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RESEARCH ARTICLE

# Molecular Mechanisms of Immune Regulation and Evasion in Hepatocellular Carcinoma: Insights into Therapeutic Interventions

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Abstract: Hepatocellular carcinoma (HCC), the most common form of primary liver cancer, remains a significant clinical challenge due to its high mortality rate, frequent late-stage diagnosis, and poor responsiveness to conventional treatment modalities. Recent studies have highlighted the important influence of the tumor microenvironment (TME) in promoting immune evasion, supporting tumor growth, and contributing to therapeutic resistance in HCC. This review presents a detailed assessment of the cellular and molecular components within the hepatic TME, including tumor-associated macrophages (TAMs), regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), dendritic cells (DCs), and natural killer (NK) cells. Furthermore, we analyze the complex network of cytokines and chemokines such as IL-6, IL-10 and TGF-β, that mediate immunosuppressive and proinflammatory responses, as well as the roles of hypoxia and oxidative stress in impairing immune functions and enhancing tumor survival. Additionally, critical immune checkpoint pathways and molecular regulators involved in immune escape mechanisms are explored. By elucidating these immunological pathways, and identifying potential therapeutic targets this review emphasizes the promise of immunomodulatory approaches for improving treatment efficacy in HCC. A comprehensive understanding of these complex immune interactions is vital for the development of advanced immunotherapeutic strategies, and personalized interventions in liver cancer management.

Keywords: Hepatocellular; Immunosuppression; Microenvironment; Cytokines; Hypoxia; Macrophages.

## INTRODUCTION

Liver cancer, with hepatocellular carcinoma (HCC) as its main form, poses a major universal healthiness challenge, accounting for additional than 800,000 deaths annually and ranking amid the top three origins of cancer related mortality globally. HCC comprise approximately 75–85% of primary liver cancers. And it is frequently accompanied by subtle onsets, rapid disease progression and poor clinical outcomes. The highest prevalence of HCC is observed in regions such as East Asia, and sub-Saharan Africa where chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are highly endemic [1,2]. Over the recent years however, the epidemiology of liver cancer has shifted, particularly in Western populations where metabolic disorders, including nonalcoholic steatohepatitis (NASH), obesity metabolic syndrome, and alcohol related liver injury, has become increasingly prominent risk factors [3,4]. Despite the progress in diagnostic imaging and therapeutic approaches such as surgical excision liver transplantation, local ablation techniques, and systemic drug therapies, the prognosis for advanced HCC remains poor with five-year survival rates continuing to be low. The underlying causes of treatment resistance is multifactorial, involving tumor heterogeneity, intrinsic resistance to chemotherapy, and the complex interactions between cancer cells and hepatic immune microenvironment. Furthermore, HCC frequently develops against a back-drop of chronic liver inflammation and cirrhosis which foster a tumor promoting environment by enhancing immune suppression and facilitating malignant transformation [4,5].

The liver is a distinct immunological organ that functions both as a central metabolic center and an immunotolerant barrier between the gastrointestinal tract and systemic circulation. Constant exposure to antigens and microbial products from gut through the portal vein necessitate precise regulation of immune responses to ensure balance between immune defense and tolerance. This delicate immune homeostasis is maintained by a well-organized and specialized network of innate and adaptive immune cells including Kupffer cells (resident liver macrophages), dendritic cells (DCs), liver sinusoidal endothelial cells (LSECs), natural killer (NK) cells natural killer T (NKT) cells, and regulatory T (Treg) cells. Kupffer cells plays a critical role in detecting pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), thereby regulating inflammatory signaling. However, in chronic inflammatory states these cells may adopt an immunosuppressive phenotype contributing to the formation of tumor-supportive microenvironment.

Likewise, hepatic dendritic cells are characterized by limited antigen presenting capabilities and tend to induce T cell dysfunction or promote their differentiation into Tregs, reinforcing immune tolerance, and dampening anti-tumor responses [6,7].

Additionally, the liver tumor microenvironment is characterized by elevated levels of immunosuppressive cytokines including interleukin-10 (IL-10) and transforming growth factor beta (TGF-β), which suppress the activity of cytotoxic T lymphocytes (CTLs), and promote proliferation of regulatory T cells (Tregs). Collectively this immunologically tolerant state, although physiologically protective against autoimmune responses, renders the liver particularly susceptible to immune evasion by neoplastic cells [8 9]. Hepatocarcinogenesis is intricately linked to chronic inflammation, genomic instability and immune escape. During the multistep transformation from chronic liver disease to HCC tumor cells acquire molecular hallmarks that allow them evade immunosurveillance and create a microenvironment conducive to immune suppression. This involves increased expression of immune checkpoint molecules including programmed death-ligand 1 (PD-L1), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), which collectively act to suppress T cell activation and limit their proliferation. In addition, abnormal stimulation of oncogenic signaling cascades such as the Wnt/β-catenin pathway, transforming growth factor-beta (TGF-β) signaling and phosphoinositide 3-kinase (PI3K)/AKT pathway has been reported to interfere with antigen presentation, downregulate interferon-mediated responses and alter the extracellular matrix architecture thereby obstructing infiltration and function of immune cells within tumor microenvironment [10,11].

Recent studies reveal that the tumor immune microenvironment (TIME) in hepatocellular carcinoma (HCC) is highly heterogenous and constantly influenced by interactions between malignant cells, stromal components and infiltrating immune cells. Among immunosuppressive elements, myeloid-derived suppressor cells (MDSCs) and M2-like tumor associated macrophages (TAMs) have recognized as central contributors. These cells release factors such as arginase-1, nitric oxide and prostaglandin E2 which impair the cytotoxic activity of both cytotoxic T lymphocytes (CTLs), and natural killer (NK) cells. Additionally, T cells within HCC lesions frequently exhibit features of exhaustion including persistent expression of inhibitory receptors and reduced effector capabilities, reflecting prolonged antigen exposure and subsequent immune dysfunction [10,12].

A comprehensive molecular understanding of the immune regulatory networks involved in hepatocellular carcinoma (HCC) is vital for the development of targeted and effective immunotherapeutic strategies. Although immune checkpoint inhibitors (ICIs) have revolutionized the oncology treatment landscape, their therapeutic efficacy in HCC is often limited due to tumors exhibiting inherent or acquired resistance mechanisms that compromise clinical outcomes. Analysis of molecular determinants of immune escape, such as mutational burden, neoantigen landscape, immunogenicity and stromal barriers will offer crucial insights for tailoring personalized immunotherapies [13,14].

This review aims to characterize molecular and cellular mechanisms contributing to immune evasion in hepatocellular carcinoma. We examine key immunosuppressive pathways, describe interactions among diverse immune cell populations within hepatic tumor microenvironment, and assess current and novel immunotherapeutic approaches with emphasis on specific molecular targets. A deeper understanding of these intricate immune networks is essential for development of more effective and lasting treatment options for patients with liver cancer.

**Tumor Microenvironment (TME) in Hepatocellular Carcinoma**

The TME in HCC constitute a dynamic and immunologically heterogenous milieu that substantially governs tumor progression, immune escape and therapeutic resistance. It comprises an intricate network of malignant hepatocytes, immune infiltrates stromal cells, cytokines, chemokines, and extracellular matrix components (Table 1)[15,16]. The immunosuppressive landscape of hepatic the TME is particularly adept at fostering immune tolerance, and promoting tumor cell survival through diverse molecular and cellular mechanisms (Table 1) [17].

**Cellular Constituents of the Hepatic TME**

Tumor-Associated Macrophages (TAMs) are a predominant immune cell subset within the HCC microenvironment and principally skewed toward M2-like phenotype, which exhibit pro-tumoral and immunosuppressive functions. These macrophages secrete interleukin 10 (IL-10), transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF), facilitating angiogenesis extracellular matrix remodeling and suppression of cytotoxic T lymphocyte (CTL) responses [16,17]. TAM density positively correlate with tumor aggressiveness and poor prognosis in HCC patients. Regulatory T Cells (Tregs) $CD4^+CD25^+FOXP3^+$ Tregs exert profound immunosuppressive effects within the TME by inhibit effector T cell activity and secreting IL-10 and TGF-β [18,19]. Their expansion often driven by tumor derived factors and contribute to attenuation of anti-tumor immunity. Increased Treg infiltration associate with reduced survival rates and resistance to immune checkpoint blockade therapies. Myeloid-Derived Suppressor Cells (MDSCs): MDSCs constitutes a heterogeneous population of immature myeloid cells which suppress innate and adaptive immune responses

via production of arginase-1 (Arg-1), nitric oxide (NO), and reactive oxygen species (ROS) [20,21]. In HCC, MDSCs interacts with TAMs and Tregs to orchestrate a profoundly immunosuppressive TME that impair dendritic cell (DC) maturation and T cell activation. Dendritic Cells (DCs): Although DCs are potent antigen presenting cells (APCs), their function in the HCC TME is often subverted. Tumor induced maturation arrest and downregulation of co stimulatory molecules such as CD80 and CD86 impair effective antigen presentation thus compromising priming of tumor-specific T cells. The presence of tolerogenic DCs further exacerbate immune escape mechanisms (Fig 1). Natural Killer (NK) Cells: NK cells possess innate cytotoxic potential against malignant cells; however, in HCC their functionality is significantly diminished [22,23]. The down regulation of activating receptors (e.g., NKG2D) and up regulation of inhibitory ligands (e.g., PD-L1) on tumor cells contributes to NK cell anergy. Additionally, immunosuppressive milieu rich in IL-10 and TGF-β hampers NK cell cytotoxicity and cytokine production. [24,25].

**Cytokines and Chemokines in Immune Modulation**

The cytokine and chemokine networks within the HCC TME orchestrate recruitment, differentiation and functional polarization of immune cells. Interleukin-6 (IL-6): A pleiotropic cytokine with pivotal role in hepatic inflammation and oncogenesis, IL-6 promotes STAT3 activation facilitating tumor cell proliferation, survival and resistance to apoptosis [26]. Moreover IL-6 contributes to expansion of MDSCs and suppression of antigen presenting cells. Interleukin-10 (IL-10): As a key immunoregulatory cytokine IL-10 inhibits Th1 cytokine production and antigen presentation, thereby fostering immune tolerance. In the HCC TME IL-10 is secreted by TAMs and Tregs playing central role in dampening effector T cell responses. [27,28]. Transforming Growth Factor-beta (TGF-β): TGF-β serves dual roles in hepatic carcinogenesis. While acting as tumor suppressor during early stages, it promotes tumor progression in advance HCC by inducing epithelial mesenchymal transition (EMT), angiogenesis and immune evasion. TGF-β also modulates immune cells recruitment and differentiation enhancing suppressive capacity of Tregs and inhibiting cytotoxic T lymphocyte activity (Table 1) [29,30].

**Hypoxia and Oxidative Stress in the TME**

Hypoxia is a hall mark of solid tumors and exerts profound effect on immune landscape of HCC. Hypoxic regions within tumors stabilize hypoxia inducible factors (HIFs), particularly HIF-1α which transcriptionally regulate genes involved in angiogenesis (eg VEGF), glycolysis and immune modulation [31,32]. Hypoxia impairs dendritic cell maturation and T cell effector functions, while simultaneously enhances Treg recruitment and PD-L1 expression on tumor cells, thus involve in immune suppression. Oxidative stress, marked by increase levels of reactive oxygen species (ROS), disrupts redox homeostasis and augments DNA damage, lipid peroxidation and mutagenesis in hepatic tissues. Within TME, ROS also modulate signaling cascades such as NF-κB and MAPK pathways, promoting inflammation, immune suppression and tumor survival. Moreover, oxidative stress can inhibit cytotoxic immune responses by induce T cell exhaustion and apoptosis, thereby further exacerbate immune evasion in HCC (Fig. 1; Table 1) [33,34] .

**Table 1 Key immune and molecular components contribute to the tumor niche in HCC.**

| Component | Subtype/Factor | Major Functions in HCC TME | Immunological Impact | References |
|---|---|---|---|---|
| TAMs | M2-like macrophages | Secrete IL-10, TGF-β, VEGF | Enhance neovascularization, inhibit cytotoxic T cell activity, Indicate adverse prognosis | [35,36] |
| Tregs | $CD4^+CD25^+FOXP3^+$ | Secrete IL-10, TGF-β | Inhibit effector T cell activity, contribute to immune tolerance | [37,38] |
| MDSCs | Monocytic and granulocytic types | Produce Arg-1, NO, ROS | Inhibit T and NK cells, impair DC function | [20,39] |
| DCs | Immature and tolerogenic DCs | Downregulate CD80, CD86 | Impaired antigen-mediated T cell activation | [40,41] |
| NK Cells | $CD56^+CD16^+$ cytotoxic cells | Reduced NKG2D, increased PD-L1 | Loss of cytotoxicity, reduced IFN-γ secretion | [42,43] |
| IL-6 | Pleiotropic cytokine | Activates STAT3, promotes proliferation | Enhances tumor growth and suppresses APCs | [44,45] |

| | | | | | |
|---|---|---|---|---|---|
| | IL-10 | Anti-inflammatory cytokine | Secreted by Tregs, TAMs | Inhibits Th1 responses, dampens CTL activation | [46,47] |
| | TGF-β | Multifunctional cytokine | Induces EMT, angiogenesis | Promotes immune evasion, enhances Treg activity | [48,49] |
| | Hypoxia | HIF-1α | Induces VEGF, PD-L1, glycolysis | Impairs DC and T cell function, enhances immune suppression | [50,51] |
| stress | Oxidative | ROS | Activates NF-κB, MAPK | Promotes inflammation, T cell exhaustion and apoptosis | [52,53] |
| es | Chemokin | CCL2, CXCL12, CXCL9/10 | Recruit immune and stromal cells | Facilitate infiltration of Tregs, MDSCs; modulate immune landscape | [54,55] |
| Cells | Stromal | Cancer-associated fibroblasts (CAFs) | Secrete ECM components and cytokines | Support tumor growth, modulate immune infiltration | [56,57] |

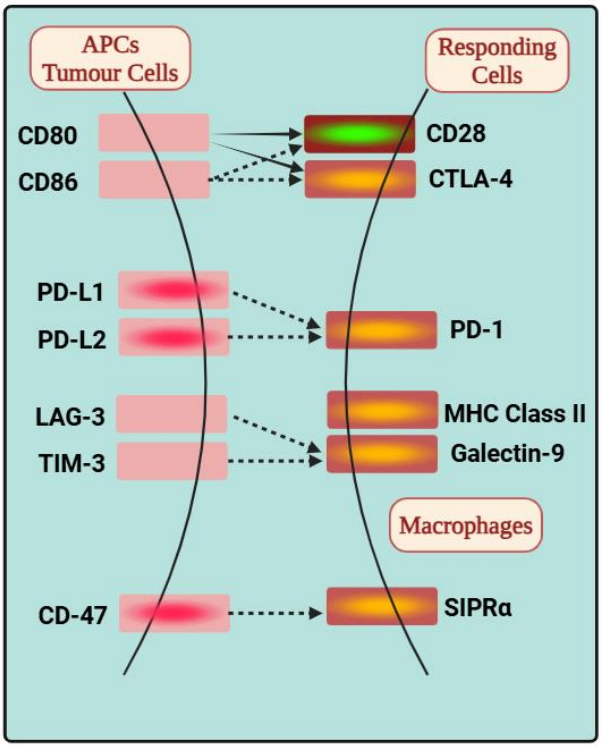


Fig. 1. Schematic representation of immune checkpoint interactions between antigen-presenting cells (APCs)/tumor cells and responding immune cells. Key inhibitory pathways include CD80/CD86–CTLA-4, PD-L1/PD-L2–PD-1, LAG-3–MHC class II, TIM-3–Galectin-9, and CD47–SIRPα, which collectively suppress antitumor immunity and contribute to immune evasion in the tumor microenvironment.

**Molecular Mechanisms of Immune Evasion in HCC**

Hepatocellular carcinoma (HCC) exemplifies a malignancy that skilfully subverts immune surveilance through a multifaceted network of suppressive pathways [22]. The complex interactions between immunoregulatory checkpoints, oncogenic signaling pathways, and epigenetic alterations establish an immunosuppressive TME in HCC that fosters tumor progression and resistance to therapy. This section focuses on the principal molecular pathways that facilitate immune

evasion, including the PD-1/PD-L1 and CTLA-4 axes, Wnt/β-catenin signaling, TGF-β–driven immunosuppression, and epigenetic regulation of immune checkpoints (Table 2; Fig. 2) [58,59].

**PD-1/PD-L1 and CTLA-4 Pathways**

PD-1, expressed on activated T cells, binds to PD-L1 and PD-L2, often upregulated in HCC tumor cells and infiltrating immune cells, transmitting inhibitory signals that impair TCR signaling and induce T cell exhaustion. Elevated PD-L1 correlates with poor prognosis, higher tumor grade, and reduced cytotoxic T cell infiltration (Fig. 2; Table 2). Similarly, CTLA-4 on Tregs and activated T cells binds B7 ligands (CD80/CD86) on APCs, blocking CD28 co-stimulation and enhancing Treg-mediated immunosuppression. Together, PD-1 and CTLA-4 overexpression creates an immunologically "cold" TME, reducing HCC responsiveness to conventional immunotherapies [60,61].

**Wnt/β-Catenin Signaling and Immune Exclusion**

Dysregulated Wnt/β-catenin signaling, commonly driven by mutations in CTNNB1 or AXIN1 in HCC, represent a key mechanism underlying immune exclusion. Activation and nuclear localization of β-catenin promote transcriptional programs that impair dendritic cell (DC) recruitment and maturation, limiting the priming of antigen-specific T cells. Tumors with active Wnt/β-catenin signaling frequently lack $CD103^+$ cross-presenting DCs, resulting in reduced infiltration of cytotoxic $CD8^+$ T cells and a diminished immune presence within the tumor microenvironment (Fig. 2). This immune-desert phenotype is a major factor contributing to the resistance of certain HCC cases to immune checkpoint blockade therapies (Table 2) [62,63].

**TGF-β Signaling and Immune Suppression**

Transforming growth factor-beta (TGF-β), a multifunctional cytokine with context-specific effects, is a key regulator of immune tolerance within the hepatic tumor microenvironment. In hepatocellular carcinoma, TGF-β facilitates the conversion of naïve $CD4^+$ T cells into $FOXP3^+$ regulatory T cells while concurrently suppressing the cytotoxic functions of $CD8^+$ T cells and natural killer (NK) cells. It also hampers antigen presentation by reducing MHC class I expression and co-stimulatory molecules on dendritic cells. Mechanistically, TGF-β engages both SMAD-dependent and SMAD-independent pathways, including MAPK and PI3K/AKT signaling, leading to the transcription of immunosuppressive genes. Additionally, this pathway promotes epithelial-mesenchymal transition (EMT), contributing to tumor invasiveness and resistance to immune-mediated cell killing. Targeting TGF-β signaling or its downstream effectors is being explored as a strategy to improve the efficacy of immunotherapies in HCC (Fig. 2) [64,65].

**Epigenetic Regulation of Immune Checkpoints**

Epigenetic modifications encompassing DNA methylation, histone post-translational modifications, and non-coding RNAs constitute another critical layer of immune modulation in HCC. Tumor cells exploit these mechanisms to silence genes encoding tumor-associated antigens (TAAs), major histocompatibility complex (MHC) molecules, and pro-inflammatory cytokines, thereby evade immune recognition. Hypermethylation of promoter regions in genes such as CD8A, IFNG, and HLA class I molecules contributes to functional impairment of tumor-infiltrating lymphocytes (TILs). Additionally, histone deacetylase (HDAC)-mediated chromatin remodeling has been evidenced to counteract PD-L1 transcription, paradoxically rendering tumors resistant to PD-1 blockade while maintaining a suppressive milieu. Non-coding RNAs, such as microRNAs (e.g., miR-146a, miR-23a) and long non-coding RNAs (e.g., lncRNA SNHG20), further modulate immune checkpoint expression and T cell function through post-transcriptional regulation. Therapeutic potential of epigenetic reprogramming, used singly or combined with immune checkpoint therapies, hold promise in overcoming the immune inertia characteristic of HCC.

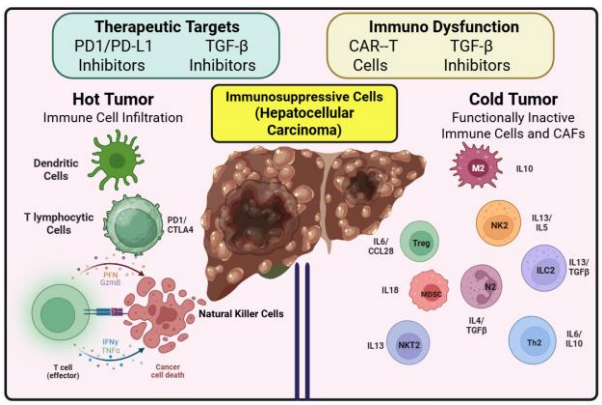


Fig. 2. Immune landscape of hepatocellular carcinoma (HCC) showing hot and cold tumor microenvironments. Hot tumors exhibit immune cell infiltration (dendritic cells, T lymphocytes, natural killer cells) that promote cancer cell death, whereas cold tumors harbor immunosuppressive cells (M2 macrophages, Tregs, MDSCs, NK2, NKT2, Th2, ILC2) and CAFs that

release cytokines (IL-6, IL-10, IL-13, IL-18, TGF-β) to drive tumor progression. Therapeutic targets include PD-1/PD-L1 and TGF-β inhibitors, and CAR-T cell therapy.

**Table 2: Key molecular mechanisms of immune evasion in HCC**

| Pathway | Key Molecules/Components | Mechanism of immune evasion | Effect on immune system | Clinical implications | References |
|---|---|---|---|---|---|
| PD-1/PD-L1 & CTLA-4 | PD-1, PD-L1, PD-L2, CTLA-4, CD80/CD86, SHP2 | Inhibitory checkpoint signaling attenuates TCR activation; suppresses T-cell proliferation and cytokine production | ↓ $CD8^+$ T cell activity, ↑ Tregs, ↓ IFN-γ and IL-2 | Resistance to immunotherapy, poor prognosis | [66] |
| Wnt/β-Catenin Signaling | Wnt ligands, Frizzled receptors, β-Catenin, CCL5 | Nuclear β-catenin represses chemokines essential for DC recruitment; immune cell exclusion | ↓ Dendritic cell infiltration, ↓ T-cell priming, "cold" tumor phenotype | Poor response to immune checkpoint blockade | [67] |
| TGF-β Signaling | TGF-β, TGFBR1/2, SMAD2/3 | Inhibits cytotoxic immune cell function; promotes Treg and MDSC recruitment | ↓CTL/NK cell cytotoxicity, ↑ Tregs/MDSCs, ↓ antigen presentation | EMT induction, metastasis, immunotherapy resistance | [8,68] |
| Epigenetic Regulation | DNMTs, HDACs, microRNAs (miR-200, miR-34), histone modifiers | Alters transcription of immune checkpoints and antigen presentation machinery | ↓MHC expression,↑ PD-L1/CTLA-4 expression, ↓ immunogenicity | Epigenetic therapies may enhance immunotherapy efficacy | [69] |
| JAK/STAT3 Pathway | IL-6, STAT3, SOCS3 | Promotes immunosuppressive cytokine production and PD-L1 expression | ↑ T-cell exhaustion, ↑ suppressive cytokines, ↓ antigen presentation | Linked with inflammation-driven immune escape and therapy resistance | [70,71] |
| Hypoxia-Induced Immunosuppression | HIF-1α, VEGF, Adenosine A2A receptor | Induces PD-L1 expression and suppresses APCs under low oxygen conditions | ↓ Dendritic cell activation, ↑ Tregs and MDSCs | Stimulates neovascularization and contributes to immune evasion in hypoxic tumor niches. | [72] |
| IDO Pathway | IDO1, Tryptophan, Kynurenine | Tryptophan catabolism suppresses effector T-cell proliferation | ↑ T-cell anergy, ↑ Tregs, ↓ CTL response | Correlates with immune tolerance and escape mechanisms in HCC | [73] |

| | | | | | |
|---|---|---|---|---|---|
| Autophagy and Antigen Presentation | LC3, ATG proteins, MHC I/II | Obstructs the processing and loading of antigens, reducing their display. | ↓ T-cell recognition and activation | Contributes to immune evasion and reduced immunotherapy response in advanced tumors | [74] |

**Immunotherapy in HCC: Molecular Approaches**

The emergence of immunotherapy approaches have transformed the treatment paradigms for hepatocellular carcinoma (HCC), a cancer that have long been refractory to standard systemic treatments. At the molecular level, HCC exhibits a highly immunosuppressive tumor microenvironment (TME), highlight the need for novel strategies to reinstate effective antitumor immune responses. Recent advancements, including immune checkpoint inhibitors, chimeric antigen receptor T-cell (CAR-T) therapies, neoantigen-targeted vaccines, and combination treatments with precision-targeted drugs, have demonstrated encouraging therapeutical outcomes [75].

**Immune Checkpoint Blockade Therapy**

Immune checkpoints are inhibitory signaling pathways that control the strength and duration of immune responses, playing crucial role in maintaining self-tolerance and preventing autoimmune reactions. In hepatocellular carcinoma (HCC), however, the upregulation of checkpoint molecules such as programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) facilitates immune escape by promoting T-cell dysfunction and exhaustion. Nivolumab, a fully human IgG4 monoclonal antibody that targets the PD-1 receptor, has shown sustained therapeutic responses and an acceptable safety profile in advanced HCC, especially among patients who did not respond to sorafenib (Fig. 3) [76]. Similarly, pembrolizumab, another anti-PD-1 monoclonal antibody, has exhibited promising anti-cancer effects along with tolerable adverse events in the KEYNOTE clinical trials. Atezolizumab, a humanized IgG1 antibody against PD-L1, has been effectively combined with bevacizumab (an anti-VEGF antibody), demonstrating the potential benefits of concurrently targeting immune checkpoints and angiogenesis pathways [77].

**CAR-T Cell Therapy Targeting GPC3**

Adoptive cell transfer has been revolutionized by CAR T-cell therapy, which allows T cells to be engineered to target tumor-associated antigens (TAAs) without reliance on major histocompatibility complex presentation. In HCC, glypican-3 (GPC3), a heparan sulfate proteoglycan that is highly expressed on cancerous liver cells but not on healthy liver tissue, serves as a precise antigen target. CAR-T cells directed against GPC3 have shown potent tumor-killing effects in both preclinical studies and early clinical trials for HCC [78]. These engineered T cells exhibit enhanced proliferation, cytokine release, and tumor infiltration, although their efficacy is frequently curtailed by immunosuppressive TME. Therefore, strategies such as armored CAR-Ts and the synergistic potential of immune checkpoint inhibitors alongside other treatments are being evaluated to optimize patient outcomes [79].

**Personalized Neoantigen Vaccines**

Neoantigens, derived from tumor-specific somatic mutations, represent an attractive class of immunogenic peptides that can trigger highly specific cytotoxic T-cell responses. Personalized neoantigen vaccines exploit next-generation sequencing (NGS) and bioinformatic algorithms to identify patient-specific mutation-derived epitopes, which are subsequently synthesized and administered to induce a targeted immune response [80,81]. Although HCC has relatively low mutational burden compared to other solid tumors, certain driver mutations and viral epitopes (in HBV- and HCV-related HCC) have been identified as viable targets. Preliminary data suggest that neo-antigen based immunization can potentiate antigen-specific T-cell-mediated responses, especially when paired with checkpoint inhibition, enhance anti-tumor activity underscoring the relevance of individualized immunotherapeutic strategies [82].

**Combination Therapy with Molecular Targeted Agents**

The rationale for combinatorial regimens lies in the multidimensional interplay between oncogenic signaling pathways and immune regulation. Tyrosine kinase inhibitors (TKIs) such as sorafenib, lenvatinib, and regorafenib, initially employed for their antiangiogenic and antiproliferative properties, have demonstrated immunomodulatory effects, including depletion of regulatory T cells, attenuation of MDSCs, and enhancement of antigen presentation. Sorafenib, for instance, inhibits RAF kinases and VEGFR, leading to normalization of tumor vasculature and improved immune cell infiltration. In conjunction with immune checkpoint blockade, this two-pronged approach works synergistically to recondition the tumor microenvironment, enhancing T-cell responses and restoring immune competence. The IMbrave150 trial exemplifies the clinical relevance of this approach, where atezolizumab plus bevacizumab exhibited superior overall survival and progression-free survival compared to sorafenib alone [83,84].

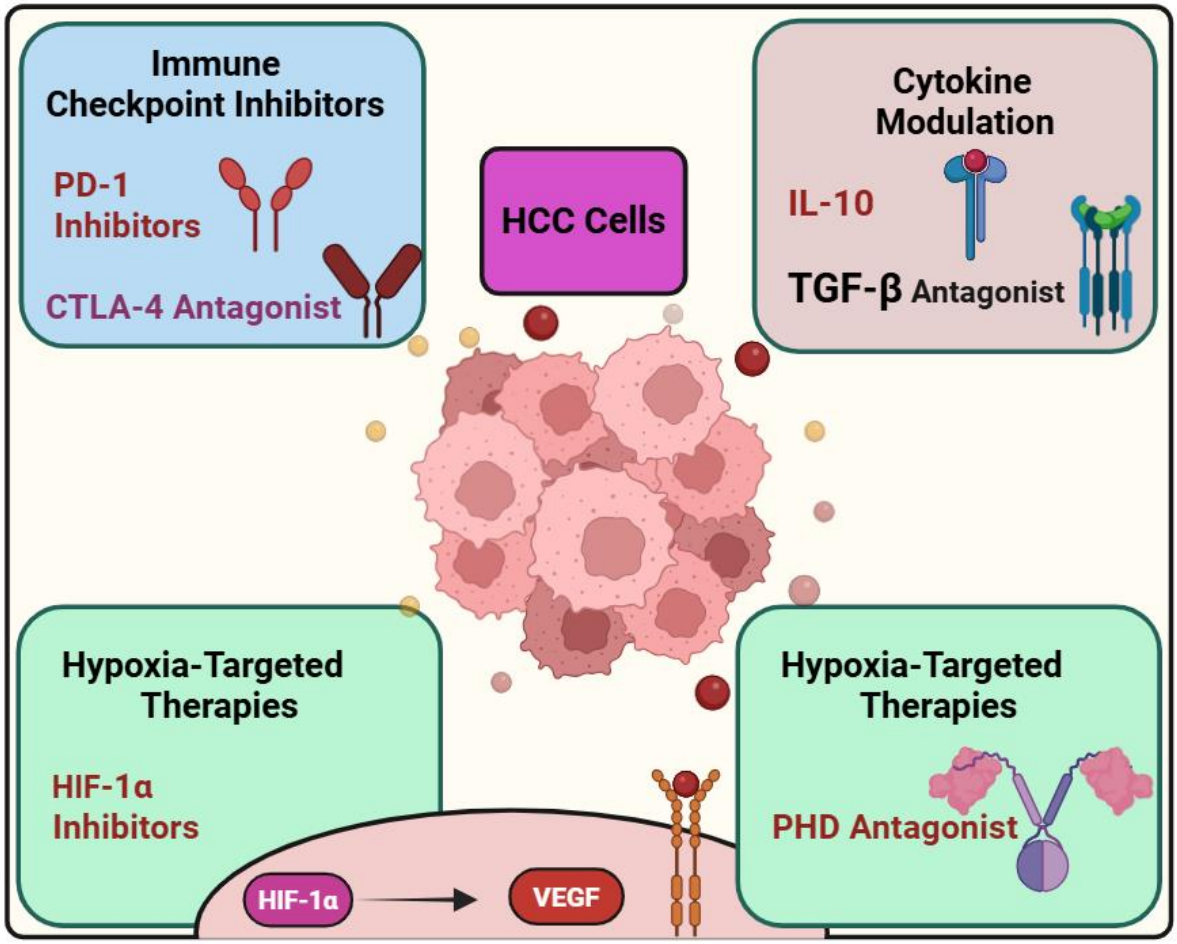


Fig. 3. Schematic representation of therapeutic strategies for hepatocellular carcinoma (HCC), including immune checkpoint inhibitors (PD-1, CTLA-4), cytokine modulation (IL-10, TGF-β), and hypoxia-targeted therapies (HIF-1α, PHD antagonists) to suppress tumor progression and enhance treatment efficacy.

# PROSPECTIVE DIRECTIONS IN HCC IMMUNO-INTERVENTIONS

**Biomarkers for Response Prediction**

The heterogeneity nature of HCC presents considerable challenges in predicting immunotherapy success. There remains a continuous need to identify reliable biomarkers capable of forecasting patient responses [85]. Programmed death-ligand 1 (PD-L1) expression, while employed in various malignancies, has shown inconsistent predictive value in HCC due to its dynamic expression and contextual modulation within the tumor microenvironment (TME). Tumor mutational burden (TMB) and microsatellite instability (MSI) are emerging as potential predictors in other cancers; however, their prevalence and prognostic importance in HCC are limited [86,87]. Recent investigations have emphasized the relevance of immune-related gene signatures, such as interferon-gamma (IFN-γ)-related transcripts, and the intratumoral ratio of effector T cells to regulatory T cells (Tregs) as prospective biomarkers. Additionally, circulating tumor DNA (ctDNA), exosomal microRNAs, and immune exclusion—linked to this pathway via its negative effects on dendritic cell infiltration and antigen presentation—are under evaluation for their prognostic and predictive significance. A multiparametric approach integrating genomic, transcriptomic, and immunophenotypic datasets may provide a more robust framework for stratifying patients [88,89].

**Mechanisms of Resistance to Immunotherapy**

Despite the transformative promise of immune checkpoint inhibitors (ICIs), a significant subset of HCC patients exhibit primary or acquired resistance. Multiple molecular and immunological mechanisms underlie this resistance. Constitutive activation of the Wnt/β-catenin signaling pathway has been implicated in immune exclusion by impairing dendritic cell recruitment and antigen presentation [90,91]. Moreover, upregulation of alternative immune checkpoints, including TIM-3, LAG-3, and VISTA, can bypass PD-1/PD-L1 blockade. The immunosuppressive milieu, characterized by elevated transforming growth factor-beta (TGF-β), indoleamine 2,3-dioxygenase (IDO), and prostaglandin E2 (PGE2), further suppresses cytotoxic T lymphocyte (CTL) function. Epigenetic alterations and aberrant metabolic reprogramming within the TME—such as hypoxia-driven adenosine accumulation and lactate-mediated T cell anergy—also contribute to immune

evasion. Overcoming these challenges requires combinatorial therapeutic strategies targeting multiple immune regulatory axes [92].

**Role of Gut Microbiota and the Liver Immune Axis**

The gut-liver axis plays a crucial role in regulating systemic and liver-specific immune responses, being continually influenced by microbial metabolites and pathogen-associated molecular patterns (PAMPs) delivered through the portal circulation. Dysbiosis, an imbalance in gut microbiota, has been associated with chronic liver disease development and hepatocarcinogenesis. Certain bacterial species, including Akkermansia muciniphila and Faecalibacterium prausnitzii, correlate with improved responsiveness to immune checkpoint inhibitors (ICIs), whereas overgrowth of gram-negative bacteria may exacerbate systemic inflammation and promote T cell dysfunction [93]. Microbial components such as lipopolysaccharide (LPS) modulate Toll-like receptor (TLR) signaling, influencing activation of hepatic antigen-presenting cells and cytokine release. Consequently, the gut microbiome represents a modifiable factor impacting immunotherapy efficacy. Interventions including fecal microbiota transplantation (FMT), probiotic supplementation, and dietary modifications are under investigation to enhance immunotherapeutic outcomes by reshaping gut-liver immune interactions (Table 3) [94,95].

**Emerging Molecular Targets and Gene Editing Tools**

The advancing field of immuno-oncology has identified novel molecular targets beyond classical immune checkpoints. Molecules such as TIGIT, BTLA, and CD73 are gaining prominence as next-generation immunomodulatory candidates [96]. Furthermore, tumor-associated antigens including Glypican-3 (GPC3), alpha-fetoprotein (AFP), and heat shock proteins (HSPs) are being explored for vaccine development and chimeric antigen receptor T-cell (CAR-T) therapy applications. Concurrently, breakthroughs in gene editing technologies, notably CRISPR/Cas9, have substantially enhanced precise modification capabilities of both immune cells and tumor genomes. CRISPR-mediated disruption of immune checkpoint genes in T cells or upregulation of antigen presentation pathways in cancer cells offers promising avenues to potentiate antitumor immunity (Table 3) [97,98]. Additionally, epigenetic editing tools targeting enzymes involved in DNA methylation and histone modifications are being designed to reverse immune-evasive cancer phenotypes. Although predominantly in preclinical or early clinical phases, these approaches present transformative opportunities to overcome immunotherapy resistance and personalize cancer treatment strategies [99].

**Table 3: Key challenges and future directions in liver cancer immunotherapy.**

| Focus area | Key issues | Examples / insights | Future directions | References |
|---|---|---|---|---|
| Biomarkers | Lack of predictive markers | PD-L1, TMB, Wnt/β-catenin | Immune gene panels, T-cell profiling | [100,101] |
| Resistance | Therapy non-responsiveness | T-cell exhaustion, IL-10, TGF-β | Combination and sequential therapies | [102,103] |
| Gut–Liver Axis | Microbiota impact on immunity | SCFAs, dysbiosis | Microbiome modulation, FMT | [104,105] |
| Gene Editing | Need for precision therapy | CRISPR-PD-1 KO, neoantigen targeting | CRISPR-edited T cells, liver-specific targets | [97,106] |
| Tumor Heterogeneity | Varying immune profiles | Immune “hot” vs. “cold” tumors | Personalized immunotherapy | [107,108] |
| Immune Suppressive TME | Inhibits T cell function | Tregs, MDSCs, hypoxia | TME reprogramming, TAM targeting | [109,110] |
| Checkpoint Redundancy | Multiple inhibitory pathways | TIM-3, LAG-3 | Dual/multi-checkpoint blockade | [111,112] |
| Limited Clinical Response | Low response rates in trials | <25% response to monotherapy | Early biomarkers, better trial designs | [113,114] |

# CONCLUSION

Immunotherapy has emerge as a revolutionary strategy in the managment of hepatocellular carcinoma (HCC), offering alternative therapeutic options beyond traditional modalities such as chemotherapy, surgical resection, and targeted molecular therapies. By modulating the patient’s immune system,

immunotherapeutic interventions—including immune checkpoint blockade, adoptive cell therapies, and therapeutic cancer vaccines—have demonstrated promising clinical outcomes, especially in patients with advanced liver cancers. Nevertheless, despite these progresess, a considerable proportion of patients either show initial nonresponse or develop acquired resistance, underscoring the urgent necessity for a deeper comprehension of immune-related mechanisms in HCC. The liver's intrinsically immunotolerant environment, shaped by persistent exposure to microbial and dietary antigens derived from the gut, presents a substantial challenge for effective immune activation against tumor cells. This complexity is further compounded by the dynamic interplay between malignant cells and immunosuppressive components within the tumor microenvironment (TME), including tumor-associated macrophages (TAMs), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs). These immune cells, along with suppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), form a complex network that promotes tumor immune evasion and facilitates disease progression. In this context, integration of molecular biology with immunological investigations provides valuable insights into mechanisms underpinning immune escape and offers opportunities for the identification of novel therapeutic targets. High-throughput techniques such as genomic, transcriptomic, and epigenetic analyses have revealed critical alterations in pathways including Wnt/β-catenin, PI3K/AKT, and TGF-β signaling, which not only contribute to tumorigenesis but also regulate immune response modulation. The incorporation of these molecular findings into immunotherapy design could enhance patient stratification, improve prediction of therapeutic responses, and support development of personalized and more efficacious treatment approaches.

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The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Acknowledgment

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Availability of data and materials

This published article and its supplementary materials include all data generated or analyzed during this study.

**Ethical Approval**
Not applicable.

**Consent to participate**
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**Consent to publication**

The author read and approved the final content

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