




# Hepatocellular carcinoma tumor immune microenvironment: heterogeneity and therapeutic strategies

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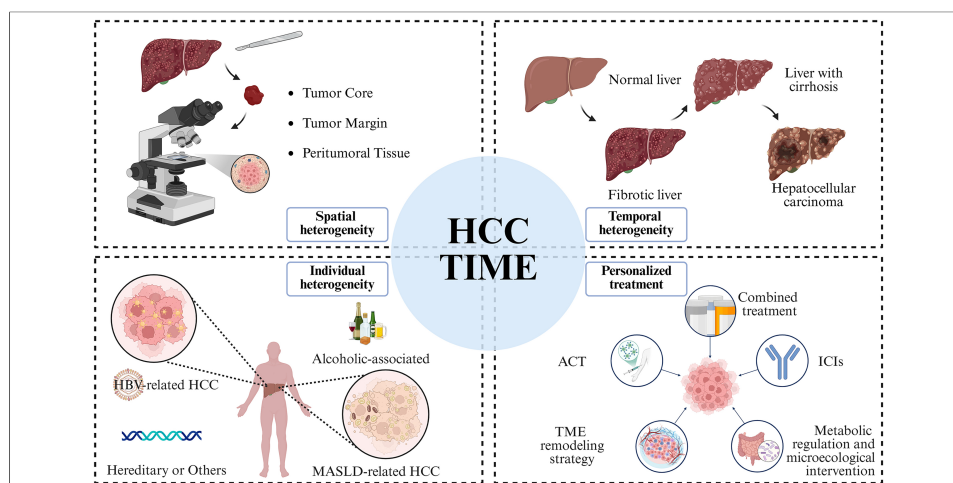
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## Abstract

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, with immunotherapy emerging as a pivotal strategy for advanced disease. However, treatment responses remain highly variable, largely due to the complex and dynamic tumor immune microenvironment (TIME). This review systematically examines the composition, heterogeneity, and function of the TIME of HCC, focusing on the interplay between immunosuppressive cells - such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) - and functional immune cells, including CTLs, NK cells, and DCs. We highlight the spatial and temporal heterogeneity of the TIME, shaped by underlying HCC etiologies, which critically influences immune evasion and therapeutic outcomes. We also evaluate current immunotherapeutic approaches, particularly immune checkpoint inhibitors (ICIs), adoptive cell therapies, and

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strategies targeting metabolic and microbial remodeling of the TIME. Finally, we discuss emerging combination therapies and future directions aimed at overcoming immunosuppressive barriers to enhance personalized treatment and improve clinical outcomes in HCC. A deeper understanding of TIME biology is essential for developing more effective immunotherapeutic strategies.

## INTRODUCTION

Hepatocellular carcinoma (HCC) remains a leading cause of global cancer mortality, largely due to its frequent late diagnosis and high recurrence rate following curative treatments<sup>[1,2]</sup>. In recent years, immunotherapy has emerged as a pivotal advancement in managing advanced HCC, with immune checkpoint inhibitors (ICIs) demonstrating notable clinical promise, particularly in combination regimens<sup>[3-6]</sup>.

However, the efficacy of immunotherapy in HCC is highly variable, and only a subset of patients achieves a durable response. A major determinant of this heterogeneous treatment outcome is the complex and dynamic tumor immune microenvironment (TIME)<sup>[7]</sup>. The TIME of HCC is characterized by an intricate network of immune cells, stromal components, cytokines, and signaling molecules that collectively promote tumor progression, immune evasion, and therapeutic resistance<sup>[7]</sup>. Its significant spatial and temporal heterogeneity - influenced by etiology, disease stage, and host factors - plays a critical role in shaping immunosuppressive landscapes and limiting the success of immunotherapies<sup>[8,9]</sup>.

To address these challenges, we herein propose an Integrated Three-Dimensional Heterogeneity Model that reframes the HCC TIME through the interplay of Etiology, Spatial Topology, and Temporal Evolution. Specifically, distinct pathogenic drivers dictate the foundational “immunological soil” and exhaustion trajectories; the physical stromal barriers and metabolic compartmentalization between the tumor core and invasive margin construct complex spatial networks of immune exclusion; and the TIME functions as a highly plastic system undergoing continuous adaptive remodeling during natural progression and clinical interventions.

By synthesizing these dimensions into a comprehensive conceptual framework, this review systematically elucidates how therapeutic strategies can be anchored to specific microenvironmental profiles - namely, through etiology-specific stratification, combination therapies designed to dismantle spatial barriers, and the strategic identification of “therapeutic windows” in immune remodeling. Ultimately, this multidimensional roadmap aims to provide a theoretical blueprint for reversing immune tolerance and achieving next-generation precision immunotherapy in HCC.

## OVERALL COMPOSITION OF THE MICROENVIRONMENT OF HCC

From a literal standpoint, the tumor microenvironment (TME) broadly defined refers to the milieu surrounding a tumor. It comprises all non-malignant host cells in the vicinity, such as endothelial cells, diverse immune cells, cancer-associated fibroblasts (CAFs), and the signaling molecules - including growth factors, cytokines, chemokines, and extracellular vesicles - secreted by these cells. Furthermore, it encompasses the extracellular matrix (ECM) as well as the vascular and lymphatic networks<sup>[10]</sup>. Tumor formation, growth, invasion, and metastasis all rely on the TME. Conversely, the TME itself responds to extracellular signals released by the tumor, resulting in a dynamic, bidirectional interaction between the two<sup>[11]</sup>.

The liver is a unique immune organ characterized by a distinct dual blood supply, which predisposes it to heightened susceptibility to viral/bacterial infections, tumors, and sterile tissue injury. Upon infection, the liver's inherent immune tolerance is disrupted, triggering inflammatory responses. A rapid and potent innate immune response is first activated to clear pathogens. However, in cases of chronic infection such as hepatitis B Virus (HBV), the interplay between host adaptive immunity and viral immune-evasion mechanisms can lead to persistent, lifelong disease<sup>[12,13]</sup>.

Hepatic immunity involves both resident and recruited immune cells. The liver harbors the body's largest populations of natural killer (NK) cells and Kupffer cells (KCs), the latter being the largest group of tissue-resident macrophages. It is also enriched with natural killer T cells (NKT cells), which collectively form a robust innate immune compartment. Nevertheless, many immune responses in the liver depend on the recruitment of circulating immune cells, particularly neutrophils and monocytes<sup>[14-16]</sup>.

Under physiological conditions, the liver maintains a state of immune tolerance, exhibiting low immune reactivity. This is partly due to continuous low-level exposure to gut-derived pathogen-associated molecular patterns (e.g., lipopolysaccharides), which dampen KC-mediated lymphocyte activation and help prevent excessive immune-mediated tissue damage. In pathological states - such as chronic viral hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease - this normal immune tolerance becomes fragile and easily breached<sup>[17-19]</sup>.

Research indicates that persistent chronic stimulation, leading to irreversible processes like liver fibrosis or cirrhosis, can promote HCC development even after the primary stimulus (e.g., HCV) is eliminated. In such settings, HCC often arises in the context of pre-existing fibrosis or cirrhosis, suggesting that virus clearance may not fully reverse long-term alterations in the hepatic microenvironment, leaving the tissue prone to subsequent oncogenic events<sup>[17-19]</sup>. These changes persist through the formation of a precancerous microenvironment, which disrupts the balance between immune activation and suppression. A shift in the ratio of pro-inflammatory to anti-inflammatory cytokines is a hallmark of this stage, fostering a sustained pro-inflammatory milieu.

As the precancerous microenvironment evolves, malignant transformation occurs, and the liver's immune-suppressive environment begins to protect nascent tumor cells. Immune evasion, tissue remodeling, and chronic inflammation persist, driving the transition from a precancerous to a tumor-supportive microenvironment<sup>[20]</sup>. Concurrently, immune cell recruitment leads to the infiltration of immunosuppressive populations - such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs) - into TME. These cells further promote an immunosuppressive niche that facilitates cancer progression.

## **THE ROLE OF IMMUNOSUPPRESSIVE CELL POPULATIONS IN HCC**

In HCC, beyond the intrinsic malignant properties of tumor cells, TIME actively enables immune evasion, allowing tumors to escape host immune surveillance and destruction. Key to this process are specific immunosuppressive cell populations within the TIME, most notably TAMs, MDSCs, and Tregs. These cells promote tumor progression, metastasis, and therapy resistance by secreting immunosuppressive factors, reshaping the immune landscape, and directly inhibiting effector immune cells such as T cells and NK cells.

### TAMs/M2-like macrophages

TAMs predominantly exhibit an M2-like phenotype, primarily arising from the phenotypic transformation of resident KCs during tumor progression. Unlike pro-inflammatory, anti-tumor M1 macrophages, M2-polarized TAMs secrete anti-inflammatory factors such as Interleukin-10 (IL-10), transforming growth factor- $\beta$  (TGF- $\beta$ ), and arginase-1 (Arg1), which suppress immune cell activity and foster an immunosuppressive microenvironment conducive to tumor growth<sup>[21]</sup>. Furthermore, M2 TAMs promote tumor progression by secreting angiogenic factors like vascular endothelial growth factor (VEGF) and chemokines such as CCL2, thereby stimulating vasculature formation and enhancing the metastatic potential of cancer cells<sup>[21,22]</sup>.

In summary, TAMs are extensively infiltrated within HCC, and their polarization state is closely linked to tumor aggressiveness, immune evasion, and patient prognosis. Through the secretion of immunosuppressive factors, metabolic reprogramming, promotion of angiogenesis, and facilitation of metastasis, M2-like TAMs serve as a central driver of immune tolerance and malignant progression in HCC, making them a pivotal target for immunotherapy<sup>[23-26]</sup>.

### Source of TAMs and regulation of M2 polarization

TAMs in HCC originate from diverse sources, primarily including bone marrow-derived monocytes, splenic hematopoietic stem cell populations, and the local activation and phenotypic conversion of resident liver macrophages (KCs)<sup>[26-28]</sup>. These precursor cells are recruited to the tumor tissue by chemotactic signals within the TIME and undergo M2-like polarization under the influence of various regulatory factors.

This polarization is driven by both cytokine and metabolic cues. Cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and macrophage colony-stimulating factor (M-CSF) in the TME are key inducers of the M2 phenotype<sup>[25,29,30]</sup>. Concurrently, tumor-derived metabolic products (e.g., lactate and lipid intermediates) activate signaling pathways involving peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) and hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), which reinforce M2 polarization<sup>[31-34]</sup>. For instance, lactate produced by tumor cells can mediate histone lactylation in TAMs, upregulating genes like *NUPR1* to enhance their immunosuppressive function<sup>[35]</sup>.

### TAMs mediated immune suppression mechanism: factor secretion and signal metabolism regulatory network

Among the immunosuppressive factors secreted by TAMs, IL-10, TGF- $\beta$ , and the chemokine CCL2 are key molecules that play a central role, forming an “inhibition-enhancement-feedback loop” in the immunosuppressive chain<sup>[36,37]</sup>.

IL-10 directly inhibits the proliferation and cytotoxic function of effector T cells [including CD8<sup>+</sup> Cytotoxic T Lymphocytes (CTLs)] and reduces the antigen-presenting efficiency of dendritic cells (DCs), ultimately inducing local immune tolerance in tumors<sup>[36]</sup>. TGF- $\beta$  suppresses the activation and function of CD4<sup>+</sup> helper T cells and CD8<sup>+</sup> effector T cells, and its upregulation of SOX18 promotes Treg accumulation while enhancing tumor cell invasiveness via EMT induction<sup>[38,39]</sup>. CCL2 recruits peripheral monocytes to replenish the TAM pool and attracts other immunosuppressive cells, forming a positive feedback loop of immune suppression<sup>[40,41]</sup>. Additionally, M2-like TAMs express programmed death ligand-1 (PD-L1), which binds to programmed death-1 (PD-1) on CD8<sup>+</sup> T cells to directly induce T cell exhaustion<sup>[42,43]</sup>.

The secretion of IL-10, TGF- $\beta$ , and M2 polarization is primarily regulated by classical signaling pathways such as nuclear factor kappa B (NF- $\kappa$ B), signal transducer and activator of transcription 3 (STAT3), and Phosphoinositide 3-Kinase/Protein Kinase B (PI3K/AKT)<sup>[44-46]</sup>. Recent studies confirm that metabolic

reprogramming is a key mechanism sustaining TAM immunosuppressive function, with targeting relevant pathways enhancing immunotherapy effectiveness<sup>[33]</sup>.

## MDSCs

MDSCs are a heterogeneous population of immature bone marrow-derived cells with immunosuppressive functions. They originate from myeloid progenitor cells that fail to differentiate normally under pathological conditions such as cancer, expanding as abnormal neutrophils and monocytes<sup>[47]</sup>. Initially lacking immunosuppressive activity<sup>[48]</sup>, these precursor cells are converted into functionally immunosuppressive MDSCs within TME under the influence of inflammatory and immunosuppressive cytokines including sTNF, TGF- $\beta$ , IL-10, and IL-1 $\beta$ <sup>[49]</sup>.

### *Types of MDSCs*

MDSCs are primarily divided into two categories: polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs/G-MDSCs) and monocytic myeloid-derived suppressor cells (M-MDSCs), distinguished by their granulocyte or monocyte lineage origin. PMN-MDSCs are phenotypically and morphologically similar to neutrophils, while M-MDSCs resemble monocytes. In mice, they are defined as PMN-MDSCs (CD11b<sup>+</sup>GR1<sup>+</sup>Ly6G<sup>+</sup>Ly6C<sup>lo</sup>) and M-MDSCs (CD11b<sup>+</sup>GR1<sup>+</sup>Ly6G<sup>-</sup>Ly6C<sup>hi</sup>)<sup>[50,51]</sup>. In humans, PMN-MDSCs are HLA-DR<sup>+</sup>CD11b<sup>+</sup>CD14<sup>-</sup>CD15<sup>+</sup>CD33<sup>Mid</sup>, and M-MDSCs are HLA-DR<sup>+</sup>CD11b<sup>+</sup>CD14<sup>+</sup>CD15<sup>-</sup>CD33<sup>high</sup>, with Lin<sup>-</sup>HLA-DR<sup>-/lo</sup>CD33<sup>+</sup> cells identified as immature eMDSCs<sup>[52]</sup>. These subpopulations represent different differentiation states within the same lineage.

### *The immunosuppressive function of MDSCs*

MDSCs exert immune evasion through multiple mechanisms, including inhibiting T cell function, inducing Tregs, and suppressing immune responses via ligand-receptor interactions. M-MDSCs accumulate in cirrhotic patients and exhibit stronger T cell suppressive activity<sup>[53]</sup>. The most common mechanism involves releasing Arg1 and NO to inhibit T cell proliferation, while secreted TGF- $\beta$  suppresses T cell and NK cell functions. MDSCs recruit and enhance Treg activity through the C-X-C motif chemokine ligand 16 (CXCL16)-C-X-C motif chemokine receptor 6 (CXCR6) axis interaction, with Tregs secreting TGF- $\beta$  to induce M-MDSC proliferation and immunosuppressive activity<sup>[54-56]</sup>. MDSCs also upregulate PD-L1 under hypoxic conditions via HIF-1 $\alpha$ <sup>[57]</sup>, directly binding to PD-1 on T cells to inhibit activation and promote tumor metastasis and drug resistance.

### *MDSCs in TIME of HCC*

The occurrence of HCC is accompanied by chronic inflammation and liver fibrosis, with MDSC accumulation further promoting an immunosuppressive hepatic microenvironment. E-twenty-six-specific sequence variant 5 (ETV5) promotes S100A9 secretion in liver cancer cells, recruiting and activating MDSCs that secrete inhibitory cytokines and highly express PD-L1 to inhibit cytotoxic CD8<sup>+</sup> T cells<sup>[58]</sup>. SLC7A2 deficiency may upregulate CXCL1 and recruit MDSCs through the PI3K/Akt/NF- $\kappa$ B pathway<sup>[59]</sup>. In HBV-related HCC, the frequency of CD14<sup>+</sup>HLA-DR<sup>-/low</sup> MDSCs is elevated, inducing Tregs and suppressing CD8<sup>+</sup> T cells via high arginase activity and the extracellular signal-regulated kinase (ERK)/interleukin-6 (IL-6)/STAT3 pathway<sup>[60]</sup>. During metabolic dysfunction-associated steatohepatitis (MASH) progression, urokinase-type plasminogen activator receptor (uPAR) expression shifts from hepatic stellate cells (HSCs) in early stages to MDSCs in advanced disease, marking inhibitory TIME establishment<sup>[61]</sup>.

## Tregs

Tregs are a subset of T cells characterized by Foxp3, CD25, and CD4 expression, playing a crucial role in immune suppression. They maintain immune tolerance, prevent autoimmune diseases, and regulate the tumor immune environment by suppressing unnecessary immune responses.

### *Types of Tregs*

Thymus-derived Tregs (tTregs) develop in the thymus and acquire self-tolerance through selection, expressing the Foxp3 transcription factor<sup>[62]</sup>. Peripheral Tregs (pTregs) are induced from peripheral CD4<sup>+</sup> T cells in specific microenvironments such as chronic inflammation, infection, or tumor settings<sup>[63]</sup>. Both subsets suppress effector T cell activity to maintain immune system balance.

### *The immunosuppressive function of Tregs*

Tregs exert immune suppression through secretion of immunosuppressive cytokines, cell contact-dependent mechanisms, and metabolic pathway regulation. TGF- $\beta$ 1 is the predominant immune-related subtype, directly inhibiting effector T cell proliferation and function, and activating tumor cell-derived TGF- $\beta$ 1 via GARP and  $\alpha$ v $\beta$ 8 integrins in TME to inhibit CD8<sup>+</sup> T and NK cells and promote M2 macrophage polarization<sup>[64]</sup>. IL-10 inhibits antigen presentation by macrophages and DCs, amplifies its own secretion via autocrine signaling, and induces myeloid cells to upregulate PD-L1<sup>[65]</sup>. IL-35 works with IL-10<sup>+</sup> Tregs via the STAT3/STAT1/STAT4-BLIMP1 axis to induce CD8<sup>+</sup> tumor-infiltrating lymphocytes (TIL) exhaustion<sup>[66]</sup>. Tregs express Cytotoxic T Lymphocyte-Associated Protein-4 (CTLA-4), which mediates CD80/CD86 consumption, releasing free PD-L1 to bind PD-1 on effector T cells, exerting dual immunosuppressive effects<sup>[67]</sup>. Lymphocyte activation gene 3 (LAG-3), selectively expressed in inducible Tregs, is crucial for their suppressive activity independent of the Foxp3 pathway<sup>[68]</sup>.

### *Tregs in TIME of HCC*

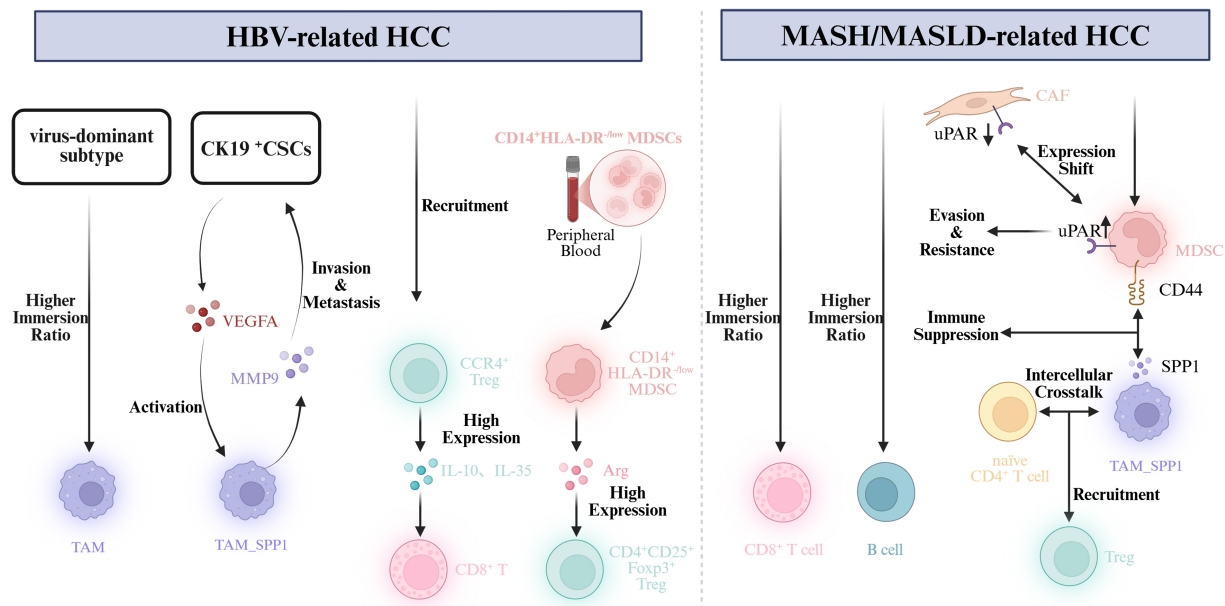
LT $\beta$ R downregulates PRDM1 via N-glycosylation to initiate FOXP3 transcription, with HCC cell overexpression of glycolysis-related enzymes hindering LT $\beta$ R N-glycosylation to promote Foxp3<sup>+</sup> Treg infiltration<sup>[69]</sup>. TGF- $\beta$ 1 upregulates SOX12 via the TGF $\beta$ R1-Smad2/3/4 pathway and SOX18 via the Smad2/3 complex, enhancing Treg infiltration and immunosuppressive activity while reducing CD8<sup>+</sup> T cell function<sup>[38,70]</sup>. In HBV-related HCC, CCR4<sup>+</sup> Tregs are dominant, expressing higher immunosuppressive cytokines and exhibiting stem cell-like characteristics to significantly inhibit CD8<sup>+</sup> T cell proliferation and cytotoxicity. Tumor cells secrete CCL22 to recruit CCR4<sup>+</sup> Tregs, creating an immunosuppressive environment that induces PD-1 expression on CD8<sup>+</sup> T cells and promotes macrophage and MDSC accumulation<sup>[71]</sup>.

### **Heterogeneity of immunosuppressive cells in HCC**

The heterogeneity of M2-like TAMs exhibits distinct etiological and spatial specificity. In HBV-related HCC, the bacteria-dominant intratumoral microbial subtype is associated with higher M2 infiltration and specific metabolic reprogramming<sup>[72]</sup>, while the CK19<sup>+</sup> subtype specifically enriches secreted phosphoprotein 1-positive (SPP1<sup>+</sup>) TAMs<sup>[23]</sup>. During nonalcoholic fatty liver disease (NAFLD) progression, the NAFLD-mSII subtype linked to high cirrhosis risk shows mixed M1/M2 infiltration<sup>[73]</sup>. Spatially, M2 macrophages are enriched at the tumor margin, forming a metabolic feedback loop with tumor cells to maintain immunosuppression<sup>[74]</sup>.

MDSC phenotype and function evolve with underlying liver disease. In HBV-related HCC, CD14<sup>+</sup>HLA-DR<sup>low</sup> MDSCs are elevated, while during MASH progression, uPAR expression shifts to MDSCs in advanced stages<sup>[61]</sup>. Infiltration of MDSCs and SPP1<sup>+</sup> macrophages correlates with poor prognosis via interactions like SPP1-CD44<sup>[61]</sup>. Tregs are integrated in the immunosuppressive network, with their expansion and activation driven by MDSCs in contexts like HBV-related HCC, collaborating with other cells to weaken anti-tumor immunity<sup>[60,75-77]</sup>.

In summary, M2 macrophages, MDSCs, and Tregs are shaped by HCC etiology and tumor molecular subtypes, forming a heterogeneous immunosuppressive ecosystem [Figure 1]. Deciphering this heterogeneity is essential for understanding immune evasion and developing precision immunotherapies.



**Figure 1.** Comparative differences in immune microenvironment characteristics of HCC with different etiologies. Created in BioRender. HBV: Hepatitis B virus; HCC: hepatocellular carcinoma; CK19: cytokeratin 19; CSCs: cancer stem cells; VEGFA: vascular endothelial growth factor A; TAM: tumor-associated macrophage; MMP9: matrix metalloproteinase 9; CCR4: C-C chemokine receptor type 4; Treg: regulatory T cell; SPP1: secreted phosphoprotein 1; HLA-DR: human leukocyte antigen-DR isotype; MDSC: myeloid-derived suppressor cell; Arg: arginase; Foxp3: forkhead box P3; uPAR: urokinase-type plasminogen activator receptor; MASH: metabolic dysfunction-associated steatohepatitis; MASLD: metabolic dysfunction-associated steatotic liver disease.

## FUNCTIONAL IMMUNE CELLS AND ANTI-TUMOR IMMUNITY

In the TIME, the functional activity of immune cells directly impacts anti-tumor immunity. Functional immune cells such as CD8<sup>+</sup> CTLs, CD4<sup>+</sup> helper T cells, B cells, NK cells, and DCs play key roles in immune surveillance, anti-tumor responses, and immune tolerance<sup>[71,78,79]</sup>.

### CTLs: antigen recognition, functional exhaustion, and recovery

CD8<sup>+</sup> CTLs are key anti-tumor effector cells in HCC TIME, exerting cytotoxic effects by recognizing tumor antigens presented via major histocompatibility complex-I (MHC-I) molecules. Their functional status directly affects tumor progression and immunotherapy efficacy.

Tumor cells reduce MHC-I expression via lysosomal degradation, weakening antigen presentation and inhibiting CTL recognition<sup>[80]</sup>. Tumor-associated neutrophils (TANs) highly express PD-L1, inhibiting CD8<sup>+</sup> T cell activation and cytotoxicity while promoting exhaustion<sup>[81]</sup>. High tumor metabolism causes nutrient depletion, leading to CTL functional exhaustion with reduced secretion of cytotoxic molecules and anti-tumor cytokines<sup>[82]</sup>. LCFAs accumulation induces mitochondrial dysfunction in CD8<sup>+</sup> T cells, suppressing fatty acid catabolism and function<sup>[82]</sup>. Additionally, tumor cell secretion of immunosuppressive factors, recruitment of inhibitory cells, and high PD-L1 expression contribute to CTL exhaustion via PD-1/PD-L1 pathway activation<sup>[83]</sup>.

Stem cell-like CD8<sup>+</sup> T cells (TCF1<sup>+</sup> TIM3<sup>-</sup> CD28<sup>+</sup>) exhibit strong proliferative potential, while terminally differentiated cells (TCF1<sup>-</sup> TIM3<sup>+</sup> PD-1<sup>+</sup>) lack proliferation ability and show typical exhaustion characteristics<sup>[84]</sup>. Functional reprogramming is coordinated by the JAK-STAT pathway, with IL-2/IL-15 activating STAT5, IL-21 activating STAT3, and IL-6 activating STAT3/STAT1 heterodimers. In chronic hepatitis B patients, STAT3 phosphorylation correlates with CTL functional recovery<sup>[85,86]</sup>.

### **CD4<sup>+</sup> helper T cell subsets: cytokine network**

Different CD4<sup>+</sup> helper T cell subsets affect tumor immunity via specific cytokines, with Th1 and Th17 playing complex roles in HCC. Th1 cells secrete Interferon- $\gamma$  (IFN- $\gamma$ ), IL-2, and TNF- $\alpha$  to promote CD8<sup>+</sup> T and NK cell activation and enhance anti-tumor immunity. Their differentiation depends on IFN- $\gamma$ -STAT1-T-bet signaling, with T-bet regulating chemokine expression and migration<sup>[87]</sup>. IFN- $\gamma$  activates macrophages and enhances DC antigen presentation. Probiotic BLE may enhance liver Th1 function by upregulating T-bet and promoting cytokine secretion<sup>[88]</sup>. Th17 cells secrete IL-17, activating NF- $\kappa$ B and STAT3 pathways to promote tumor development<sup>[87]</sup>. Differentiated from naïve CD4<sup>+</sup> T cells under IL-6 and TGF- $\beta$ 1 induction, Th17 cells are regulated by retinoic acid-related orphan nuclear receptor  $\gamma$ t (ROR $\gamma$ t), with IL-23 enhancing ROR $\gamma$ t expression via STAT3 to promote expansion and stabilization<sup>[89-91]</sup>.

### **B cells and TABs: anti-tumor vs. immunosuppression**

B cells play a dual role in TIME, both promoting anti-tumor immunity and mediating suppression. Tumor-infiltrating B cells (TIL-Bs) and tumor-associated B cells (TABs) exhibit significant heterogeneity. B cell presence in liver cancer correlates with improved patient survival, with activated subsets enhancing CD8<sup>+</sup> T cell activation<sup>[92]</sup>.  $\mu$ MT mice lacking B cells show aggravated HCC progression and impaired CD8<sup>+</sup> T cell activation<sup>[92]</sup>. B cells participate in tertiary lymphoid structures (TLS) formation, promoting local immune memory and effector cell generation<sup>[93,94]</sup>.

B cells also mediate immune suppression by secreting IL-10 or expressing PD-L1. In HCC, TABs are recruited by TAMs via the CXCL12/CXCR4 axis, expressing PD-L1 to inhibit immune effector cells<sup>[95]</sup>. B cell-derived GABA reprograms macrophages to induce an immunosuppressive environment<sup>[96]</sup>. IgG<sup>+</sup> plasma cells promote tumor-promoting macrophage formation in HCC, while IgA<sup>+</sup> plasma cells induce MDSC activation in colorectal cancer liver metastasis<sup>[97]</sup>. B cell abundance and activation status predict survival and immunotherapy response, with high infiltration indicating better prognosis and stronger anti-tumor immunity<sup>[98,99]</sup>.

### **NK cells: suppressed by NKG2A and TIGIT signaling mechanisms**

NK cell activity is regulated by activating and inhibitory receptors, with natural killer group 2A (NKG2A) and T Cell Immunoreceptor with Ig and ITIM Domains (TIGIT) pathways crucial for function inhibition. In HCC patients, NKG2A upregulation reduces NK cell anti-tumor effects, while blocking NKG2A restores activity and enhances antibody-dependent cellular cytotoxicity (ADCC)<sup>[100]</sup>. In HBV-related HCC, TIGIT<sup>+</sup> TIM-3<sup>+</sup> NK cells exhibit exhaustion with weakened killing function, reduced cytokine secretion, and impaired proliferation<sup>[101]</sup>. Fibrinogen-like protein 1 (FGL1) inhibits CD8<sup>+</sup> T and NK cells via LAG-3 binding, promoting HCC progression<sup>[102]</sup>. LCACs accumulation induces iNKT cell senescence, weakening immune surveillance<sup>[103]</sup>. Enhanced STAT3 activity and PRDM10 downregulation in liver NK cells contribute to dysfunction and exhaustion<sup>[104,105]</sup>. Soluble MHC Class I Chain-Related A (sMICA) released by tumor cells disrupts NKG2D signaling, impairing NK cell activation<sup>[106]</sup>. These mechanisms collectively lead to NK cell exhaustion and immune evasion.

### **DCs and antigen presentation defects**

DCs are key antigen-presenting cells, cross-presenting tumor antigens to activate naïve T cells and induce anti-tumor immunity. After capturing antigens, DCs present them to CD8<sup>+</sup> T cells via MHC-I molecules, promoting CTL differentiation and tumor cell killing<sup>[81,107]</sup>. In TME, DCs often exhibit antigen-presenting defects. In HCC, DC dysfunction is characterized by reduced maturity, decreased CD80/CD86 expression, and reduced IL-12 secretion, preventing effective T cell activation and exacerbating immune tolerance<sup>[108,109]</sup>.

The JAK2-STAT3 pathway activation negatively impacts DC maturation and function<sup>[109]</sup>. Tumor-associated exosomes (TAEs) alter DC cytokine secretion, reducing IL-12 and increasing IL-10 and TGF- $\beta$  to impair immune activation<sup>[110,111]</sup>.

### Heterogeneity of functional immune cells in HCC

CD8<sup>+</sup> T cell infiltration and function are pivotal in HCC. HBV-related HCC shows higher infiltration and tumor reactivity, while metabolic dysfunction-associated steatotic liver disease (MASLD)-related HCC exhibits T cell dysfunction due to tumor glucose consumption via NSUN2-mediated metabolic reprogramming<sup>[112,113]</sup>. HBV/MASLD co-existing HCC has elevated precursor-exhausted CD8<sup>+</sup> T cells, associated with better immunotherapy responses<sup>[112]</sup>, while Wnt-non-mutant metastatic sites show terminal CD8<sup>+</sup> T cell exhaustion mediated by immunosuppressive B cells<sup>[114]</sup>. In alcohol-associated HCC, reduced CD69<sup>+</sup> CD4<sup>+</sup> T cell frequency with elevated PD-L1 suggests impaired immune surveillance<sup>[115]</sup>. In MASH-HCC, naïve CD4<sup>+</sup> T cell crosstalk with SPP1<sup>+</sup> macrophages via macrophage migration inhibitory factor (MIF) signaling promotes Treg infiltration<sup>[116]</sup>. In MASLD-driven HCC, activated B cell subsets correlate with favorable prognosis, co-localizing with CD45RO<sup>+</sup> CD8<sup>+</sup> T cells in TLS<sup>[92,117]</sup>. However, in Wnt-non-mutant metastatic microenvironments, B cells adopt an immunosuppressive phenotype to drive CD8<sup>+</sup> T cell exhaustion<sup>[114]</sup>. HBV-related HCC shows increased “adaptive NK cells” with limited anti-tumor activity<sup>[118,119]</sup>. In MASH-HCC, tumor cell metabolism-associated genes (e.g., ACSL4) modulate NK cell-mediated clearance via ferroptosis regulation<sup>[120]</sup>. DC heterogeneity in HCC is still insufficient. In summary, immune cell abundance, function, and spatial distribution are regulated by etiology, tumor genetics, and local signaling networks, shaping TIME characteristics and treatment responses.

### THE INTERACTION NETWORK BETWEEN INHIBITORY CELLS AND FUNCTIONAL CELLS

The HCC TIME operates as an integrated network where stromal, immune, and tumor cells mutually reinforce immunosuppression. CAFs and HSCs act as primary architects, driving monocyte differentiation into MDSCs via IL-6/STAT3 and SDF-1/CXCR4 signaling<sup>[121]</sup>. Beyond cytokines, the physical environment - specifically Neutrophil Extracellular Traps (NETs) - induces TLR4-mediated mitochondrial oxidative phosphorylation to reshape myeloid metabolic profiles<sup>[33,122]</sup>.

This network is sustained by “amplification loops”: MDSCs secrete CCL2 to replenish the TAM pool, while lipid-laden TAMs recruit and differentiate Tregs via the CCL20/CCR6 and S1P axes<sup>[40,78]</sup>. Unique TAM subsets further refine this niche; TREM2<sup>+</sup> TAMs modulate CXCL9/Galectin-1 expression, while FABP5<sup>+</sup> and XOR-deficient TAMs drive suppressive metabolite accumulation<sup>[123]</sup>. Non-classical regulators also play vital roles: Bregs impair CD8<sup>+</sup> T-cell function via IL-21R-STAT1 or PD-L1 expression, and IgA<sup>+</sup> B cells promote fibrosis<sup>[124,125]</sup>. In HBV-related HCC, B cells communicate with CAFs through the pleiotrophin (PTN)-syndecan-1 (SDC1)/nucleolin (NCL) axis to accelerate progression<sup>[126]</sup>. Meanwhile, endothelial cells secrete CXCL12 to recruit MDSCs and directly arrest the differentiation of naïve CD8<sup>+</sup> T cells, creating an immunosuppressive vascular barrier<sup>[127]</sup>.

Ultimately, this crosstalk facilitates evasion by targeting both innate and adaptive killers. Beyond exhaustion, MDSCs suppress NK cells via Nkp30 binding, while tumor-derived TGF- $\beta$  flips NK cells into dysfunctional group 1 innate lymphoid cells (ILC1)-like phenotypes<sup>[121]</sup>. Specific HCC markers like EpCAM and CD155 further confer resistance to cytotoxicity. Moreover, high-lactate and high-lipid conditions expand regulatory DCs, impairing essential DC-T cell crosstalk<sup>[128,129]</sup>. These interactions are highly etiology-specific: In NAFLD/MASH, lipid dysfunction and MATR3 deficiency accelerate CD4<sup>+</sup> T cell apoptosis, whereas HBV-related HCC establishes an “exhaustion hub” via itaconate and SOX18 signaling to resist NK-cell-mediated lysis<sup>[33,38,127,130]</sup>.

**Table 1. Immune features, tumor phenotypes and therapeutic responses of HCC by etiological subtypes**

Etiological subtype	Core immune features	Tumor phenotype	Therapeutic response characteristics	Ref.
HBV-related HCC	Dysfunctional/exhausted HBV-specific T cells; Increased Tregs infiltration	“Warm” tumor (partial immune infiltration)	Moderate response to ICIs; Dependent on functional T cell activation	[112,132]
MASLD-related HCC	Abundant CD8 <sup>+</sup> T cell infiltration; Enhanced tumor vascular normalization	“Cold-to-warm” transition phenotype	Variable ICI efficacy; Responsive to metabolic intervention	[112,133,134]
HBV <sup>+</sup> MASLD <sup>+</sup> coinfecting HCC	Enriched precursor-exhausted CD8 <sup>+</sup> T cells; Optimized immune cell crosstalk	Immune-responsive phenotype (“Hot” tumor)	Immunotherapy offers the greatest benefits.	[112,134]
Non-HBV/non-MASLD-related HCC	Weak antigen-specific T cell response; Balanced inhibitory/effector immune cell ratio	Heterogeneous phenotype (A predominantly “cold” tumor microenvironment)	Intermediate ICI response	[112,135]

HBV: Hepatitis B virus; HCC: hepatocellular carcinoma; MASLD: metabolic dysfunction-associated steatotic liver disease; Treg: regulatory T cell; ICIs: immune-checkpoint inhibitors.

## HETEROGENEITY OF TIME IN HCC: AN INTEGRATED THREE-DIMENSIONAL PERSPECTIVE

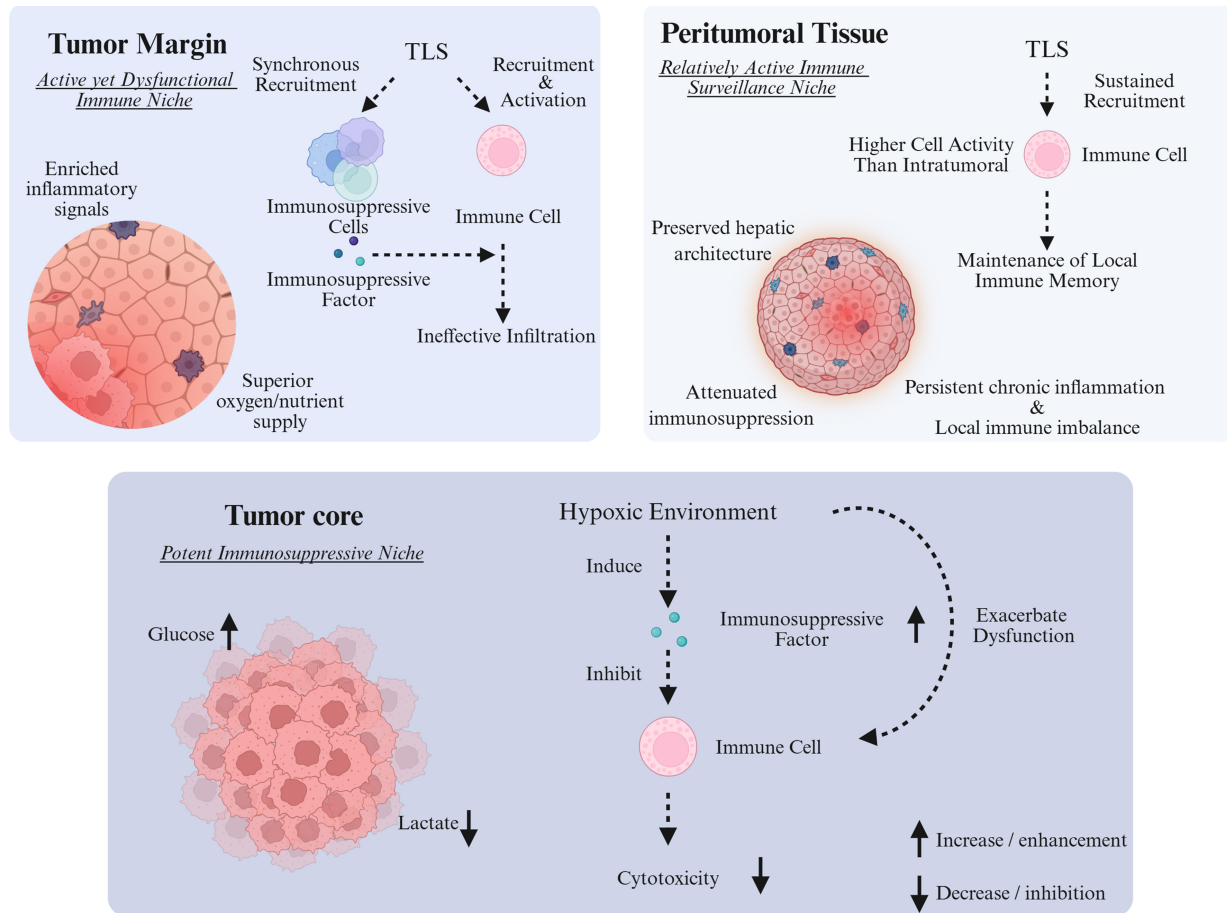
The preceding sections have delineated the core cellular components of the HCC TIME and their intricate intercellular crosstalk. However, these elements do not exist in a static or uniform state; rather, they exhibit profound heterogeneity that acts as a primary driver of immune evasion and a fundamental determinant of variable treatment responses. To systematically decode this complexity, we map these dynamic variations onto our proposed Integrated Three-Dimensional Heterogeneity Model. The following discussion will elucidate how this heterogeneity manifests and is mechanistically regulated across three interconnected axes: variations among individual patients driven by distinct pathogenic backgrounds (Etiology), localized compartmentalization across discrete tumor niches (Spatial Topology), and phased immunological shifts throughout disease progression (Temporal Evolution).

### Heterogeneity among individual patients

The heterogeneity of the immune microenvironment across individual patients is likely a key determinant of variable responses to immunotherapy. Patients exhibit inherent differences in their immune systems, reflected in baseline variations in immune responsiveness, immune evasion mechanisms, and immune tolerance.

Beyond these intrinsic factors, the immunological profile of liver cancer patients is shaped by a combination of genetic, environmental, and lifestyle influences. Underlying liver disease etiologies - such as hepatitis virus (HBV or HCV) infection, alcoholic liver disease, and nonalcoholic fatty liver disease - further remodel the immune landscape in which the tumor develops<sup>[131]</sup>. The main heterogeneity of immune features in HCC caused by different etiologies is summarized in [Table 1](#).

In addition to these contextual factors, differential response rates to immunotherapy in HCC are closely linked to the composition and functional states of specific immune cell populations within the TME. The activity, abundance, and immunosuppressive factor secretion of various cell types - including Tregs, NK cells, DCs, MDSCs, TAMs and CAFs - collectively influence the capacity for immune evasion and ultimately shape the efficacy of immunotherapeutic interventions<sup>[7,60,76,136]</sup>.



**Figure 2.** Spatial heterogeneity of the immune microenvironment in different local regions of HCC. Created in BioRender. HCC: Hepatocellular carcinoma; TLS: tertiary lymphoid structure.

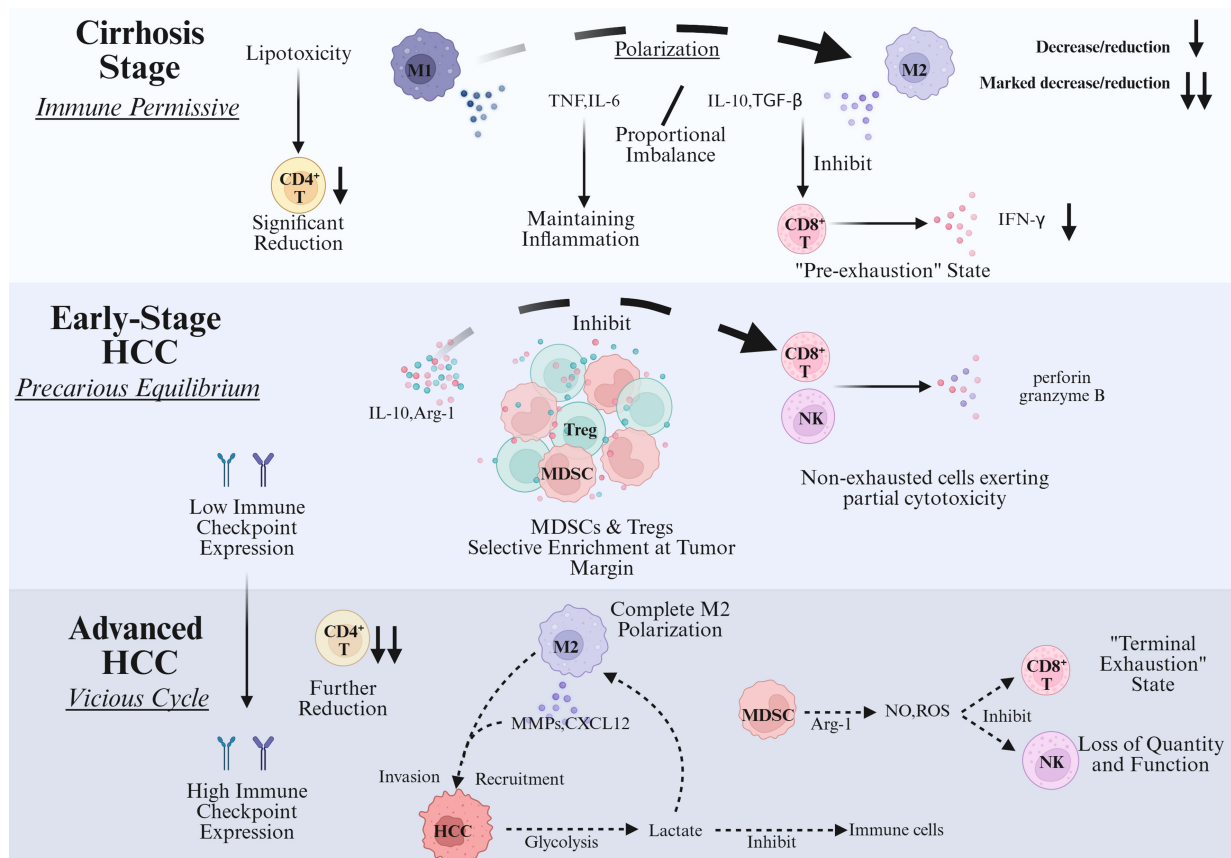
### Intratumoral heterogeneity

HCC is characterized by pronounced local progression. Different tumor regions - such as the core, invasive margin, and peritumoral area - display marked variations in immune cell infiltration, metabolic profiles, and mechanisms of immune evasion.

#### *Spatial heterogeneity: tumor core vs. margin vs. peritumoral tissue*

The infiltration and function of immune cells within HCC exhibit substantial spatial heterogeneity across different tumor regions [Figure 2]. The tumor core is typically characterized by a hypoxic microenvironment, which drives tumor cells to adopt adaptive mechanisms that sustain their growth and survival. This hypoxic state not only enhances the production of immunosuppressive factors, such as TGF- $\beta$  and IL-10, but also upregulates immune checkpoint molecules like PD-L1, thereby impairing the cytotoxic activity of immune cells. Consequently, the tumor core represents the most immunosuppressive region, marked by reduced immune cell infiltration and compromised immune function<sup>[137-139]</sup>.

By contrast, the tumor margin and peritumoral tissues generally display more active immune engagement. These regions often show increased infiltration of immune cells. Studies have shown that peritumoral tertiary lymphoid structures (pTLS) help recruit B cells, T cells (including CD3<sup>+</sup>, CD8<sup>+</sup>, and CD20<sup>+</sup> subsets), and DCs to the invasive margin and surrounding stroma. Furthermore, NKs in the peritumoral compartment tend to exhibit stronger antitumor activity compared to their intratumoral counterparts<sup>[119,140,141]</sup>.



**Figure 3.** Dynamic evolution of immune status across stages of HCC progression. Created in BioRender. HCC: Hepatocellular carcinoma; Treg: regulatory T cell; MDSC: myeloid-derived suppressor cell; TNF: tumor necrosis factor; TGF- $\beta$ : transforming growth factor-beta; Arg-1: arginase-1; MMPs: matrix metalloproteinases; CXCL12: C-X-C chemokine ligand 12; NO: nitric oxide; ROS: reactive oxygen species.

Nevertheless, immune function in these areas is frequently modulated by tumor-associated immunosuppressive cells - such as Tregs and MDSCs - which create a locally imbalanced immune milieu<sup>[142,143]</sup>. Consequently, although immune cell density is higher at the tumor margin and in peritumoral tissue, their effector functions are often restrained, enabling the tumor to evade immune surveillance.

### *Temporal heterogeneity: phased manifestations in the immune evolution of HCC*

The temporal heterogeneity of immune evolution in HCC unfolds across the continuum from cirrhosis (a pre-neoplastic stage) through early HCC to advanced disease [Figure 3]. This progression exhibits a bidirectional shift, characterized by an “increasing gradient of immune suppression” alongside a “decreasing gradient of anti-tumor immunity”.

During the cirrhosis stage, the immune microenvironment is characterized by chronic inflammation that drives the establishment of immune tolerance, thereby laying the groundwork for an immune-permissive milieu conducive to liver cancer development. The function of innate immune cells becomes dysregulated: KCs shift from an anti-tumor M1 phenotype toward a pro-inflammatory, pro-fibrotic M2-like state. These cells sustain chronic inflammation through the secretion of TNF and IL-6, while simultaneously inhibiting T-cell activation via TGF- $\beta$ . HSCs, activated by inflammatory signals, differentiate into myofibroblasts that

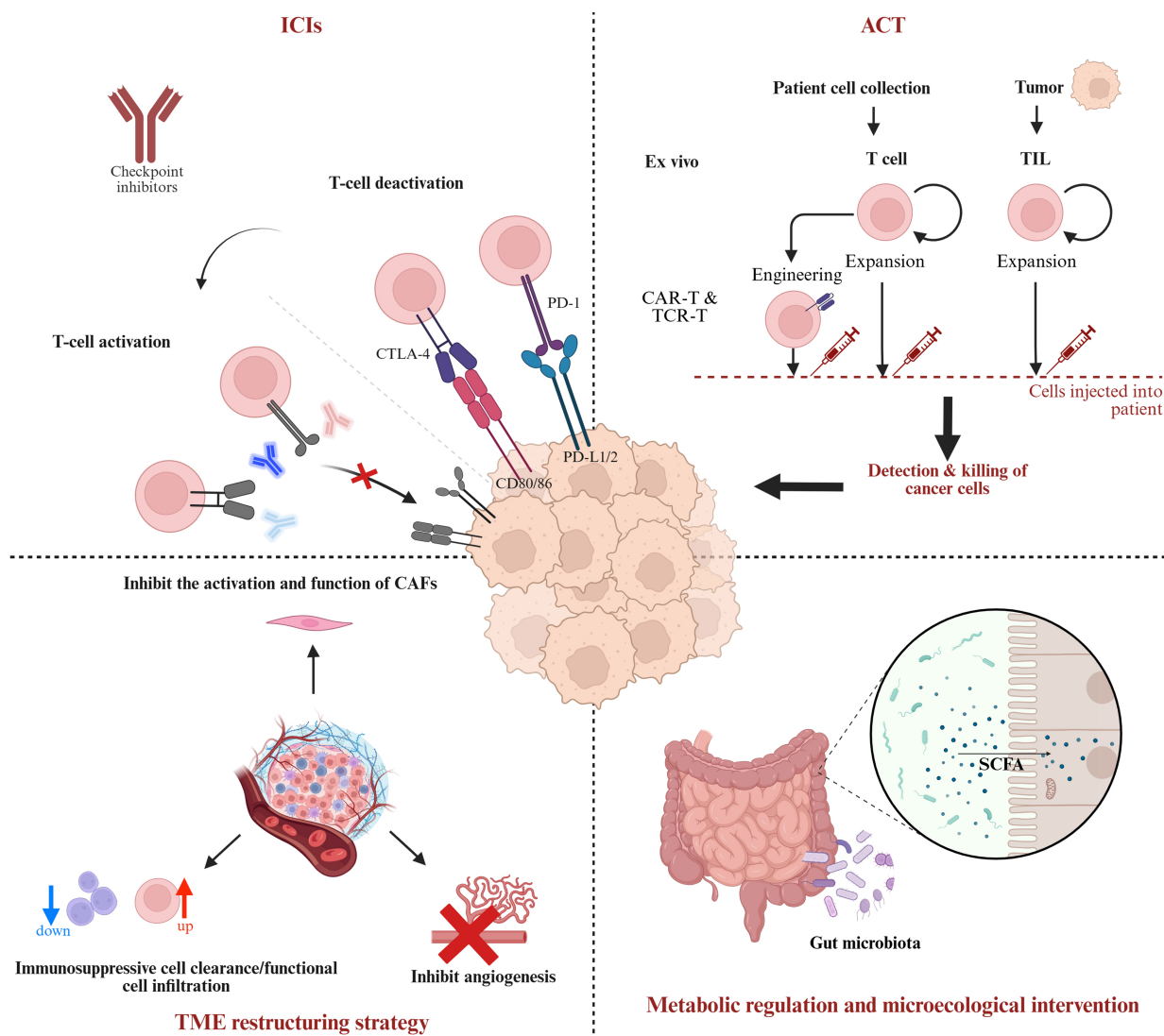
not only promote fibrosis through collagen deposition but also foster the accumulation of Tregs by expressing PD-L1 and secreting IL-10<sup>[144]</sup>. Adaptive immunity undergoes selective attrition. In metabolic-associated cirrhosis, such as non-alcoholic steatohepatitis (NASH), linoleic acid-mediated lipotoxicity leads to a pronounced loss of CD4<sup>+</sup> T cells. Although CD8<sup>+</sup> T cells are not significantly depleted, they enter a “pre-exhausted” state marked by diminished IFN- $\gamma$  secretion and cytotoxicity<sup>[130]</sup>. At the molecular level, the immune network exhibits a procancer imbalance, with a skewed ratio of proinflammatory cytokines (e.g., TNF, IL-6) to antiinflammatory factors (e.g., IL-10, TGF- $\beta$ ). Chronic inflammation continuously induces DNA damage and the accumulation of pre-mutagenic alterations in hepatocytes, while anti-inflammatory mediators suppress immune-mediated clearance of mutated cells. This results in a paradoxical state of “inflammation-driven carcinogenesis coupled with immune tolerance”, enabling the emergence and persistence of potentially malignant clones<sup>[145]</sup>.

In early-stage HCC, where tumor burden remains relatively low, the immune landscape displays a precarious equilibrium between local immunosuppression and anti-tumor immunity. Anti-tumor immune cells retain partial functionality: liver-resident NK cells (particularly the CD49a<sup>+</sup>DX5<sup>-</sup> subset) and a subset of non-exhausted CD8<sup>+</sup> T cells exert cytotoxic effects via perforin and granzyme B secretion. Although DC numbers are reduced, they can still present tumor antigens in limited quantities, sustaining a weak but detectable anti-tumor response<sup>[146]</sup>. Concurrently, immunosuppressive cells begin to accumulate locally. Tregs and MDSCs are selectively enriched at the tumor margin, where they inhibit CD8<sup>+</sup> T cell and NK cell function through IL-10 secretion and arginase release. TAMs further polarize toward an M2-like phenotype, promoting early angiogenesis via VEGF secretion. Immune structures and molecular profiles undergo early remodeling. Intratumoral tertiary lymphoid structures (iTLS) - composed of B cells, T cells, and DCs - form in approximately 30% of early HCC cases and can locally activate adaptive immunity, temporarily restraining tumor progression. Meanwhile, HCC cells initiate early immune evasion, though expression of immune checkpoint molecules such as PD-L1 and CTLA-4 remains low and not yet sufficient to fully block anti-tumor immunity<sup>[145,147]</sup>.

In advanced HCC, a marked increase in tumor burden drives the formation of a multidimensional immunosuppressive network and highly evolved immune evasion mechanisms. Adaptive immune cells become profoundly exhausted. CD8<sup>+</sup> T cells enter a terminally exhausted state, characterized by high coexpression of immune checkpoint molecules (PD-1, TIM-3, LAG-3), loss of proliferative capacity and cytotoxicity, and inability to secrete IFN- $\gamma$  or granzymes. CD4<sup>+</sup> T cell numbers decline further, with Tregs expanding to constitute over 40% of the remaining pool. Tregs enhance suppression by sequestering IL-2 and inhibiting dendritic cell maturation<sup>[148]</sup>. Innate immune function is also severely paralyzed. NK cells are diminished due to IL-15 deprivation and MDSC-derived reactive oxygen species (ROS), losing their cytotoxic capacity. MDSCs, particularly the granulocytic subset, accumulate extensively and suppress CD8<sup>+</sup> T and NK cells by depleting arginine and releasing nitric oxide (NO) and ROS. TAMs fully polarize into a pro-metastatic M2-like phenotype, secreting matrix metalloproteinases (MMPs) to promote invasion, CXCL12 to recruit tumor cells, and expressing PD-L1 to directly inhibit T-cell activation<sup>[149]</sup>. A self-reinforcing “vicious cycle” dominates TIME. Tumor cells enhance glycolysis, depleting local glucose and starving CD8<sup>+</sup> T cells of energy. Concurrently, lactate secreted by tumor cells and TAMs acidifies the microenvironment, further impairing Tcell function and promoting MDSC enrichment. High expression of immune checkpoint molecules (PD-L1, CTLA-4) on both tumor and suppressive immune cells establishes multiple, overlapping layers of immune blockade<sup>[148,149]</sup>.

## **THERAPEUTIC STRATEGY TARGETING TIME OF HCC**

We learned that the etiology heterogeneity of HCC is closely related to immune cells heterogeneity, and this complex relationship will have a significant impact on therapeutic response variation [Figure 4]. Given the



**Figure 4.** Multidimensional regulatory strategies for HCC immunotherapy and tumor. Created in BioRender. ICI: Immune-checkpoint inhibitor; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; PD-1: programmed cell death protein 1; PD-L1: programmed cell death ligand 1; TIL: tumor-infiltrating lymphocyte; CAR-T: chimeric antigen receptor T cell; TCR-T: T-cell receptor-engineered T cell; TME: tumor microenvironment; ACT: adoptive cell therapy; SCFA: short-chain fatty acid; CAFs: cancer-associated fibroblasts.

heterogeneous features of the TIME of HCC - such as inter-patient differences in immune cell composition and microenvironmental variations between the tumor core and margin - single-target therapies are inadequate to cover all contexts. Overcoming the therapeutic resistance imposed by this heterogeneity requires a multidimensional targeting strategy. Recent years have witnessed significant advances in therapeutic strategies targeting the TIME of HCC, notably in immunotherapy, exemplified by ICIs.

### ICIs

ICIs have emerged as a pivotal tool in HCC immunotherapy, achieving substantial clinical progress and becoming an indispensable treatment strategy. ICIs function primarily by blocking negative regulatory signals between tumor cells and immune cells, thereby restoring T cell-mediated anti-tumor activity and reversing tumor immune evasion. The most extensively utilized ICI targets in clinical practice include PD-1 and its ligand PD-L1, as well as CTLA-4. Additionally, emerging targets such as LAG-3, TIGIT, TIM-3, V-Domain Ig Suppressor of T Cell Activation (VISTA), and Siglec-9 are garnering increasing research interest.

Monoclonal antibodies targeting the PD-1/PD-L1 pathway, such as the anti-PD-1 agents nivolumab and pembrolizumab and the anti-PD-L1 agent atezolizumab, have demonstrated therapeutic efficacy in advanced HCC across multiple clinical trials. The IMbrave150 trial established atezolizumab combined with the anti-angiogenic bevacizumab as a first-line standard, significantly improving overall survival (OS) and progression-free survival (PFS)<sup>[150]</sup>. Single-cell sequencing data suggest differential benefits across molecular subgroups of advanced HCC with this regimen. Real-world evidence, such as from the AB-real study ( $n = 296$ ), reported a median OS of 15.7 months, a median PFS of 6.9 months, and an objective response rate (ORR) of 30.8%<sup>[151]</sup>.

Additionally, the combination of nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) has shown significant clinical activity with durable responses, particularly in sorafenib-pretreated patients. In the second-line setting, this combination achieves an ORR of approximately 30% and a median OS of 22 months, demonstrating superior anti-tumor efficacy<sup>[151]</sup>.

CTLA-4 is another key immune checkpoint, which primarily mediates immunosuppression by regulating the activation of naïve T cells. Anti-CTLA-4 monoclonal antibodies, such as tremelimumab and ipilimumab, have demonstrated efficacy in HCC treatment. Their combination with PD-1/PD-L1 inhibitors can yield synergistic effects, enhancing the overall response rate. Emerging immune checkpoint targets, including LAG-3, TIGIT, and VISTA, have attracted growing interest<sup>[151,152]</sup>. These molecules are involved in modulating T cell activity and maintaining immune tolerance. Preclinical and early clinical studies suggest that inhibitors targeting these pathways may help overcome resistance to PD-1/PD-L1 blockade, whether used alone or combined with anti-CTLA-4 therapy, offering potential for new advances in HCC immunotherapy.

Although ICIs have shown considerable promise in HCC, only a subset of patients achieve a meaningful response, while challenges like therapeutic resistance and immune-related adverse events (irAEs) remain common. The complex TIME of HCC, rich in immunosuppressive cells and signaling pathways, facilitates immune evasion and contributes to low ICI response rates. Additional factors, including tumor heterogeneity, loss of tumor antigen expression, and impaired antigen presentation, further limit efficacy. Consequently, significant research efforts are focused on developing ICI-based combination strategies to overcome resistance and improve outcomes. These include combinations with anti-angiogenic tyrosine kinase inhibitors (TKIs), other targeted molecular therapies, local-regional treatments [e.g., Transarterial Chemoembolization (TACE)], and multi-checkpoint inhibition<sup>[153-156]</sup>.

The advancement of precision immunotherapy in HCC will increasingly depend on biomarker screening to identify responsive patients. Studies suggest that potential predictive markers for ICI efficacy include the density of tumor-infiltrating CD8<sup>+</sup> T cells, expression of immune-related genes, serum levels of inflammatory cytokines (e.g., IL-8), and liver function-related indicators [e.g., alpha-fetoprotein (AFP), neutrophil-to-lymphocyte ratio (NLR)]<sup>[157-159]</sup>. Furthermore, a deeper understanding of tumor genomics and the TIME will help explain the differential ICI responses between immunologically “hot” and “cold” tumors, thereby providing a foundation for individualized treatment strategies<sup>[160,161]</sup>.

### **TME remodeling strategy**

Tregs are highly enriched in HCC. Notably, PD-1-expressing Treg subsets demonstrate potent immunosuppressive activity within both the tumor and peripheral blood, effectively hindering effector T cell activation and anti-tumor immunity<sup>[162]</sup>. Key pathways governing Treg function include immune checkpoints

such as PD-1/PD-L1 and LAG-3, whose blockade can attenuate Treg-mediated suppression. Furthermore, Treg recruitment into the TME is critically regulated by chemokine signaling. For example, the CXCL12/CXCR4 axis promotes Treg infiltration in HCC, and its inhibition can reduce Treg accumulation and alleviate local immunosuppression<sup>[38,55]</sup>.

MDSCs, a major class of immunosuppressive cells in the TIME of HCC, promote tumor immune evasion by secreting factors that impair cytokine production and effector T cell function<sup>[121]</sup>. The metabolic characteristics and signaling pathways underpinning MDSCs and TAMs are being elucidated. For instance, in liver metastasis, they exhibit upregulated CD36-mediated lipid metabolism, which enhances fatty acid uptake and augments their immunosuppressive activity. Targeting CD36 can thereby suppress the tumor-promoting functions of them<sup>[163,164]</sup>.

Current strategies to deplete or inhibit Tregs and MDSCs are inherently synergistic. For example, ICIs such as anti-CTLA-4 antibodies can restore effector T cell function while simultaneously suppressing Treg activity<sup>[67]</sup>. Furthermore, targeting chemokine receptors like CXCR4 - which is overexpressed in HCC and TME cells - holds promise. In liver cancer, a “dual-hit therapy” using CXCR4 antagonists can block the recruitment of both Tregs and MDSCs to the tumor, thereby alleviating immunosuppression within the TME<sup>[55]</sup>.

Nanodrug delivery systems enable the targeted delivery of siRNA or small-molecule inhibitors. For example, silencing key regulators such as protein tyrosine phosphatase non-receptor type 2 (PTPN2) can promote the polarization of TAMs toward an M1 phenotype and enhance tumor cell immunogenicity<sup>[165,166]</sup>. Moreover, small molecules like vitamin B3 can modulate the GPR109A signaling pathway, inhibiting the immunosuppressive polarization of myeloid cells while boosting the cytotoxicity of CD8<sup>+</sup> T cells<sup>[167]</sup>.

The integration of nanotechnology with radiotherapy, chemotherapy, or targeted therapy can further elevate oxidative stress within the TME. This enhances immune cell infiltration and reduces the recruitment of immunosuppressive cells, creating a synergistic effect that ultimately improves the efficacy of immunotherapy<sup>[168-171]</sup>. These strategies extend beyond directly targeting immunosuppressive cells; they also remodel the tumor immune landscape by modulating metabolic reprogramming, cellular polarization, and intercellular communication. This multifaceted approach can effectively convert immunologically “cold” tumors into “hot” ones, thereby improving treatment responses in patients with HCC.

### **Adoptive cell therapy (ACT)**

Chimeric antigen receptor T cell (CAR-T) cell therapy is under active investigation for HCC, leveraging its high specificity and potent cytotoxicity<sup>[172]</sup>. Tumor-associated antigens such as AFP and glypican-3 (GPC3) serve as key targets. AFP-targeted CAR-T cells demonstrate selective cytotoxicity against HCC cells<sup>[173]</sup>. Furthermore, GPC3-directed CAR-T therapies have progressed to clinical trials, showing favorable safety and preliminary antitumor activity<sup>[174-176]</sup>.

Combining AFP- and GPC3-targeted CAR-T cells, or engineering T cells to secrete bispecific T-cell engagers (BiTEs), may enhance anti-tumor immune responses<sup>[177]</sup>. However, CAR-T therapy in solid tumors such as HCC remains challenged by the immunosuppressive TME, insufficient T cell infiltration, and tumor antigen heterogeneity, which collectively promote immune evasion and limit therapeutic efficacy<sup>[174,178,179]</sup>.

TIL therapy involves isolating lymphocytes from a patient’s tumor, expanding them *ex vivo*, and reinfusing them to enhance anti-tumor immunity. This approach shows particular promise in HCC patients with “hot”, immune-infiltrated tumors, which provide a supportive microenvironment for TIL activity. However, its

efficacy in HCC is limited by several factors. The TIME, rich in Tregs and MDSCs, can inhibit reinfused TILs. Furthermore, TIL manufacturing is complex, labor-intensive, and exhibits considerable interpatient variability. These challenges - coupled with the need for personalized protocols - currently restrict the broader clinical application of TIL therapy for HCC<sup>[180-182]</sup>.

NK cell therapy is increasingly explored for HCC due to its role in anti-tumor immunity. NK cells can recognize and eliminate MHC-I-deficient tumor cells independently of antigen presentation. Specialized subsets such as  $\gamma\delta$ T cells also hold potential for liver cancer immunotherapy, and modulating NK cell function has been shown to enhance anti-tumor responses<sup>[183]</sup>. However, NK cell therapy faces challenges within the inhibitory TIME. A key limitation is the lack of sustained proliferative signals after infusion, which curtails long-term efficacy. Strategies such as cytokine engineering or lymphodepletion prior to infusion are being investigated to prolong NK cell persistence and activity, thereby overcoming the suppressive microenvironment and maximizing therapeutic potential<sup>[184,185]</sup>.

Cellular therapies for HCC remain in early clinical development, facing several key challenges including the inhibitory TIME, antigen heterogeneity and loss, manufacturing complexity, and treatment-related toxicity. Looking forward, a novel approach involves nanoscale lipid vesicles (30-150 nm) derived from CAR-T, CAR-NK, or CAR-M cells, termed CAR-Exosomes. These particles inherit the tumor-targeting specificity of their parental cells while leveraging the intrinsic advantages of exosomes. By combining these strengths, CAR-Exosomes represent a promising strategy to overcome current limitations and may provide a breakthrough in next-generation cancer immunotherapy<sup>[186,187]</sup>.

In summary, adoptive cell therapies - such as CAR-T, TIL, and NK cell-based treatments - represent promising therapeutic approaches for HCC<sup>[188]</sup>. However, their efficacy is limited by the inhibitory TIME and significant tumor heterogeneity. Advances in cellular engineering, rational combination with immunomodulatory agents, and active remodeling of the TIME are therefore critical to enhancing the safety and efficacy of these therapies and improving patient outcomes.

### **Metabolic regulation and microecological intervention**

Lactate and its derived lactylation modifications significantly reshape the immune landscape within TIME. Lactate drives the polarization of TAMs toward an immunosuppressive M2 phenotype while impairing the effector functions of CD8<sup>+</sup> T cells, thereby suppressing immune surveillance<sup>[189-191]</sup>. Furthermore, lactate can activate signaling pathways that upregulate immune checkpoint expression, further dampening anti-tumor immunity.

Interventions targeting lactate metabolism - such as inhibiting lactate dehydrogenase (LDH) or modulating lactate transporters (e.g., SLC16A3 knockdown or SLC16A4 upregulation) - have shown promise in alleviating this immunosuppression and enhancing immunotherapy efficacy<sup>[192-194]</sup>. Given its pivotal role in the HCC TIME, inhibiting lactate production and transport represents a promising strategy that simultaneously modulates tumor metabolism and remodels the immune landscape. Combining such approaches with immune checkpoint blockade may offer a synergistic strategy to overcome immune evasion and improve treatment responses in HCC patients<sup>[195]</sup>.

Modulating the gut microbiota represents a promising frontier in liver cancer immunotherapy. As a key regulator of metabolism and immunity, the gut microbiota profoundly shapes the hepatic immune microenvironment and influences HCC development. Through the gut-liver axis, microbial metabolites - such as short-chain fatty acids (SCFAs), bile acids, and lipopolysaccharides - travel via the portal system to modulate hepatic immune homeostasis and inflammatory responses, thereby affecting TIME<sup>[196,197]</sup>.

Studies indicate that HCC patients exhibit reduced gut microbial diversity, characterized by a decline in beneficial bacteria and an expansion of pro-inflammatory species. This dysbiosis contributes to local immune dysfunction and tumor immune evasion, correlating with altered immune cell infiltration, checkpoint expression, and variable responses to immunotherapy<sup>[198,199]</sup>. Interventions aimed at restoring microbial balance - including probiotics, prebiotics, fecal microbiota transplantation (FMT), or antibiotics - can reshape the TIME and enhance therapeutic efficacy<sup>[200,201]</sup>. For instance, FMT has shown potential to reverse immune tolerance and improve outcomes in patients resistant to anti-PD-1 therapy<sup>[202,203]</sup>.

Furthermore, the gut microbiota regulates bile acid metabolism, which in turn influences the activity and metabolic state of hepatic immune cells and modulates immune checkpoint signaling<sup>[204,205]</sup>. Thus, targeting the gut microbiome not only helps explain heterogeneity in immunotherapy responses but also offers a novel strategy to overcome resistance. Future combinations of microbiome-based interventions with immune checkpoint blockade hold significant potential to improve survival and therapeutic outcomes in HCC.

In summary, dysregulated lactate metabolism drives immunosuppression in the HCC microenvironment, while the gut microbiota modulates the TIME through metabolic and immunologic crosstalk. Targeting lactate pathways and modulating the microbiota represent promising strategies to overcome current therapeutic limitations, enhance anti-tumor immunity, and improve outcomes in HCC, holding considerable translational potential.

### **Combined treatment trend: multidimensional targeted TIME**

The combination of ICIs with molecular targeted agents is now a cornerstone of first-line therapy for HCC. A key regimen, atezolizumab (anti-PD-L1) plus bevacizumab (anti-VEGF), demonstrated superior efficacy over sorafenib monotherapy in the phase III IMbrave150 trial, achieving an ORR of 30% and a complete response rate of 8%. This strategy works synergistically by concurrently inhibiting angiogenesis and remodeling TIME, effectively converting immunologically “cold” tumors into “hot” ones to enhance immune cell infiltration and activity<sup>[206]</sup>. The positive correlation between VEGF pathway activity and immunosuppressive gene expression further supports the strong rationale for this combination, highlighting its significant potential to improve treatment outcomes in HCC<sup>[207]</sup>.

Combining immunotherapy with local treatments such as TACE and radiotherapy represents a promising strategy for HCC<sup>[208]</sup>. TACE combined with systemic therapy has become a recommended regimen for advanced HCC, as it enhances the release of tumor-associated antigens and inflammatory cytokines, thereby activating the cancer-immunity cycle and remodeling the TIME to increase immunotherapy sensitivity<sup>[209,210]</sup>. Similarly, radiotherapy combined with ICIs can profoundly modulate the TIME, augment T cell-mediated anti-tumor immunity, and has shown encouraging efficacy in clinical settings for refractory HCC<sup>[211,212]</sup>. Other local modalities, including radiofrequency ablation, also demonstrate synergistic potential with immunotherapy in preclinical and early clinical studies. This integrated approach offers a promising avenue to improve outcomes for HCC patients<sup>[213,214]</sup>.

The combination of multi-targeted TKIs with immunotherapy is an increasingly studied strategy in HCC<sup>[215]</sup>. TKIs not only directly inhibit tumor growth and angiogenesis but also remodel TIME by enhancing immune cell activity and suppressing immunosuppressive functions, thereby potentially sensitizing tumors to immunotherapy<sup>[210,216]</sup>. For instance, the phase III COSMIC-312 trial, evaluating cabozantinib plus atezolizumab in treatment-naïve advanced HCC, shows promising translational potential<sup>[210]</sup>. Similarly, the combination of lenvatinib with ICIs has demonstrated robust anti-tumor activity, prompting ongoing research into mechanisms of resistance and optimization of personalized treatment strategies<sup>[217]</sup>.

Combination therapies targeting key components of the TIME, such as the gut microbiota and TAMs, are an emerging focus. For example, quercetin combined with anti-PD-1 therapy can remodel the TIME by modulating both the gut microbiome and macrophage polarization, potentially enhancing immunotherapy efficacy<sup>[218]</sup>. Furthermore, preclinical strategies co-targeting MDSCs and Tregs have shown promise in boosting anti-tumor immunity. These approaches aim to overcome immune evasion by simultaneously disrupting multiple immunosuppressive axes within the TME<sup>[219,220]</sup>.

Finally, emerging technologies such as nanoparticle-based delivery systems offer innovative platforms for combinatorial treatment. By enabling targeted and synchronized drug release, nanocarriers can enhance immunogenic cell death, promote antigen release, and activate immune cells, thereby improving the precision and efficacy of immunotherapy. For example, an integrated nanoparticle platform combining ultrasound contrast with chemotherapy, photothermal, and immunotherapy has been shown in animal models to potently activate antitumor immunity and suppress metastasis<sup>[221]</sup>.

Additionally, in clinical practice, the safety and tolerability of combination therapies must be carefully considered. While combining radiotherapy with other treatments has significantly improved efficacy, it also increases the risk of hematologic and liver toxicity, necessitating close monitoring and management<sup>[222]</sup>. Furthermore, factors such as sarcopenia, which is associated with treatment prognosis, indicate that combination therapy regimens should be individualized<sup>[223]</sup>. This personalized matching approach highly aligns with the etiological heterogeneity of the immune microenvironment in HCC. For instance, in HBV<sup>+</sup> MASLD<sup>+</sup> HCC, the presence of high CD8<sup>+</sup> T cell infiltration and an enriched population of precursor-exhausted T cells suggests that this subgroup of patients may derive the greatest benefit from immunotherapy<sup>[113]</sup>. The mechanisms, clinical advantages, and inherent limitations of these diverse combination regimens and other emerging strategies are systematically summarized in [Table 2](#).

## CONCLUSION

This review highlights that the inhibitory TIME of HCC acts as one of the key drivers of disease progression and a major obstacle to effective immunotherapy. Actively shaped by a network of suppressive cells, including TAMs, MDSCs, and Tregs - the TIME impairs effector lymphocyte function and promotes immune evasion through diverse molecular and metabolic mechanisms. Crucially, the substantial heterogeneity of this immunosuppression, which manifests across divergent etiological backgrounds, spatial topologies, and temporal evolutionary trajectories, partly underlies the highly variable patient responses to treatments such as ICIs.

While therapeutic strategies increasingly aim to target and remodel the TIME - with combination therapies demonstrating notable clinical success - resistance, which is partly rooted in the adaptability of this immunosuppressive network, remains a major clinical challenge. Currently, research on the HCC TIME is transitioning from macroscopic, static descriptions of cellular populations to microscopic, dynamic analyses of spatial interactions. However, critical gaps in knowledge persist. These include that the distinct immune heterogeneity driven by different etiologies remains incompletely understood; precise characterization mapping the spatial topology and dynamic evolutionary trajectories of the TIME requires further exploration; and the metabolic-immune interactive network, particularly the metabolic competition between immune cells within the hypoxic microenvironment, remains incompletely deciphered.

Addressing these gaps and unlocking the full therapeutic potential of immunotherapy would benefit from a paradigm shift in future research. Efforts should focus on leveraging cutting-edge spatial multi-omics technologies to construct high-resolution, three-dimensional maps of the TIME. Furthermore, identifying robust predictive biomarkers is urgently needed to help precisely guide clinical interventions. Future

**Table 2. Classification, mechanisms, and challenges of immunotherapy strategies targeting the HCC TME**

Therapeutic category	Mechanism of action	Specific regimen/targets	Associated heterogeneity dimension	Key advantages	Major limitations and challenges	Ref.
I. Immune checkpoint inhibitors						
Standard ICIs	Nivolumab, Pembrolizumab, Durvalumab	PD-1/PD-L1	Temporal (Advanced stages)	Established first/second-line standard; durable response in subsets	Low ORR (15%-30%); primary/acquired resistance; irAEs	[224]
	Ipilimumab, Tremelimumab	CTLA-4				
Emerging ICIs	Inhibits alternative co-inhibitory receptors to prevent terminal exhaustion	LAG-3, TIGIT, VISTA, Siglec-9	Etiology (HBV-specific exhaustion)	[H/M] Potential to overcome PD-1 resistance; synergistic with anti-PD-1	Mostly in early clinical phases; efficacy as monotherapy is limited	[225-227]
II. TME remodeling strategies						
Recruitment inhibition	Disrupts chemotactic signals to block suppressive cell infiltration	CXCR4/CXCL12 axis antagonists	Spatial (Invasive margin)	[H/M] Reduces "immune-excluded" niches; clears path for CTL infiltration	Redundancy in chemokine pathways; potential systemic side effects	[38,228]
Polarization modulation	Reprograms TAMs/MDSCs from suppressive to pro-inflammatory phenotypes	PTPN2 silencing, Vitamin B3 (GPR109A)	Spatial (Metabolic barrier)	[M] Directly dismantles the core immunosuppressive barrier; enhances ICD	[M] Precise liver delivery challenges; metabolic adaptability of TAMs	[229,230]
III. Adoptive cell therapy						
Engineered T cells	Redirects T cells to recognize tumor-specific antigens independently of MHC	CAR-T (GPC3, AFP), BiTEs	Etiology (Antigen-specific)	High specificity; potent lysis of antigen-positive HCC cells	Antigen loss/heterogeneity; poor penetration into solid tumor core	[231,232]
Native/Infiltrating cells	Exploits broad antigen recognition of tumor-resident or innate killers	TILs, NK cells, T cells	Spatial (Tumor infiltration)	Better management of tumor heterogeneity than CAR-T; low GVHD risk	Labor-intensive expansion; rapid exhaustion within the inhibitory TME	[233-235]
Cell-derived vesicles	Leverages engineered exosomes for targeted delivery of cytotoxic cargo	CAR-Exosomes	Spatial (Deep penetration)	[M] Superior tissue penetration; lower risk of cytokine storm than live cells	[M] Standardization of isolation; short circulation half-life	[236,237]
IV. Metabolic and microbiome interventions						
Metabolic regulation	Normalizes TME pH and nutrient availability to reinvigorate effector cells	LDH inhibitors, SLC16A3 knockdown	Spatial (Hypoxic core)	[M] Reverses metabolic-driven immune silencing in the tumor core	[M] Potential interference with systemic glucose metabolism	[74,238]
Gut-liver axis modulation	Restores microbial diversity to activate hepatic immunity via portal system	FMT, Probiotics, SCFAs	Etiology (Metabolic-related)	Systematic and non-invasive; highly relevant for MASH-related HCC	High inter-individual variability; difficult to standardize across cohorts	[239,240]
V. Multidimensional combination strategies						
ICI + Anti-angiogenic	Normalizes vasculature to increase oxygenation and CTL infiltration	Atezolizumab + Bevacizumab	Spatial (Vascular barrier)	Current gold standard; simultaneously addresses hypoxia and exclusion	Risk of bleeding/hemorrhage; vascular pruning may lead to secondary hypoxia	[241,242]
ICI + Local-treatment	Triggers ICD to release "in situ" vaccines	TACE, Radiotherapy, RFA	Temporal (Window of activation)	Creates a temporal window for priming; converts "cold" tumors to "hot"	Optimal sequencing remains unclear; potential treatment-induced toxicity	[243]

[M]: Demonstrated in mouse models or preclinical research; [H/M] signifies emerging strategies primarily validated in preclinical/murine models or currently under clinical investigation; HCC: hepatocellular Carcinoma; TIME: tumor immune microenvironment; ICIs: immune-checkpoint inhibitors; PD-1: programmed cell death protein 1; PD-L1: programmed cell death ligand 1; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; ORR: objective response rate; irAEs: immune-related adverse events; LAG-3: lymphocyte-activation gene 3; TIGIT: T cell immunoreceptor with Ig and ITIM domains; VISTA: V-domain immunoglobulin suppressor of T cell activation; Siglec-9: sialic acid-binding immunoglobulin-like lectin 9; TME: tumor microenvironment; CXCR4: C-X-C chemokine receptor type 4; CXCL12: C-X-C chemokine ligand 12; CTL: cytotoxic T lymphocyte; TAMs: tumor-associated macrophages; MDSCs: myeloid-derived suppressor cells; PTPN2: protein tyrosine phosphatase non-receptor type 2; GPR109A: G protein-coupled receptor 109A; ICD: immunogenic cell death; CAR-T: chimeric antigen receptor T cell; GPC3: glypican-3; AFP: alpha-fetoprotein; BiTEs: bispecific T-cell engagers; TILs: tumor-infiltrating lymphocytes; NK cells: natural killer cells; GVHD: graft-versus-host disease; LDH: lactate dehydrogenase; SLC16A3: solute carrier family 16 member 3; FMT: fecal microbiota transplantation; SCFAs: short-chain fatty acids; MASH: metabolic dysfunction-associated steatohepatitis.

translational research should also systematically investigate the mechanisms of microenvironmental remodeling designed to reverse “cold tumors” through rational combined local-regional therapies and immunomodulators. Simultaneously, exploring strategies that target innate immunity, investigate novel immune checkpoints, and develop microbiome-based interventions via the gut-liver axis will be important. Ultimately, deciphering the dynamic regulation of the TIME may provide a robust theoretical foundation for developing next-generation combinatorial approaches, sustainably reinvigorating anti-tumor immunity, and facilitating the realization of personalized precision medicine in HCC.

## DECLARATIONS

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### Authors' contributions

Developed the concept and designed the review: Mao T, Jiang J, Liu P

Independently screened the full texts of eligible studies to confirm compliance with inclusion criteria, assess study quality, and extract data: Mao T, Jiang J, Liu P, Zhao Y, Zhong L, Zhang Z, Wu K, Xia L

Provided overall supervision and revision of the manuscript: Xia L

Contributed to manuscript drafting and revision, and approved the final version for submission: Mao T, Jiang J, Liu P, Zhao Y, Zhong L, Zhang Z, Wu K, Xia L

### Availability of data and materials

Not applicable.

### AI and AI-assisted tools statement

Not applicable.

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### Conflicts of interest

Wu K and Xia L are the Associate Chief Editors of the journal *Hepatoma Research*. They were not involved in any stage of the editorial process, including reviewer selection, manuscript handling, or decision-making. The other authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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