¹⁰ Hepatitis B and/or C virus infection, alcoholism, metabolic dysfunction-associated steatotic liver disease (MASLD), obesity, and metabolic syndrome are the major reasons for HCC. Chronic HBV infection activates and maintains chronic non-resolving inflammation. HBV cccDNA forms double-stranded DNA (dsDNA) in the cytoplasm or forms cyclic extrachromosomal DNA (ecDNA) with human genomic DNA fragments. In the process of inflammation-cancer transition,

Graphical Abstract



dsDNA promotes the activation of non-classical nuclear factor-kappa B (NF- κ B) and produces cytokines such as transforming growth factor beta-1 (TGF- β 1), plasminogen activator inhibitor-1 (PAI1), and helper T cell 2 (Th2), which recruit inhibitory immune cells such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and tumor-associated neutrophils (TANs). These inhibitory immune cells inhibit the antiviral and anti-tumor immune activities of cytotoxic CD8⁺ T cells (CTLs), natural killer cells (NK cells), and dendritic cells (DCs) by secreting interleukin-10 (IL-10), TGF- β 1 and producing reactive oxygen species (ROS), forming an immunosuppressive TME that promotes the evolution and development of HCC.¹¹ The complex TME provides more options for the treatment of HCC.

Targeted therapy and immunotherapy toward TME have provided a promising avenue for advanced or metastatic HCC. Targeted therapy mainly includes multi-target tyrosine kinase inhibitors (like Lenvatinib, Regorafenib, and Sorafenib), vascular endothelial growth factor receptor (VEGFR) antagonists (such as Apatinib and Axitinib), and VEGF/VEGFR monoclonal antibodies (such as Bevacizumab and Ramelimumab).¹² Various immune checkpoint inhibitors have been developed, with anti-programmed cell death protein 1 (PD-1) and anti-programmed cell death ligand 1 (PD-L1) agents showing significant potential as adjuvant treatments for early-stage HCC, leading to notable extensions in survival.^{13,14} These treatments not only achieve more durable and robust efficacy across genders but also confer significant benefits in terms of quality of life for patients.¹⁵ The complex TME promotes the evolution and development of HCC and also provides potential molecular targets for targeted therapy and immunotherapy in HCC. In this review, we focus on the compositions of TME, the interaction between TME and HCC cells, and various therapeutic approaches to TME, with a view to providing a reference for efficient treatments of HCC.

The Composition of TME in HCC

As a dynamic system, TME is closely related to the occurrence, development, and metastasis of HCC. TME mainly comprises extracellular matrix (ECM), helper cells, immune cells, cytokines, chemokines, and growth factors.¹⁶ Helper cells in TME mainly include cancer-associated fibroblasts (CAFs), hepatic stellate cells (HSCs), and vascular endothelial cells. The immune cells are TAMs, TANs, Tregs, inhibitory B cells, MDSCs, DCs, CTLs, and NK cells. CTLs, NK cells, DCs, and helper T cell 1 (Th1) cytokines participate in anti-tumor as well as anti-viral immunity and inhibit tumor metastasis; TAN, Treg, helper T cell 17 (Th17), M2-TAMs, CAFs, and Th2 cytokines facilitate immune escape and HCC metastasis.⁶ Cancer cells and immune cells express co-inhibitory receptors, including cytotoxic T cell-associated antigen 4 (CTLA4), programmed death 1 (PD-1), T cell immunoglobulin domain and mucin domain-3 (TIM-3), and lymphocyte activation gene 3 (LAG3), which maintain the stability and immunosuppressive characteristic of TME in HCC.¹⁷

Pro-Tumor Cells in TME

TAMs

TAMs play a “double-edged sword” role in the occurrence and development of HCC. M1-TAMs can kill tumor cells, while M2-TAMs promote tumor development. The polarization of TAM is deeply associated with TME, and the polarization of macrophages to M1 and M2 is reversible and adjustable. With the progression of HCC, M1-TAMs gradually polarize to M2-TAMs, and the increase in the number of M2-TAMs also indicates a poor prognosis.¹⁸ M1-TAMs are stimulated by interferon gamma (IFN- γ), granulocyte-macrophage colony stimulating factor (GM-CSF), or lipopolysaccharide (LPS). Cytokines secreted by Th2 cells and tumor cells, including interleukin (IL)-4, IL-10, IL-13, colony stimulating factor 1 (CSF1), chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-X-C motif) ligand 12 (CXCL12), and connective tissue growth factor (CTGF), promote the polarization of macrophages to M2.¹⁹ The upregulation of Wnt2b expression in macrophages promotes the polarization of TAMs from M1 to M2 by activating the Wnt2b/ β -catenin/c-Myc signaling pathway.²⁰

As shown in Figure 1, the tumor-promoting effects of M2-TAMs mainly include: secreting cytokines (such as IL-6, CXCL8, and IL-10), blocking the inducible nitric oxide synthase (iNOS) pathway, reducing the synthesis of nitric oxide (NO);²¹ inhibiting the activation of CTLs and NK cells and reducing their killing effects on HCC cells by secreting IL-10

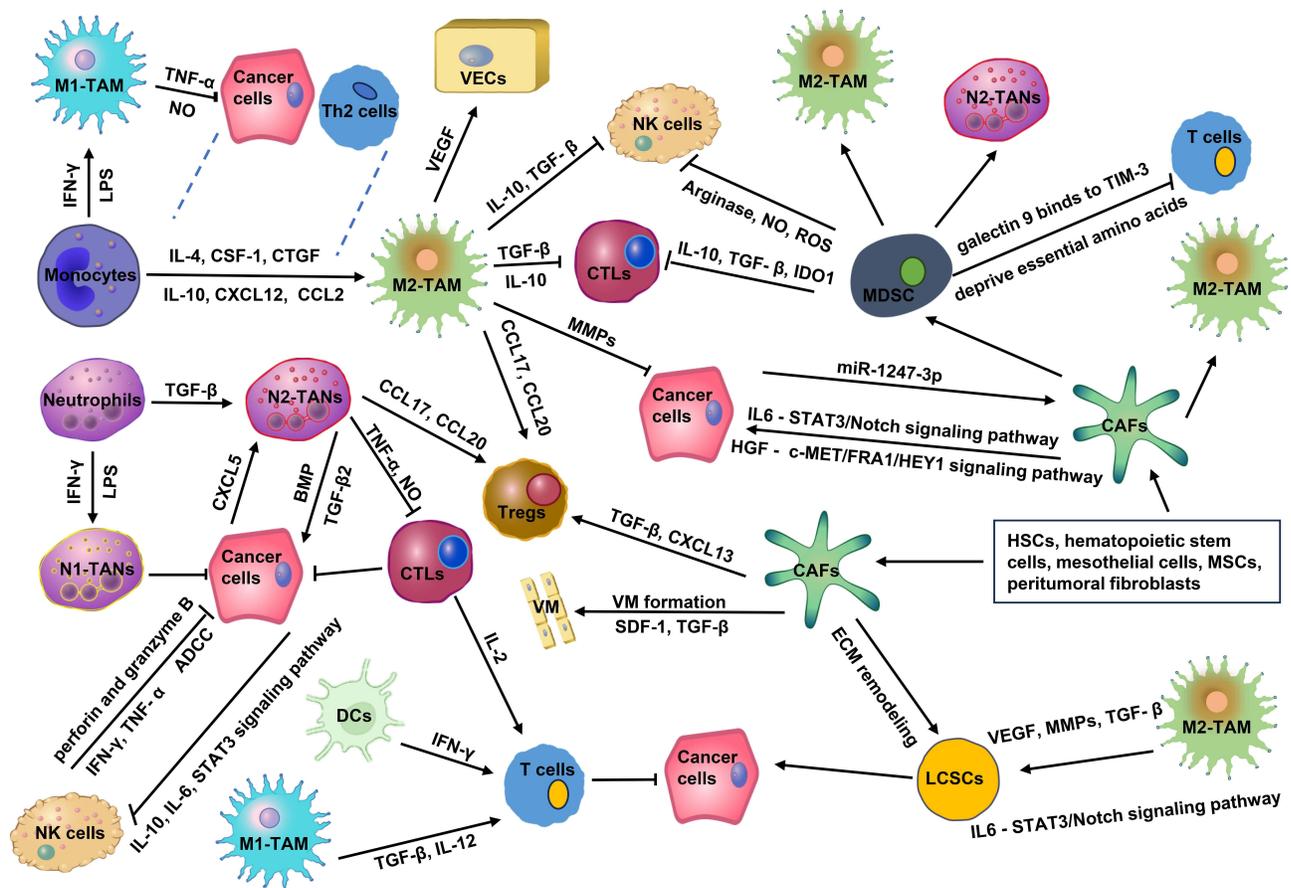


Figure 1 The complex network of interactions between cellular components in TME. In TME, pro-tumor cells promote angiogenesis, ECM remodeling, and immune escape via secreting various cytokines and chemokines, such as VEGF, MMPs, CCL17, CXCL20, and so on. Meanwhile, pro-tumor cells also inhibit the function of anti-tumor cells through the release of several cytokines, such as IL-10, NO, TGF- β , ROS, and so on. The imbalance between pro-tumor strength and anti-tumor strength forms an immunosuppressive TME, which significantly promotes the malignant progression as well as the recurrence, metastasis, and drug resistance of HCC.

Abbreviations: IL-4, interleukin-4; IL-10, interleukin-10; CSF-1, colony-stimulating factor 1; CXCL12, chemokine (C-X-C motif) ligand 12; CTGF, connective tissue growth factor; CCL2, chemokine (C-C motif) ligand 2; CXCL8, chemokine (C-X-C motif) ligand 8; IL-6, interleukin-6; TGF- β , transforming growth factor- β ; VEGF, vascular endothelial growth factor; CCL17, chemokine (C-C motif) ligand 17; CCL20, chemokine (C-C motif) ligand 20; MMPs, matrix metalloproteinases; IFN- γ , interferon- γ ; LPS, lipopolysaccharide; TNF- α , tumor necrosis factor- α ; NO, nitric oxide; BMP2, bone morphogenetic protein 2; IDO1, indoleamine 2,3-dioxygenase 1; ROS, reactive oxygen species; TIM-3, T cell immunoglobulin domain and mucin domain-3; HSCs, hepatic stellate cells; MSCs, mesenchymal stromal cells; SDF-1, stromal cell-derived factor-1; VM, vasculogenic mimicry; HGF, hepatocyte growth factor; STAT3, signal transducer and activator of transcription 3; c-MET/FRA1/HEY1, cellular-mesenchymal epithelial transition factor/FOS-related antigen 1/hes related family bHLH transcription factor with YRPW motif 1.

and TGF- β ,^{22,23} promoting HCC cells neovascularization by secreting vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), IL-17, matrix metalloproteinase 2 (MMP2), and MMP9; promoting HCC cells invasion and metastasis by secreting of MMPs, cathepsins, and serine proteases;²⁴ triggering the recruitment of Tregs to exert immunosuppressive effects by secreting CCL17, CCL18, CCL20 and CCL22; promoting immune evasion by secreting prostaglandin E2, MMP7 and other mediators.²⁵ Hypoxia-inducible factor-1 α (HIF-1 α) produced by HCC promotes the release of IL-1 β from TAMs, and IL-1 β induces epithelial–mesenchymal transition (EMT) in HCC.²⁶ Chemokine (C-C motif) ligand 15 (CCL15) is significantly enriched in the core of cancerous areas, which promotes an immunosuppressive microenvironment by recruiting and polarizing M2-TAMs.²⁷ Low expression of cluster of differentiation 86⁺ (CD86⁺) TAMs and high expression of CD206⁺ TAMs were significantly associated with an aggressive tumor phenotype, poor overall survival (OS), and a shorter time to recurrence.²⁸ Carbonic anhydrase XII (CA12) mediates TAMs survival in the acidic TME and induces TAMs to secrete C-C motif chemokine ligand 8 (CCL8), which promotes EMT and metastasis in HCC.²⁹

TANs

TANs can be divided into subtype N1 (anti-tumor) and subtype N2 (pro-tumor).³⁰ High levels of TANs infiltration and neutrophil/lymphocyte ratio (NLR) are correlated with the poor prognosis of HCC.³¹ LPS and IFN- γ promote N1 polarization, while TGF- β drives the acquisition of the N2 phenotype.³² N2-TANs predominantly produce chemokines such as CCL20 and CCL17, which recruit Tregs and TAMs to shape the immunosuppressive TME and promote HCC progression as well as sorafenib resistance.³³ TANs secrete TNF- α and NO, kill CD8⁺ T cells, and inhibit their anti-tumor effect on HCC.³¹ TANs secrete high levels of bone morphogenetic protein 2 (BMP2) and TGF- β 2 and induce the abnormal expression of microRNA -301b-3p in HCC cells, which promotes HCC cells to acquire “stem” characteristics. These cancer stem-like cells excessively produce chemokine (C-X-C motif) ligand 5 (CXCL5) and sustain elevated activity of the NF- κ b signaling pathway. This, in turn, recruits even more TANs into the tumor, creating a vicious cycle that significantly exacerbates the malignancy of HCC (Figure 1).³⁴

MDSCs

MDSCs represent a diverse group of immature myeloid cells known for their pronounced immunosuppressive capabilities.³⁵ MDSCs mainly inhibit the antitumor function of CTLs and NK cells through arginase, iNOS, indoleamine 2,3-dioxygenase 1 (IDO1), reactive oxygen species (ROS), TGF- β , and IL-10.³⁶ MDSCs can induce the differentiation and expansion of Tregs, deprive T cells of essential amino acids, promote oxidative stress, and promote the polarization of M2-TAMs and N2-TANs.^{37,38} MDSCs express galectin 9 and bind to TIM-3 on T cells to trigger T cell apoptosis.³⁹ A specific subtype of MDSCs, characterized as CD11b⁺ CD33⁺ HLA-DR⁻, effectively hampers the proliferation of CD8⁺ T cells in HCC (Figure 1).⁴⁰ Overexpression of ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2/CD39L1) mediated by HIF-1 converts extracellular ATP to 5'-AMP, which interdicts the differentiation of MDSCs.⁴¹ Upregulation of apolipoprotein B mRNA editing enzyme catalytic subunit 3B (APOBEC3B) in HCC inhibits global H3K27me3 abundance through interaction with polycomb repressor complex 2 (PRC2) and reduces H3K27me3 occupancy at the chemokine CCL2 promoter, thereby recruiting a large number of TAMs and MDSCs.⁴² G9a-mediated downregulation of solute carrier family 7 member 2 (SLC7A2) promotes CXCL1 secretion through the phosphatidylinositol 3-kinases (PI3K)/protein kinase B (PKB)/NF- κ B pathway. Upregulation of SLC7A2 recruits MDSCs, which fosters a pro-tumor TME.⁴³ Prior to the metastatic colonization of HCC cells into target tissues, cancer cells secrete several soluble factors, including CXCL17, G-CSF, osteopontin, CXCL12, TNF- α , TGF- β , and VEGF-A. These factors play key roles in the development of the immune system by influencing CD11b⁺/Gr-1⁺MDSC-mediated neo-angiogenesis, inducing inflammatory responses, remodeling the ECM, and recruiting immune cells, creating an immunosuppressed but nutrient-rich TME.⁴⁴

CAFs

In HCC, CAFs mainly originate from HSCs and hematopoietic stem cells. Mesothelial cells, mesenchymal stromal cells (MSCs), and peritumoral fibroblasts can also be transformed into CAFs.⁴⁵ In terms of their effects on cancer, CAFs can be broadly categorized into cancer-promoting CAFs (pCAFs) and cancer-restraining CAFs (rCAFs).⁴⁶ The pCAFs enhance the “stemness” of CD24⁺ HCC by secreting hepatocyte growth factor (HGF) acting on the cellular-mesenchymal epithelial transition factor/

FOS-related antigen 1/hes related family bHLH transcription factor with YRPW motif 1 (c-MET/FRA1/HEY1) signaling pathway^{47,48} or by secreting HGF and IL-6 acting on the phosphorylated signal transducer and activator of transcription 3 (STAT3)/Notch signaling pathway.⁴⁹ HCC cells activate CAFs by secreting the exosome miR-1247-3p, which upregulates inflammatory factors such as IL-1, IL-6, and IL-8 and contributes to the resistance to sorafenib.⁵⁰ Hypoxia also activates CAFs, induces angiogenesis, and promotes HCC cell proliferation by producing proangiogenic factors such as VEGF, MMP2, and MMP9.⁵¹ A peculiar phenomenon known as vasculogenic mimicry (VM) is observed when aggressive tumor cells mold themselves to establish vascular-like channels through ECM remodeling. This unique framework acts as an alternative blood supply route for malignant tumors. Freshly isolated CAFs from HCC specimens have shown the capability to maintain tumor blood supply. They achieve this by inducing VM formation in HCC cells, driven by the paracrine factors TGF- β and stromal cell-derived factor-1 (SDF-1).^{52,53} HCC cells and CAFs induce M2 polarization of TAMs. This process involves upregulating the mRNA expression of markers CD163 and CD206 in macrophages while concurrently diminishing IL-6 expression and secretion.⁵⁴ CAFs promote the proliferation of Tregs by secreting TGF- β and CXCL13.⁵⁵ CD36⁺ CAFs recruit CD33⁺ MDSCs by expressing macrophage migration inhibitory factor (MIF) via the lipid peroxidation/p38/CEBPs axis (Figure 1).⁵⁶

Tregs

As a subset of CD4⁺ T cells, Treg is one of the pivotal factors in maintaining immune tolerance. Tumor-infiltrating Tregs promote tumor immune escape through both contact-dependent and contact-independent mechanisms. The proportion and absolute number of CD4⁺CD25⁺ T cells increased significantly in the vicinity of HCCs.⁵⁷ Huh7 culture supernatant seems to promote the proliferation of CD4⁺CD25⁺ T cells.⁵⁸ In the hypoxic TME, Tregs are significantly enriched and interact with type 2 conventional dendritic cells (cDC2), leading to the loss of antigen-presenting HLA-DR on cDC2.⁵⁹ In naive T cells, CTLA-4 is localized in the intracellular space and expressed on the cell surface after receiving stimulation signals. Contrastingly, CTLA-4 is constitutively expressed in Tregs, which is associated with immunosuppressive functions.⁶⁰ Long noncoding epidermal growth factor receptor (lnc-EGFR) specifically binds EGFR and promotes Tregs differentiation, which connects immunosuppressive states with HCC.⁶¹

Liver Cancer Stem Cells

Liver cancer stem cells (LCSCs), a class of cells capable of self-renewal and differentiation to form tumors, initiate HCC and are at the root of HCC recurrence.⁶² Intriguingly, the ECM modulates the expression of malignant markers in LCSCs, notably CD44 and CD133. ECM also plays a role in intercellular communication and acts as a catalyst for HCC.⁶³ Multiple cytokines in TME (such as HIF-1 α , BMP, OSM, MMP2, and MMP9) interact synergistically with ECM remodeling, EMT, and tumor neovascularization networks to activate Notch, TGF- β , STAT3, and Hedgehog signaling pathways, enhance LCSCs stemness and chemoresistance, and positively impact HCC progression.^{64–66} Endothelial cells secrete HIF-1 α and VEGF, increase LOXL2 expression, induce EMT and VM formation, and synergize with ECM remodeling to promote LCSC maintenance and self-renewal.^{67,68} TAMs can activate the IL-6/STAT3 signaling pathway through the release of VEGF, MMP, the secretory proteins S100A9, TGF- β , and TNF- α , which, together with tumor neovascularization, alter LCSCs phenotype and function.⁶⁹ TANs secrete BMP2 and TGF β to induce LCSCs production, and LCSCs increase, in turn recruiting more TANs, creating positive feedback in HCC.⁷⁰ Furthermore, CAFs indulge in ECM remodeling, primarily through collagen synthesis. They also release a cocktail of cytokines, including CCL2, CCL5, CXCL1, and IL-6. These factors activate Notch, TGF- β , Hedgehog, and STAT3 signaling pathways, significantly amplifying the stemness characteristics of LCSCs.⁷¹

Anti-Tumor Cells in TME

NK Cells

It is reported that 30–50% of the lymphocytes in the liver are NK cells, and the proportion of NK cells in the liver is five times higher than that in the spleen or peripheral blood.⁷² NK cells can directly release cytotoxic substances such as perforin and granzyme B or secrete cytokines such as TNF- α and IFN- λ to kill HCC cells. Another strategy employed by NK cells is antibody-dependent cell-mediated cytotoxicity (ADCC), which can also result in the destruction of HCC cells.⁷³ However, HCC cells can circumvent the recognition and killing of NK cells in TME. Hypoxia-inducible gene 2

(HIG-2) promotes IL-10 release from HCC cells, activates the STAT3 signaling pathway of NKs, and inhibits the killing activity of NKs.⁷⁴ HCC cells also evade the killing activity of NK cells by reducing the expression of tumor-associated antigens and increasing the expression of major histocompatibility complex class I (MHC-I)-related molecules, prompting the inactivation of NK cells.⁷⁵

DCs

DCs can capture and phagocytose HCC cells and transfer them to lymph nodes while presenting tumor-associated antigens (TAAs) to T cells. T cells migrate and infiltrate into cancerous tissues, recognize T-cell receptors (TCRs) on HCC cells, and then bind to them to kill them.⁷⁶ TNF- β and IL-12 induced by M1-TAM, IFN- γ produced by NKs, and IL-2 secreted by CTLs further activate T cells. Lysosome-associated membrane glycoprotein 3⁺ (LAMP3⁺) DCs are higher expressed in tumor tissues than paracancerous liver tissues with highly phagocytic activity. LAMP3⁺ DCs stimulate the immune response of CD8⁺T, CD4⁺ T cells, and NK cells in tumor-draining lymph nodes by receptor and ligand binding.⁷⁷

Other Factors in TME

ECM

The ECM is a complex network comprising collagen, non-collagen fibers, elastin, proteoglycans, and aminoglycans. As HCC progresses, the ECM undergoes dynamic remodeling. While it serves as a physical shield, obstructing direct interaction between immune cells and tumor cells, the ECM also modulates the functions of immune cells.⁷⁸ Research has identified six ECM-associated genes (namely *SPPI*, *ADAMTS5*, *MMP1*, *BSG*, *LAMA2*, and *CDH1*) that correlate with a dismal prognosis in HCC patients.⁷⁹ ECM is gradually remodeled by cancer cells in TME, which facilitates tumor development and metastasis.⁸⁰ MMPs are a class of zinc-dependent endoproteases that disrupt the ECM by destroying the structure of different proteins within it. Overexpression of different MMPs can lead to severe ECM catabolism as well as a significant elevation in EMT.⁸⁰ CAFs have a central role in shaping the ECM. They deposit major ECM proteins like collagen, fibronectin, and laminin. Moreover, they release ECM-degrading enzymes, including MMPs, which not only bolster CAF migration but also expedite ECM degradation, setting the stage for tumor cell infiltration.⁸¹ HSCs are activated when the liver is damaged or subjected to extracellular stimuli. Activated HSCs participate in ECM remodeling by synthesizing ECM molecules and secreting MMPs.⁸²

Growth Factors

HGF plays key roles in endothelial cell generation, tissue and organ regeneration, and cellular malignancy. Growth factor receptor-binding protein 2 (Grb-2), Grb2-associated binding protein 1 (Gab1), and EGFR promote HCC occurrence and progression by activating the C-stromal-epithelial transformation receptor (HGF/c-Met) axis.⁸³ Insulin-like growth factor (IGF)/IGF-1 receptor (IGF-1R) signaling modulate stem cell differentiation and pluripotency during embryonic development. Dysregulated IGF/IGF1R signaling enhances tumor “stemness” and promotes drug resistance as well as tumor recurrence in hepatitis B virus (HBV)-related HCC (HBV-HCC).⁸⁴ TGF- β maintains homeostasis in the normal liver, inhibits early-stage HCC, and promotes the progression of advanced HCC. TGF- β modulates the activity of various TME-associated cells (such as HSCs, CAFs, endothelial cells, and NK cells) and promotes the progression of HCC as well as the immune evasion of malignant cells.⁸⁵ The functions and the targets of various growth factors are listed in [Table 1](#).

Additional Mediators in TME

Beyond the primary cellular constituents and factors of the TME, there exist several other potential mediators that significantly influence the environment. It was reported that upregulated expression of the 4-gene inflammatory signature (including lymphocyte-activating gene 3 (LAG-3), signal transducer and activator of transcription 1 (STAT1), CD8, and PD-L1) was associated with immunotherapy responses in HCC. However, only the percentage of LAG-3⁺ CD8⁺ cells was notably linked with the immune-checkpoint blockade (ICB) response. This indicates that the efficacy of ICB therapy in HCC can potentially be predicted by analyzing pre-treatment levels of LAG-3 and CD8 in the TME.⁹⁰ Neuropilins (NRPs) are the receptors of multiple proteins involved in pivotal signaling pathways related to HCC progression. NRP1 and NRP2 were reported as potential therapeutic targets and biomarkers for HCC.⁹¹ Defined as the percentage of tumor

Table 1 The Functions and the Targets of Various Growth Factors in TME of HCC

Growth Factors/ Cytokines	Drugs	Targets	Functions
HGF	Rilotumumab; Ficlatuzumab; Onartuzumab	HGF/c-Met	Endothelial cell generation, tissue and organ regeneration, and cellular malignancy ⁸³
IGF	Ganitumab	IGF/IGF1R	Modulates stem cell differentiation and pluripotency; enhances tumor "stemness" and promotes drug resistance; promotes HCC recurrence ⁸⁴
TGF- β	Pirfenidone; Fluorofenidone	TGF- β /SMAD	Maintains homeostasis in normal liver, inhibits early-stage HCC, and promotes the progression of advanced HCC; promotes immune escape of HCC cells ⁸⁵
VEGF VEGFR	Bevacizumab Sorafenib; Lenvatinib; Regorafenib; Cabozantinib	VEGF/VEGFR	Promotes neovascularization, increases vascular permeability, and promotes immunosuppression in TME ⁸⁶
IL-6	Aspirin	IL-6/STAT3	Promotes the occurrence, development, invasion and metastasis of HCC ⁸⁷
HIF-1 α	Histone deacetylase 6 specific inhibitors		Promotes angiogenesis and tumor invasion and metastasis; maintains tumor cell metabolism ⁸⁸
MMP	FR (EtOH)	MMP/ TIMP	Promotes ECM degradation; promotes HCC growth, invasion and metastasis ⁸⁹

cells within the TME, tumor purity was associated with the occurrence and development of HCC. Genes like *AarF Domain Containing Kinase 3 (ADCK3)*, *Hexokinase-3 (HK3)*, and *palmitoyl-protein thioesterase 1 (PPT1)* were associated with tumor purity in HCC. Elevated expression of *ADCK3* and reduced expression of *HK3* and *PPT1* resulted in high levels of tumor purity and were related with a better prognosis.⁹² It was found that mucosal-associated invariant T (MAIT) cells secrete TNF to activate TNFR2 on regulatory T cells, which forms an immunosuppression TME in HCC.⁹³ Furthermore, as a naturally occurring flavonoid compound, Oroxylin A has been demonstrated to hinder the progression of HCC by reshaping the immune landscape of the TME. Oroxylin A achieves this by inducing M1-like polarization of macrophages through the release of apoptosis-related extracellular vesicles. Additionally, it reduces the M2-like macrophage population while bolstering T cell infiltration within the TME.⁹⁴

Molecular Bases of Immunotherapy and Targeted Therapy

As compared with adjacent tissue, HCC tissues express higher levels of immunosuppressive molecules such as PD-L1, CTLA4, LAG3, and TIM3.⁹⁵ The expression of these molecules is negatively correlated with the infiltration of IFN- γ ⁺ T lymphocytes in TME. The blocking antibody of these inhibitory molecules can enhance the proliferation of CD4⁺ and CD8⁺ tumor-infiltrating T lymphocytes and the production of cytokines.⁹⁶ In the microenvironment of chronic inflammation, the immune checkpoints CTLA4 and PD1 are upregulated. PD1 binds to PD-L1 to prevent TCR signaling, block T cell proliferation, and induce T cell exhaustion. Tregs constitutively express CTLA4 and block immune responses. CTLA4 binds CD80/CD86 and competes with CD28 to block T cell activation.^{97,98} Another molecule of significance is TIM3, which displays markedly increased expression in the peripheral blood monocytes and TAMs of individuals with HCC. The cytokine TGF- β plays a role in this elevation, as it encourages TIM3 expression and concurrently drives the alternative activation of macrophages.²²

Over-expression of tumor-associated antigens (TAAs) and new tumor antigens formed in the process of somatic mutation in HCC cells stimulate specific T lymphocyte immune responses, which is the theoretical basis of tumor vaccine. TAAs mainly include tumor-overexpressed antigens (TERT, Wilms tumor antigen 1 (WT1)), carcinoembryonic antigens (AFP, glypican-3 (GPC3)), and cancer-testicular antigens (melanoma-associated antigens A (MAGE-A), synovial sarcoma X family member 2 (SSX-2), and New York esophageal squamous cell carcinoma 1 (NY-ESO-1)).⁹⁹ Furthermore, there is an intriguing cell group referred to as cytokine-induced killer cells. These cells belong to the peripheral blood mononuclear cells and exhibit dual-surface markers: the T lymphocyte marker CD3 and the NK cell marker CD56. These cells, when subjected to activation via IFN, anti-CD3 antibody, and IL-2 in a controlled

environment, gain a robust ability to target and kill a wide array of tumor cells. Impressively, they carry out this function with minimal side effects, making them an attractive option for potential therapeutic strategies.¹⁰⁰

Targeting angiogenic factors and their related signal pathways has become a hot topic in the research on targeted therapy for HCC. Common HCC targets for targeted therapy include cellular-mesenchymal epithelial transition factor (c-Met), CD 24, CD 147, GPC3, fibroblast growth factor receptor (FGFR), VEGFR, EGFR, and mammalian target of rapamycin (mTOR).¹⁰¹ Signaling pathways involved in cell differentiation (Wnt), proliferation (EGF, IGF, HGF/C-MET, RAF/MEK/ERK), survival (Akt/m-TOR), and angiogenesis (VEGF, PDGF, FGF) contribute to HCC growth and metastasis and also provide potential molecular targets for targeted therapies in HCC.¹⁰²

Immunotherapy and Targeted Therapy

Immunotherapy

Immunotherapy mainly includes immune checkpoint inhibitors (ICIs), tumor vaccines, and immune cell therapy. Nivolumab is the first ICI approved by the US Food and Drug Administration (FDA) for the treatment of HCC. An open-label, non-comparative, dose escalation and expansion trial (NCT01658878) was carried out to evaluate the efficacy of Nivolumab as second-line therapy for HCC, and it indicates that the objective response rate (ORR) was 20% and the disease control rate (DCR) was 64%.¹⁰³ Recent trial (NCT02576509) indicates Nivolumab may be considered a therapeutic option for patients who are contraindicated with tyrosine kinase inhibitors (TKIs) and antiangiogenic agents.¹⁰⁴ Tislelizumab is the first ICI for HCV-HCC, and its partial response (PR) rate was 17.6%, DCR was 76.4%, and progression-free survival (PFS) was 6.48 months (95% CI: 3.95–9.14).¹⁰⁵ Camrelizumab is a PD-1 inhibitor made in China. It has been confirmed that Camrelizumab has a remarkable anti-tumor effect and tolerance to advanced solid tumors.^{106,107} In patients with previously sorafenib-treated advanced HCC, Avelumab demonstrated moderate efficacy and was well tolerated (NCT03389126).¹⁰⁸ Tislelizumab also showed durable objective responses and acceptable tolerability in previously treated advanced HCC patients (NCT03419897).¹⁰⁹ Studies on the application of other ICIs (such as Toripalimab, Sintilamab, Durvalumab, and Atezolizumab) in advanced HCC are under way (Table 2).

Tumor vaccine strategies encompass various approaches, including peptides, proteins, dendritic cells (DCs), and viral vector vaccines.¹¹⁰ A meta-analysis of 35 cohort studies indicates that the ORR of DCs vaccine (19%, 95% CI 11 to 29%) is significantly higher compared to the peptide vaccine (1%, 95% CI 0 to 5%).¹¹¹ Additionally, HBV-related HCC may benefit more from tumor vaccines compared with hepatitis C virus-related HCC.¹¹¹ The personalized neoantigen vaccines have gained approval as a safe and effective approach for preventing HCC recurrence.¹¹² Immune cell therapy for HCC includes enhancing the activity of endogenous anti-HCC immune cells,¹¹³ inducing active anti-HCC immune cells in vitro,¹¹⁴ and genetically modifying HCC-specific immune cells (such as CAR-T cell therapy,¹¹⁵ and TCR-T cell therapy).¹¹⁶ The single-chain Fv recognizing TAAs and the immunoreceptor tyrosine-based activation motif are genetically reconstituted in vitro and then transfected into patient T cells, resulting in chimeric antigen receptor T (CAR-T) cells. In advanced HCCs expressing glypican-3 (GPC3), the intratumor injection of IL-7 and CCL19-secreting CAR-T cells led to complete tumor eradication after 30 days.¹¹⁷ The tumor-specific antigen receptor is introduced into T cells so that the patient's T cells express the antigen-specific T cell receptor (TCR), that is, TCR-modified T cells (TCR-T). A phase I clinical trial using CAR-T cells targeting CD133 (CART-133) indicates that the survival time of CD133-positive and advanced metastatic HCC patients treated with CART-133 is significantly prolonged.¹¹⁸ A kind of HCV1406 TCR-T cell is constructed to treat HCV-HCC.¹¹⁹ CAR-T and TCR-T cells have great potential for the treatment of HCC, and related clinical trials are also under way (Table 3).

Targeted Therapy

Molecular targeted drugs, such as sorafenib and Lenvatinib, have improved the survival of patients with advanced HCC who are ineligible for liver transplantation or resection.^{122,123} Sorafenib is an oral multi-target tyrosine kinase inhibitor (TKI) that inhibits tumor growth and angiogenesis, which extends overall survival (OS) by 2.8 months.¹²⁴ A multicenter non-inferiority trial shows that the mOS of the Lenvatinib group and the Sorafenib group is 13.8 months and 12.3

Table 2 Immune Checkpoint Inhibitors in HCC

Drugs	Targets	Status	Phase	Design Types	Participants	ClinicalTrials.gov	Results
Nivolumab	PD-1	Not recruiting	I/II	Open-label, non-comparative, dose escalation and expansion trial	262	NCT01658878	No Results Posted
Pembrolizumab	PD-1	Not recruiting	III	Multicenter, randomized, double-blinded, two-arm study	950	NCT03867084	No Results Posted
Durvalumab	PD-L1	Not recruiting	III	Randomized, double-blind, placebo-controlled, multicenter study	888	NCT03847428	No Results Posted
Tremelimumab	CTLA-4	Completed	II	Non-controlled, open-label, multicenter clinical trial	20	NCT01008358	Completed ¹⁰⁵
Avelumab	PD-L1	Completed	II	Open label, single group assignment, clinical trial	30	NCT03389126	Completed ¹⁰⁸
Toripalimab	PD-1	Not recruiting	II/III	Randomized, double-blind, placebo-controlled study	530	NCT03859128	No Results Posted
Tislelizumab	PD-1	Completed	II	Open-label, multicenter study	249	NCT03419897	Completed ¹⁰⁹
Nivolumab plus Ipilimumab	PD-1 + CTLA-4	Recruiting	II	Open label, single group assignment, clinical trial	40	NCT03510871	No Results Posted
Cobolimab (TSR-022) + Dostarlimab	TIM-3 + PD1	Recruiting	II	Open label, single group assignment, clinical trial	42	NCT03680508	No Results Posted
Nivolumab + Relatlimab	PD-1 + LAG3	Recruiting	I	Randomized, parallel assignment, open label, clinical trial	20	NCT04658147	No Results Posted

Table 3 CAR-T and TCR-T Cell Therapy

Cell Types	Targets	Phase	Participants	ClinicalTrials.gov	Start Date	Status
CAR-T	GPC3	I	20	NCT04121273	October 5, 2019	Recruiting
CAR-T	B7H3 (CD276)	I/II	15	NCT05323201	February 10, 2022	Recruiting
CAR-T	GPC3	I	38	NCT05003895	December 8, 2021	Recruiting
CAR-T	c-Met/PD-L1	I	50	NCT03672305	October 1, 2018	Not yet recruiting
CAR-T	CD147	I	34	NCT03993743	May 27, 2019	Recruiting
CAR-T	NKG2D	I	10	NCT04550663	September 25, 2020	Not yet recruiting
CAR-T	Mucin I	I/II	20	NCT02587689	October 2015	Recruiting
TCR-T	NY-ESO-1	II	11	NCT01967823	October 24, 2013	Completed ¹²⁰
TCR-T	AFP	I	9	NCT03971747	August 6, 2019	Completed ¹²¹
TCR-T	HBV	I	10	NCT04745403	May 20, 2022	Recruiting
TCR-T (SCG101)	HBV	I/II	46	NCT05417932	July 19, 2022	Recruiting
CAR-T/TCR-T	DR5	I/II	50	NCT03941626	September 1, 2019	Recruiting

months, respectively.¹²⁵ Regorafenib is a second-line treatment for HCC with Sorafenib resistance, and it inhibits tumor angiogenesis, cell proliferation, and metastasis by altering TME and targeting multi-targets.¹²⁶ Cabozantinib was approved by the FDA in January 2019 for the treatment of HCC. A double-blind clinical trial indicates that the mOS of the Cabozantinib group is 10.2 months and the median PFS is 5.2 months.¹²⁷ Ramucirumab is an IgG1 monoclonal antibody that binds to the extracellular region of vascular endothelial growth factor receptor 2 (VEGFR2), thus blocking the binding of VEGF to VEGFR and further preventing angiogenesis. The mOS of patients in the Ramucirumab group is 4.9 months longer than that in the placebo group, especially when AFP \geq 400ng/mL, the mOS of patients in Ramucirumab group is prolonged by 8.6 months.¹²⁸ Currently, Ramucirumab is recommended as a second-line treatment for advanced HCC patients with AFP levels \geq 400ng/mL. Apatinib, another TKI, inhibits angiogenesis by targeting VEGFR2 and is recommended for second-line treatment in advanced HCC.¹²⁹ Recently, small-molecule targeted drugs with more specific targets have been the focus of current clinical studies. MET-selective inhibitors (Tepotinib and Capmatinib) produce potent inhibition of advanced HCC with high MET while reducing off-target toxicity.¹³⁰ The selective FGFR4 inhibitor Fisogatinib (also known as BLU-554)¹³¹ and the TGF- β receptor 1 inhibitor Galunisertib have also been found to be efficacious in advanced HCC.¹³² In addition, targeted combination therapies provide additional treatment options for patients with advanced HCC who cannot tolerate ICB therapy.

Antibody-Drug Conjugate (ADC) and Bispecific Antibody (BsAb) Therapy for HCC

ADCs contain monoclonal antibodies for targeted delivery and cytotoxic payloads for targeted destruction of malignant cells, allowing for selective delivery of cytotoxic drugs to tumor cells in the most appropriate manner.¹³³ The transmembrane tight junction protein Claudin 6 (CLDN6) has been identified as a therapeutic target. An anti-CLDN6 monoclonal antibody conjugated with the cytotoxic agent (Mertansine) DM1 (CLDN6-DM1) has demonstrated potent antitumor effects, both as a standalone treatment and in combination with sorafenib.¹³⁴ SHR-A1403 is a novel c-Met ADC consisting of an anti-c-Met monoclonal antibody conjugated with a novel cytotoxic microtubule inhibitor which showed significant anti-tumor activity in cancer cell lines, xenograft mouse models, and HCC PDX models.¹³⁵ A humanized anti-c-Met antibody conjugated with oxaliplatin significantly improves cytotoxicity against c-Met-positive tumors.¹³⁶

Bispecific antibodies (BsAb) combine the binding specificity of two different monoclonal antibodies, one activating receptors on killer effector cells and the other binding TAAs to initiate tumor cytotoxicity.¹³⁷ In xenograft HCC models, GPC3/CD47 BsAb was superior to monotherapy or the combination of anti-CD47 and anti-GPC3 monoclonal antibodies.¹³⁸ Tetravalent BsAb h8B-BsAb against GPC3 and CD3 antigens significantly induces tumor regression in HCC xenograft mouse models.¹³⁹ In HBV-related HCC, anti-HBx/anti-CD3 BsAb was able to retarget effector cells in vitro and in vivo to lyse HBxAg-positive HCC cells.¹⁴⁰

Table 4 Immunotherapy Combined with Targeted Therapy for HCC

Drugs	Phase	Participants	ClinicalTrials.gov	Start Date	Status	Reference
Camrelizumab + Apatinib	II	20	NCT04297202	December 1, 2019	Recruiting	[143]
Cabozantinib + Atezolizumab	III	740	NCT03755791	June 10, 2018	Active, not recruiting	[144]
Regorafenib + Pembrolizumab	I	58	NCT03347292	June 18, 2018	Completed	
Regorafenib + Avelumab	I/II	482	NCT03475953	May 4, 2018	Recruiting	[145]
Cabozantinib + Nivolumab	I	15	NCT03299946	May 14, 2018	Completed	[146]
Cabozantinib + Durvalumab	I/II	117	NCT03539822	October 22, 2018	Active, not recruiting	
Ramucirumab + Durvalumab	I	85	NCT02572687	February 19, 2016	Completed	
Bevacizumab + Erlotinib	II	45	NCT01180959	April 14, 2011	Completed	[147]
Sorafenib + Nivolumab	II	24	NCT03439891	April 16, 2018	Active, not recruiting	
Tivozanib + Durvalumab	I/II	42	NCT03970616	September 30, 2019	Terminated	[148]
Tislelizumab + Lenvatinib	II	30	NCT04834986	April 30, 2021	Not yet recruiting	
Camrelizumab + Apatinib	II	40	NCT04826406	February 3, 2021	Recruiting	
Atezolizumab + Bevacizumab	III	668	NCT04102098	December 31, 2019	Active, not recruiting	[149]
Durvalumab + Tremelimumab	III	1324	NCT03298451	October 11, 2017	Active, not recruiting	[150]
Donafenib + Sintilimab	II	30	NCT05162352	December 4, 2021	Recruiting	
Camrelizumab + Apatinib	III	674	NCT04639180	April 1, 2021	Not recruiting	

Immunotherapy Combined with Targeted Therapy for HCC

Anti-angiogenesis and ICIs are pivotal components of anti-HCC therapy. A large number of studies have confirmed that the combination of the two regimens is better than the single regimen, which can significantly increase the clinical benefits of HCC. Atezolizumab combined with Bevacizumab (“T+A” combination) is the first combination regimen that goes beyond the efficacy of sorafenib and has been approved by the FDA for first-line treatment of advanced HCC.¹⁴¹ Lenvatinib combined with pembrolizumab (“cola” combination) significantly prolongs the mOS of patients with unresectable HCC.¹⁴² In patients with resectable HCC, perioperative camrelizumab plus apatinib results in promising therapeutic efficacy.¹⁴³ Table 4 lists several clinical trials related to combination therapy, which are expected to bring more treatment options to HCC.

Future Directions

Cell resistance to targeted drugs is a major challenge for targeted therapy for HCC. Multi-target drug combinations can reduce the dose of a single drug while maintaining or enhancing antitumor activity and reducing the occurrence of adverse drug reactions and drug-resistant mutations.¹⁵¹ Furthermore, the identification of new therapeutic targets is of paramount importance, as there is currently a shortage of targeted drugs for dominant mutational drivers in HCC, such as *TERT* promoter mutations,¹⁵² *CTNNB1* mutations,¹⁵³ and *TP53* mutations.¹⁵⁴ The long-term effectiveness and durability of responses to immunotherapies remain areas of uncertainty. Identifying biomarkers and molecular signatures that reliably predict responses to specific immunotherapeutic agents is imperative for personalized treatment approaches. Tumor mutation load,¹⁵⁵ circulating tumor cells,¹⁵⁶ and circulating tumor DNA¹⁵⁷ are both promising candidate biomarkers. In addition, the development of advanced immunomodulatory agents, such as CAR-T, TCR-T, therapeutic vaccines, and oncolytic viruses, holds promise for achieving deeper and more durable responses in a subset of HCC patients.

Conclusion

HCC is characterized by its aggressive and advanced-stage presentation upon diagnosis, posing significant challenges to curative interventions. TME is a complex immune system that plays an important role in promoting tumor cell proliferation, metastasis, and immune evasion. Notably, targeted therapy and immunotherapy following traditional chemotherapy, radiotherapy, and surgical treatment have achieved remarkable efficacy in advanced HCC by reprogramming the immunosuppressive TME. However, the application of these therapies is limited by the inter-individual and

even intra-tumoral heterogeneity of HCC, as well as the absence of reliable biomarkers for predicting therapeutic responses, drug resistance, and immune tolerance. In the future, new therapeutic targets, reliable predictive biomarkers, and more rational and efficient combination therapy regimens will greatly improve the survival of HCC patients, especially those with advanced HCC. A better understanding of the interactions between TME and HCC cells is essential for developing novel effective therapeutic approaches for HCC.

Abbreviations

APOBEC3B, apolipoprotein B mRNA editing enzyme catalytic subunit 3B; ADC, Antibody-drug conjugate; BsAb, Bispecific antibody; CAFs, cancer-associated fibroblasts; CCL2, chemokine (C-C motif) ligand 2; CTL, cytotoxic CD8+T cells; CTLA4, cytotoxic T cell associated antigen 4; CXCL12, chemokine (C-X-C motif) ligand 12; CXCL5, chemokine (C-X-C motif) ligand 5; DCs, dendritic cells; ECM, extracellular matrix; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSCs, hepatic stellate cells; ICIs, immune checkpoint inhibitors; IFN- γ , interferon gamma; IL-6, interleukin-6; LAG3, lymphocyte activation gene 3; LPS, lipopolysaccharide; MDSCs, myeloid-derived suppressor cells; MHR, major hydrophilic region; NAFLD, non-alcoholic fatty liver disease; NF κ B, nuclear factor κ B; NK cells, natural killer cells; PD-1, programmed death 1; PLC, primary liver cancer; TAMs, tumor associated macrophages; TANs, tumor associated neutrophils; Th1, helper T cell 1; TIM-3, T cell immunoglobulin domain and mucin domain-3; TKIs, tyrosine kinase inhibitors; TME, tumor microenvironment; Tregs, regulatory T cells; iNOS, inducible nitric oxide synthase; NO, nitric oxide; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; MMP, matrix metallo-peptidase; HIF-1 α , Hypoxia inducible factor 1 α ; EMT, epithelial–mesenchymal transition; OS, overall survival; NLR, neutrophil/lymphocyte ratio; TGF- β , transforming growth factor-beta; BMP2, bone morphogenetic protein 2; IDO1, indoleamine 2,3-dioxygenase 1; ROS, reactive oxygen species; PRC2, poly-comb repressor complex 2; SLC7A2, solute carrier family 7 member 2; TAAs, tumor-associated antigens; GPC3, glypican-3; NY-ESO-1, New York esophageal squamous cell carcinoma 1; EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin; JAK/STAT, janus kinase/signal transducer and activator of transcription; RFA, radiofrequency ablation; ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; CAR-T, chimeric antigen receptor T; TCR-T, T cell receptor-modified T cells; dsDNA, double stranded DNA; ecDNA, extrachromosomal DNA.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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