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Resistance to immune checkpoint inhibitors in hepatocellular carcinoma: mechanisms and combination strategies to overcome it

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Hepatocellular carcinoma, immune checkpoint inhibitors, drug resistance, combination therapy

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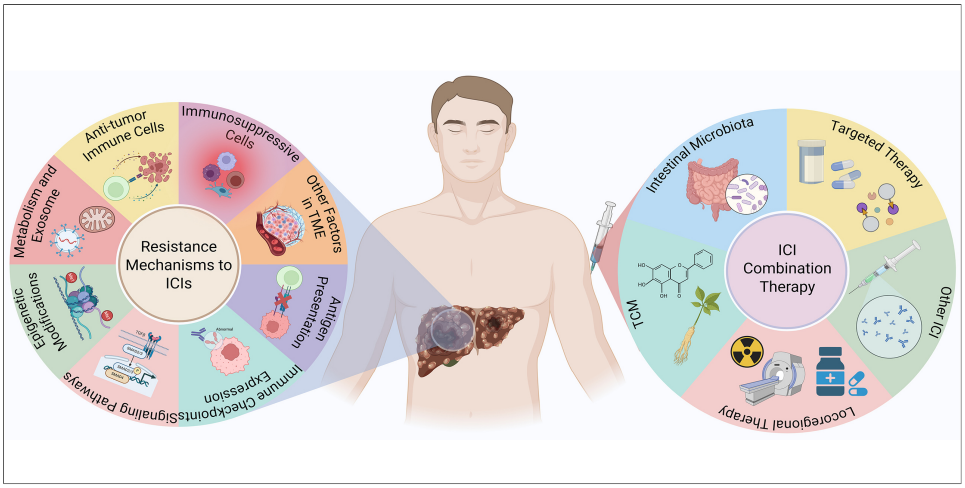

Abstract

Hepatocellular carcinoma (HCC) is predominantly diagnosed at an advanced stage and ranks as the third leading cause of cancer-related mortality worldwide. In recent years, the advent and clinical application of immune checkpoint inhibitors (ICIs) have markedly transformed the therapeutic landscape of HCC, yielding superior overall survival (OS) outcomes compared with conventional targeted therapies. Nevertheless, clinical benefit is limited to a subset of patients, largely due to drug resistance. Current evidence implicates both the tumor microenvironment and tumor cell-intrinsic factors as pivotal determinants of ICI resistance. Combination strategies have emerged as a central approach in HCC therapy, significantly enhancing the antitumor activity of ICIs. Notably, ICIs plus targeted therapy (anti-angiogenic agents), dual-ICI blockade, and ICI combined with locoregional therapy (transarterial chemoembolization) currently represent the most well-established and clinically validated approaches, being approved as first- or second-line treatments. In addition, complementary approaches, including traditional Chinese medicine and modulation of intestinal microbiota, demonstrate synergistic antitumor effects when integrated with ICI, representing promising avenues for further therapeutic optimization. This review mainly elucidates the underlying mechanisms of ICI resistance and examines

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both established and emerging combination strategies for HCC. Furthermore, it explores potential approaches to enhance the efficacy of ICI-based regimens, with the aim of improving long-term outcomes for patients with advanced HCC.

INTRODUCTION

Liver cancer remains a highly aggressive malignancy with persistently high global incidence and mortality rates, and hepatocellular carcinoma (HCC) accounts for more than 90% of all diagnosed cases[1]. Patients with HCC often face critical clinical challenges, such as late diagnosis, high rates of recurrence, and poor prognosis. With a 5-year survival rate for advanced HCC below 20%, it ranks as the third most common cause of cancer-related death globally[2]. Therefore, the development of highly effective therapeutic strategies for HCC remains a critical and urgent clinical priority.

Traditional therapeutic strategies for HCC primarily encompass surgical resection, liver transplantation, radiotherapy, chemotherapy, and targeted therapy[3]. Surgical resection is currently the preferred treatment for liver cancer, yet approximately 70% of patients experience recurrence within five years post-surgery, significantly impacting survival time[4]. Patients who underwent liver transplantation typically exhibit high survival rates and low recurrence rates. However, due to organ shortages and risk of immune rejection, only around 20% of HCC patients are eligible for liver transplantation[5]. While radiotherapy and chemotherapy can effectively inhibit tumor cell proliferation, they often damage normal tissues and generate toxic side effects due to limited target specificity[6]. Targeted therapy primarily includes multi-kinase inhibitors such as sorafenib and lenvatinib, which can effectively suppress HCC progression and reduce side effects. However, the development of drug resistance to targeted therapy makes it unsuitable for long-term use in HCC treatment[7].

Immunotherapy, represented by immune checkpoint inhibitors (ICIs), is a novel and highly effective treatment strategy for HCC. ICIs are designed to disrupt key molecular mechanisms involved in tumor immune evasion, thereby potentiating the host's immune response against cancer. As an inflammation-driven cancer, HCC represents a promising target for immunotherapeutic interventions. ICI targeting programmed cell death protein 1 or its ligand (PD-1/PD-L1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) have already been incorporated into clinical practice for advanced HCC, effectively improving patient survival. Moreover, a series of emerging immune checkpoints, including T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), lymphocyte activation gene-3 (LAG-3), T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), V-domain Ig suppressor of T cell activation (VISTA), B7-H3 (CD276), B and T lymphocyte attenuator (BTLA), and indoleamine-2,3-dioxygenase 1 (IDO1), have been identified, and blockade of these checkpoints also demonstrates encouraging therapeutic potential in HCC patients[8-14] [Figure 1 and Table 1]. Nonetheless, emerging evidence suggests that ICI therapy is susceptible to drug resistance, posing a significant challenge to HCC immunotherapy. This review will elucidate the mechanisms underlying resistance to ICIs and explore combination strategies to overcome it, with the aim of providing new insights and therapeutic avenues to enhance ICI efficacy in HCC treatment.

RESISTANCE MECHANISMS TO ICIS IN HCC

ICI therapy has achieved considerable success in the treatment of HCC. However, only 25%-40% of HCC patients benefit from this therapy, which is largely attributable to ICI resistance[15]. Based on the origin of factors contributing to ICI resistance, the underlying mechanisms primarily fall into two categories: the immunosuppressive tumor microenvironment (TME) [Figure 2] and tumor cell-intrinsic changes [Figure 3]. These mechanisms primarily involve immune system dysfunction, remodeling of the immune microenvironment, lack of neoantigens or impaired antigen presentation, dysregulated signaling pathways, metabolic reprogramming, expression of alternative immune checkpoints, and epigenetic modifications.

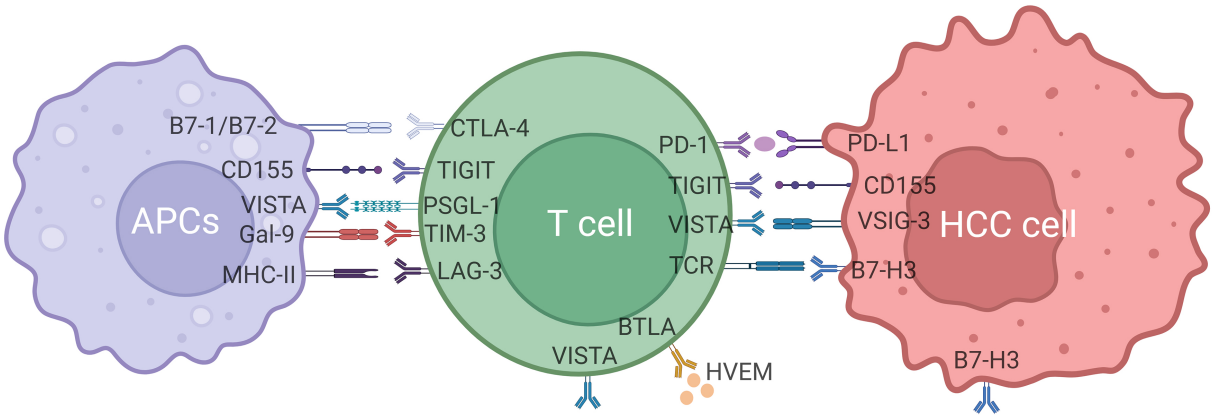

Figure 1. Immune checkpoints and their ligands inducing immune escape of HCC cells. Created with BioRender. H, T. (2025) https://BioRender.com/ko8kl51. PD-1: Programmed cell death protein 1; PD-L1: programmed cell death ligand 1; TIM-3: T-cell immunoglobulin and mucin domain containing 3; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; LAG-3: lymphocyte-activation gene-3; TIGIT: T-cell immunoreceptor with immunoglobulin and ITIM domains; VISTA: V-domain Ig suppressor of T cell activation; BTLA: B and T lymphocyte attenuator; B7-H3: CD276.

Table 1. Immune checkpoints and inhibitors used for HCC therapy

Target	Drug name	Approval status
PD-1	Nivolumab	FDA approved (2017)
	Pembrolizumab	FDA approved (2018)
	Tislelizumab	Approved in China (2024)
	Camrelizumab	Approved in China (2020)
	Sintilimab	Approved in China (2021)
	Toripalimab	In clinical trials
	Nofazinlimab	In clinical trials
PD-L1	Atezolizumab	FDA approved (2017)
	Durvalumab	In clinical trials
	Avelumab	In clinical trials
	Sugemalimab	In clinical trials
	Envafolimab	In clinical trials
	Adebelimumab	In clinical trials
	TQB2450	In clinical trials
CTLA-4	Ipilimumab	FDA approved (2020, combination therapy)
	Tremelimumab	FDA approved (2022, combination therapy)
	IBI310	In clinical trials
VISTA	CA-170	In clinical trials
TIGIT	Tiragolumab	In clinical trials
LAG-3	Relatlimab	In clinical trials
BTLA	Tifcemalimab	In clinical trials
TIM-3	Sabatolimab	In clinical trials
	Cobolimab	In clinical trials
B7-H3	Enoblituzumab	In clinical trials

PD-1: Programmed cell death protein 1; PD-L1: programmed cell death ligand 1; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; VISTA: V-domain Ig suppressor of T cell activation; TIGIT: T-cell immunoreceptor with immunoglobulin and ITIM domains; LAG-3: lymphocyte-activation gene 3; BTLA: B and T lymphocyte attenuator; TIM-3: T cell immunoglobulin and mucin-domain containing-3; B7-H3: CD276.

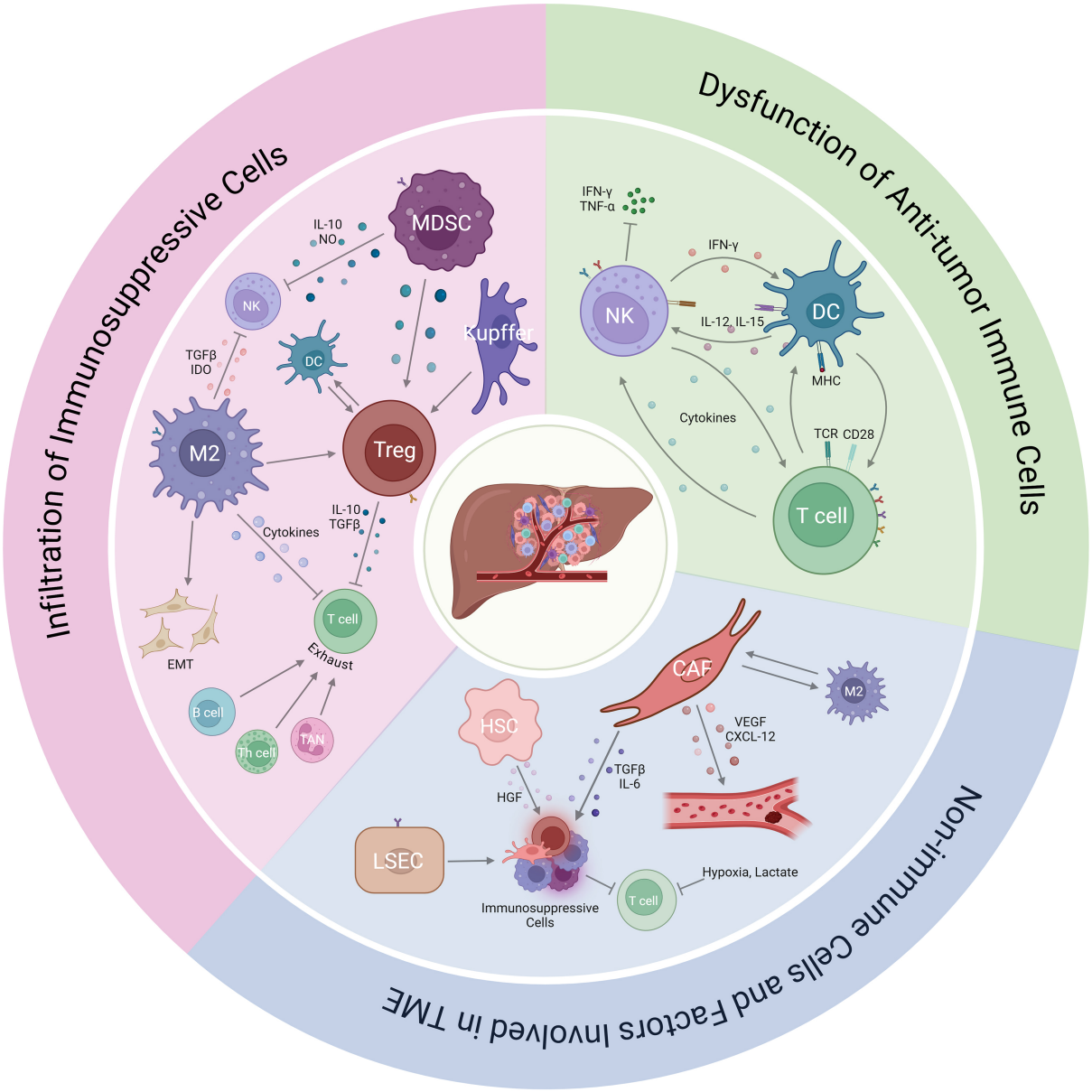

Figure 2. Immunosuppressive TME involved in ICI resistance. Created with BioRender. H, T. (2025) https://BioRender.com/1q198aq. MDSC: Myeloid-derived suppressor cells; NK: natural killer cells; Treg: regulatory T cells; DC: dendritic cells; M2: M2 macrophages; TAN: tumor-associated neutrophils; Th: helper T cells; HSC: hepatic stellate cells; LSEC: liver sinusoidal endothelial cells; CAF: cancer-associated fibroblasts; EMT: epithelial-mesenchymal transition.

IMMUNOSUPPRESSIVE TME

Dysfunction of antitumor immune cells

Among immune cells, cytotoxic $CD8^+$ T cells, dendritic cells (DCs), and natural killer (NK) cells typically exert antitumor effects, and their dysfunction significantly impairs the efficacy of immunotherapy[16]. Liver functions as a central immunomodulatory organ that generally maintains an immune-tolerant state to ensure local and systemic protection. Studies have shown that some liver cancers are "cold tumors", characterized by limited T-cell infiltration, a feature that contributes significantly to primary ICI resistance[17]. Defects in antigen-presenting cells (APCs), impaired T-cell activation, and T-cell exclusion further led to T-cell deficiency in HCC tissues[18]. Moreover, the absence of $CD8^+$ T cells in liver cancer is often accompanied by an increased frequency of exhausted $CD8^+$ T cells. These exhausted cells typically overexpress immune

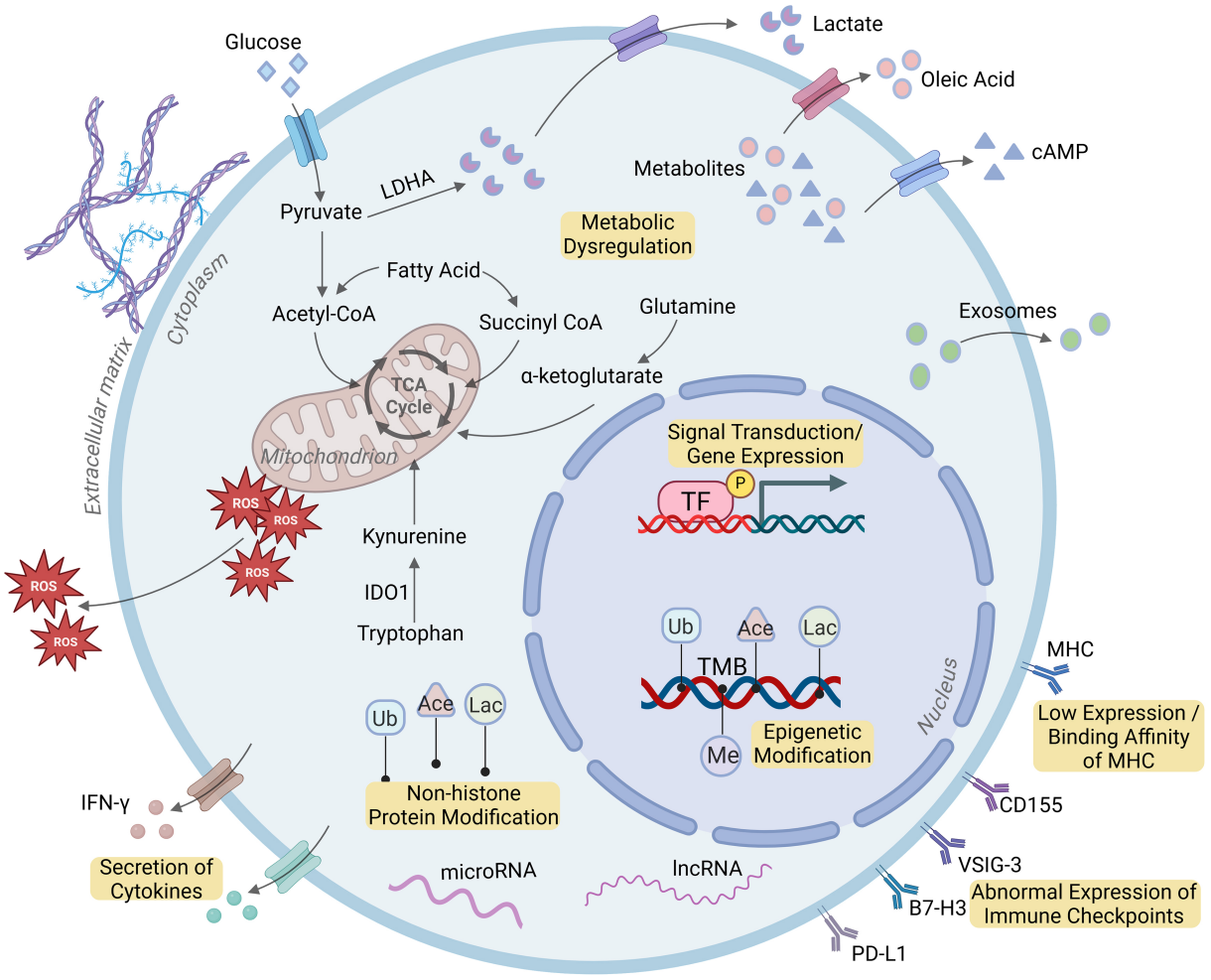

Figure 3. Tumor cell-intrinsic factors involved in ICI resistance. Created with BioRender. H, T. (2025) https://BioRender.com/aezvlck. TF: Transcription factor; Ub: ubiquitination; Ace: acetylation; Lac: lactylation; Me: methylation; cAMP: cyclic adenosine monophosphate; ROS: reactive oxygen species; TMB: tumor mutational burden; MHC: major histocompatibility complex; IDO1: indoleamine-2,3-dioxygenase 1; PD-L1: programmed cell death ligand 1; B7-H3: CD276; lncRNA: long non-coding RNA.

checkpoints such as PD-1, CTLA-4, TIM-3, LAG-3, and TIGIT, resulting in immune tolerance. DCs contribute to antitumor immunity by presenting antigens to T cells. However, immature DCs may promote tumor tolerance through the induction of regulatory T cells (Tregs) and suppression of effector T cells[19]. Even mature DCs sometimes facilitate immune evasion by promoting Treg differentiation and secreting immunosuppressive cytokines[20]. Additionally, abnormal proportions or functional impairments in NK cells also drive ICI resistance. For example, HCC patients exhibit an increased population of Siglec-9$^+$ NK cells with reduced cytotoxicity and disrupted homeostasis[21]. The expression of immune checkpoints such as TIGIT and TIM-3 on NK cells, along with diminished production of cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, further attenuates the effectiveness of ICI therapy[22].

Infiltration of immunosuppressive cells

Immunosuppressive cells within the TME represent a major mechanism underlying resistance to ICIs. These cells primarily include Kupffer cells, tumor-associated macrophages (TAMs), Tregs, and myeloid-derived suppressor cells (MDSCs). Tregs act as key negative regulators of CD8$^+$ T cells, suppressing immune responses through expression of immune-inhibitory molecules such as CTLA-4, CD39, and CD73, as well as the secretion of immunosuppressive cytokines including interleukin (IL)-10 and transforming growth factor

(TGF)-β[23]. Recent studies also indicate that Tregs can induce immunosuppression via interactions with type 2 conventional DCs[24]. Other immunosuppressive cells, including Kupffer cells, TAMs, and MDSCs, promote immune tolerance through multiple mechanisms: they facilitate the proliferation, infiltration, and activation of Tregs; upregulate checkpoint ligands (e.g., PD-L1) and immunosuppressive factors such as vascular endothelial growth factor (VEGF), arginase-1 (ARG-1), and IDO1[25,26]; inhibit the activation of $CD8^+$ T cells and NK cells; and reduce the expression of major histocompatibility complex class II (MHC-II) and co-stimulatory molecules. Additionally, TAMs have been shown to confer ICI resistance by promoting epithelial-mesenchymal transition (EMT) of HCC cells or by forming macrophage-coated tumor clusters (MCTCs) that impede the infiltration of cytotoxic T cells[27,28]. Furthermore, emerging evidence suggests that other immune cells, including B cells, monocytes, helper T cells (Th), and tumor-associated neutrophils (TANs) also contribute to ICI resistance[29]. These cells dampen antitumor immunity by expressing immune checkpoints such as PD-1, TIM-3, CTLA-4, and chemokine receptor 4/6 (CCR4/CCR6), or accelerating the exhaustion of $CD8^+$ T cells.

Non-immune cells and factors involved in TME

Besides immune cells, functional alterations in other components of TME such as hepatic stellate cells (HSCs) and liver sinusoidal endothelial cells (LSECs) also contribute to ICI resistance. Studies have shown that HSCs promote the infiltration of Tregs and MDSCs into HCC tissues through the secretion of hepatocyte growth factor (HGF). Meanwhile, LSECs can suppress immune responses by expressing PD-L1 and facilitating Treg recruitment[30]. Cancer-associated fibroblasts (CAFs) represent another critical element within the TME, which secrete various cytokines, exosomes, and other effector molecules that enable tumor cells to evade immune surveillance[31]. For instance, CAFs recruit immunosuppressive cells and inhibit T cell responses through the production of TGF-β, IL-6, and chemokine ligand (CCL)2[32,33]. Furthermore, CAFs interact with TAMs to promote M2 polarization, which supports HCC proliferation and fosters an immunosuppressive environment[34]. Additionally, other TME features such as extracellular matrix remodeling, angiogenesis, the presence of immunosuppressive soluble factors, hypoxia, and the acidic conditions within tumor tissues collectively contribute to the development of ICI resistance[35,36].

TUMOR CELL-INTRINSIC FACTORS

Defects in antigen presentation

MHC ligands on tumor cells are directly recognized by T-cell receptors (TCRs) on $CD8^+$ or $CD4^+$ T cells and play a crucial role in antitumor immune responses. Reduced expression or impaired binding affinity of MHC ligands disrupts antigen presentation, representing a key mechanism of resistance to ICIs[37]. Studies have reported that downregulation of MHC ligands leads to diminished antitumor T cell activity[38]. Furthermore, HCC patients with low expression of MHC ligands often remain unresponsive to ICIs. Additionally, tumor mutational burden (TMB), defined as the total number of somatic mutations per megabase within the coding regions of the tumor genome, serves as a quantitative biomarker for neoantigen load and mutation frequency[39]. Theoretically, a higher TMB increases the diversity and immunogenicity of neoantigens, thereby enhancing T-cell-mediated tumor killing[40]. As a result, elevated TMB is associated with improved response to ICIs, whereas tumors with low TMB frequently exhibit reduced immunogenicity, which constitutes a major mechanism of primary resistance to ICIs in HCC patients[40].

Abnormal immune checkpoint expression

Tumor cells often exploit immune checkpoints (e.g., PD-L1, CTLA-4) to evade cytotoxic T-cell-mediated antitumor immunity[41]. However, the absence or low expression of immune checkpoints leads to a lack of response to ICI therapy, a phenomenon known as primary resistance. Additionally, compensatory upregulation of alternative immune checkpoints also undermines the efficacy of ICIs, typically contributing to acquired resistance. For example, treatment of HCC patients with PD-1/PD-L1 inhibitors has been shown

to upregulate the major ligand of LAG-3, resulting in immune resistance[13]. Other immune checkpoints such as TIM-3, TIGIT, and BTLA have been reported to promote T-cell exhaustion under conditions of chronic antigen stimulation or down-regulation of IFN-γ, further enhancing resistance to PD-1/PD-L1 inhibitors in HCC[10,12,14]. Moreover, increased expression of IDO1 in HCC prevents phagocytosis of macrophages, and its inhibition enhances the efficacy of anti-PD-1 therapy[8]. Recently, two emerging immune checkpoints, including B7-H3 and VISTA (PD-1 homolog), have also been shown to promote immune escape by inhibiting the infiltration and activation of T cells in HCC[11,42].

Dysregulation of immune-associated signaling pathways

The TGF-β-induced pathway represents one of the most extensively studied mechanisms underlying resistance to ICIs in HCC. First, TGF-β1 upregulates the expression of PD-L1 and B7-H3 during EMT in HCC cells[43]. Additionally, TGF-β secreted by HCC cells not only exerts inhibitory effects on NK cells and DCs, but also suppresses $CD8^+$ T cell activity and promotes its exhaustion by downregulating IFN-γ and upregulating PD-1[44,45]. Furthermore, TGF-β enhances the immunosuppressive microenvironment within HCC tissues by promoting eosinophil and mast cell chemotaxis, inducing M2-type macrophage polarization, and amplifying Tregs activity[46,47]. Abnormal activation of the WNT/β-catenin pathway is another key contributor to ICI resistance in HCC. Approximately 30% of HCC patients harbor *catenin beta-1 gene* (*CTNNB1*) mutations (encoding β-catenin), which are associated with non-response to ICIs[48]. β-Catenin activation also reduces the expression of chemokines such as CCL1 and CCL4, impairs the infiltration of DCs and antigen-specific T cells, and suppresses NK cell activity, thereby diminishing the antitumor efficacy of ICIs[49,50]. Moreover, activation of the mitogen-activated protein kinase (MAPK) signaling pathway influences both immune-cell infiltration and immune checkpoint expression. For example, the epidermal growth factor receptor (EGFR)-p38 MAPK and MAPK/nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) axes enhance PD-L1 and CD274 expression to facilitate immune escape[51-53]. Other signaling pathways including IFN-γ/janus kinase/signal transducer and activator of transcription (JAK/STAT), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), and NF-κB have also been implicated in ICI resistance through the regulation of cytokines and immune checkpoints (e.g., TGF-β, VEGF, type I interferon, PD-L1) expression, modulation of immune-cell function [e.g., NK cells, T helper 1 (Th1) cells, $CD8^+$ T cells], and promotion of Treg accumulation[54-56].

Epigenetic modifications

Epigenetic modifications influence the efficacy of ICIs primarily by regulating the expression of effector genes, and the mechanisms include DNA and histone modifications, non-coding RNAs, and chromatin remodeling. Studies have shown that DNA methyltransferases (DNMTs)-induced hypermethylation suppresses MHC class I (MHC-I) expression, leading to impaired tumor antigen presentation[57]. Additionally, histone methyltransferases [e.g., enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), SET domain bifurcated histone Llysine methyltransferase 1 (SETDB1), protein arginine methyltransferase 5 (PRMT5), and methyltransferase like 1/3 (METTL1/3)] and demethylases [e.g., lysine demethylase 1 (KDM1)] modulate the expression of PD-L1, MHC-II, and chemokines [e.g., C-X-C motif chemokine ligand 5 (CXCL5), CXCL8, CXCL10] in HCC cells. These changes inhibit the infiltration and function of NK cells and $CD8^+$ T cells while enhancing the recruitment of MDSCs[58,59]. Furthermore, histone acetylation and deacetylation modifications mediated by enzymes such as lysine acetyltransferase 2A (KAT2A) and histone deacetylase (HDAC) 8 alter the function and abundance of Tregs and $CD8^+$ T cells within the TME through chemokine regulation, and ultimately compromise ICI efficacy in HCC. Emerging evidence indicates that post-translational modifications (PTMs) on non-histone proteins also play important roles in modulating ICI response[60]. For instance, deacetylase silent information regulator 2 (SIRT2) and HDAC2 have been reported to promote immune escape by regulating the stability of fibrinogen-like protein 1 (FGL1) and the subcellular localization of PD-L1[61,62]. Moreover, high expression of HDAC1, HDAC2, and

HDAC3 in HCC patients is associated with suppressed IFN-γ pathway activity, enhanced T-cell exclusion, and increased MDSC accumulation, contributing to ICI non-responsiveness[63]. Non-coding RNAs, including microRNAs and long non-coding RNAs (lncRNAs), represent another important class of epigenetic regulators affecting ICI efficacy in HCC. For example, miR-455 and lncRNA myocardial infarction associated transcript (MIAT) have been shown to dampen antitumor immune responses by modulating PD-L1 expression[64,65].

Tumor cell-derived metabolites and exosomes

Metabolites secreted by tumor cells influence the immune status of HCC primarily by modulating the function and infiltration of immune cells within the TME. Multiple studies indicate that enhanced aerobic glycolysis in tumor cells leads to substantial lactate release, acidifying the TME and promoting the infiltration of immunosuppressive cells. Lactate also drives M2 macrophage polarization while inhibiting the activities of $CD8^+$ T cells, NK cells, and DCs[66]. Furthermore, dysregulated lipid metabolism in HCC reduces the abundance of $CD4^+$ T cells and M1 macrophages and impairs NK cell function[67,68]. For instance, in obesity-associated HCC models, tumor-derived prostaglandin E2 (PGE2) binds to the prostaglandin E2 receptor 4 (EP4) receptor on immune cells, suppressing the expression of IFN-γ and TNF-α while inducing IL-10 and IL-6[69]. Additionally, HCC-secreted oleic acid (OA) promotes an immunosuppressive phenotype in macrophages, and its inhibition enhances the antitumor effects of anti-PD-1 therapy[70]. Clinical evidence suggests that elevated asparagine or glutamine metabolism in HCC patients is associated with increased infiltration of Tregs, macrophages, and memory B cells, fostering an immunosuppressive TME[71,72]. Inhibition of glutamine metabolism significantly improves response to ICIs in HCC[72]. Moreover, the accumulation of cyclic adenosine monophosphate (cAMP) has been shown to suppress $CD4^+$ and $CD8^+$ T cell responses while enhancing Treg activity through binding to adenosine A2A receptor (A2AR)[73]. In addition, exosomes carrying microRNAs, circular RNAs, lncRNAs, and proteins such as PD-1/PD-L1 collectively attenuate the cytotoxicity of T cells and NK cells[74]. Moreover, exosomes from tumor cells promote the infiltration of M2 macrophages, Tregs, DCs, and N2 neutrophils, as well as angiogenesis, further driving resistance to ICI therapy[75-77].

COMBINATION THERAPY FOR OVERCOMING DRUG RESISTANCE TO ICIS

Combination therapy has emerged as a key strategy to overcome resistance and improve outcomes with ICIs. Evidence demonstrates that multimodal therapeutic approaches enhance ICI efficacy through mechanisms such as counteracting immunosuppressive elements, promoting antigen release and presentation, activating and expanding effector T cells, and improving $CD8^+$ T-cell infiltration and function [Table 2][39,78-99]. Recently, combination therapy has become a foundational approach in HCC, primarily including strategies such as ICI plus targeted therapy, dual-ICI, ICI combined with locoregional therapy, ICI integrated with traditional Chinese medicine (TCM), and ICI combined with intestinal microbiota modulation.

ICIS COMBINED WITH TARGETED THERAPY

Tyrosine kinase inhibitors

The combination of ICIs with anti-angiogenic agents represents one of the most extensively studied treatment regimens and has opened new avenues for the management of advanced HCC. Among anti-angiogenic drugs, tyrosine kinase inhibitors (TKIs) such as sorafenib, lenvatinib, regorafenib, and cabozantinib have been shown to enhance the response to PD-1 inhibitors by promoting $CD8^+$ T-cell infiltration, suppressing M2 macrophage polarization, and reducing the infiltration of Th cells and Tregs[100-102]. Additionally, TKIs downregulate immunosuppressive cytokines including IL-10 and TGF-β, as well as immune checkpoints such as PD-1 and TIM-3, thereby helping to restore sensitivity to PD-1 blockade[103-105]. These mechanistic insights support the combined use of TKIs and ICIs as a novel therapeutic strategy in HCC. Recently, a growing number of clinical trials evaluating ICI-TKI combinations have been

Table 2. Combination strategies and their underlying mechanisms in promoting ICI efficacy used in clinical trials for HCC treatment

ICI	Target	Combination (target)	Sensitization mechanism	Outcome	Trial numbers (current status)
Nivolumab	PD-1	Ipilimumab (CTLA-4)	Boost the activation and proliferation of T cells	ORR: 24%, mOS: 16.4 months	NCT03222076[80] (Second-line treatment)
		Relatlimab (LAG-3) + Bevacizumab (VEGF)	Inhibit LAG-3 checkpoint, enhance T-cell function; suppress angiogenesis, promote T-cell infiltration and efficacy	Underway	NCT05337137[81]
		Lenvatinib (TKI)	Inhibit tumor angiogenesis and cancer cell proliferation, promote immune activity	ORR: 54.2%, PFS: 73.9 months	NCT04039607[82]
		Cabozantinib (TKI)	Inhibit VEGF-driven angiogenesis and reduce immunosuppressive cells, promote immune cell infiltration	ORR: 19%, 1-year OSR: 12%-38.3%	NCT03299946[83]
Pembrolizumab	PD-1	Lenvatinib (TKI)	Block tumor angiogenesis (blood vessel formation) and suppress tumor cell proliferation	mPFS: 9.3 months, mOS: 22 months	NCT03713593[84] (First-line treatment)
		Lenvatinib (TKI) + TACE	Inhibit tumor angiogenesis and proliferation; induce localized tumor necrosis and release tumor antigens	mPFS: 14.6 months, 24-month OSR: 75%	NCT04246177[85] (First-line treatment)
		Regorafenib (TKI)	Suppress tumor growth by blocking oncogenic signaling pathways and modulating the immunosuppressive TME	ORR: 29%, PFS: 6.8 months	NCT03347292[86]
Camrelizumab	PD-1	Apatinib (VEGFR inhibitor)	Normalize tumor vasculature and suppress immunosuppressive cells	mPFS: 5.6 months, mOS: 22.1 months	NCT04297202[87] (First-line treatment)
		Apatinib (VEGFR inhibitor) + TACE	Induce localized tumor ischemia and necrosis, release tumor antigens and promote inflammatory microenvironment; inhibit angiogenesis, improve immune cell infiltration	mPFS: 13.5 months, mOS: 22.1 months	NCT04559607[88]
Sintilimab	PD-1	IBI305 (VEGF inhibitor)	Suppress blood vessel growth, modulate the immune-suppressive TME	mPFS: 4.6 months	NCT03794440[89]
		IBI310 (CTLA-4 inhibitor)	Promote T-cell activation and proliferation	mPFS: 3.9 months, 6-month OSR: 93.1%	NCT04401813[90]
Penpulimab	PD-1	Bevacizumab (VEGF inhibitor)	Inhibit VEGF-driven angiogenesis, improve T-cell infiltration	ORR: 21%, PFS: 4.6 months	NCT04843943[91] (First-line treatment)
		Anlotinib (TKI)	Suppress tumor angiogenesis and proliferation	ORR: 24%, PFS: 5.4 months	NCT04344158[92]
		Tremelimumab (CTLA-4 inhibitor)	Inhibit CTLA-4, promote T-cell activation and proliferation	ORR: 33%, PFS: 19.53 months	NCT03298451[93] (First-line treatment)
Durvalumab	PD-L1	Bevacizumab (VEGF inhibitor)	Inhibit VEGF-driven angiogenesis and modulating the immunosuppressive TME	mPFS: 14.2 months	NCT02519348[94]
		Bevacizumab (VEGF inhibitor) + TACE	Normalize tumor vasculature; induce localized tumor cell death, release neoantigens and promote immune cell infiltration	Underway	NCT03778957[95]
Atezolizumab	PD-L1	Bevacizumab (VEGF inhibitor)	Inhibit VEGF to normalize tumor vasculature, reduce immunosuppression and promote T-cell infiltration	12-month OSR: 67.2%, mPFS: 6.8 months	NCT03434379[96] (First-line treatment)
		Bevacizumab (VEGF inhibitor) + TACE	Inhibit angiogenesis, reduce Treg activity and enhance DC maturation; induce localized tumor necrosis, release tumor antigens and promote immune cell infiltration	13-month OSR: 75.4%, mPFS: 7.03 months	NCT05776875[97]
		Cabozantinib (TKI)	Suppress tumor angiogenesis, proliferation and metastasis	Underway	NCT03755791[98]
		Sorafenib (TKI)	Inhibit tumor proliferation and angiogenesis	12-month OSR: 54.6%, mPFS: 4.3 months	NCT03434379[96]
Cobolimab	TIM-3	Dostarlimab (PD-1)	Enhance T-cell reactivation and proliferation	Underway	NCT03680508[99]

PD-1: Programmed cell death protein 1; PD-L1: programmed cell death ligand 1; TIM-3: T-cell immunoglobulin and mucin domain containing 3; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; LAG-3: lymphocyte activation gene 3; VEGF: vascular endothelial growth factor; TKI:

tyrosine kinase inhibitor; TACE: transarterial chemoembolization; VEGFR: vascular endothelial growth factor receptor; TME: tumor microenvironment; DCs: dendritic cells; ORR: objective response rate; PFS: progression-free survival; mPFS: median progression-free survival; OSR: overall survival rate; mOS: median overall survival.

conducted in HCC patients, with several demonstrating superior antitumor activity compared to monotherapy. For example, the combination of cabozantinib with atezolizumab prolongs progression-free survival (PFS) of HCC patients, although no significant overall survival (OS) benefit is observed[98]. In addition, PD-1 inhibitors combined with TKIs such as regorafenib and lenvatinib yield higher objective response rates (ORR), longer PFS, and improved OS in patients with unresectable HCC[106]. Notably, the combination of pembrolizumab and lenvatinib has demonstrated significant efficacy, establishing it as a first-line treatment for advanced HCC[84].

VEGF or VEGFR inhibitors

Studies have shown that inhibitors targeting VEGF or vascular endothelial growth factor receptor (VEGFR) reduce the infiltration and activity of immunosuppressive cells. In addition, anti-VEGF/VEGFR therapy inhibits M2-type macrophage polarization and downregulates the expression of immune checkpoint (e.g., PD-L1/PD-L2) and immunosuppressive factors (e.g., IL-6, IDO1, and IL-10), while promoting DC maturation and antigen presentation[107,108]. These findings indicate that VEGF/VEGFR inhibitors can alleviate immunosuppression in the TME and possibly help to overcome resistance to ICIs. Consistently, clinical trials demonstrate that combining ICIs with anti-VEGF or anti-VEGFR agents leads to improved efficacy in HCC treatment. In particular, the combination of PD-L1 inhibitor atezolizumab with VEGF inhibitor bevacizumab has been approved as a first-line treatment for HCC[109]. Another clinical trial reveals that the VEGFR inhibitor rivoceranib (apatinib) combined with PD-1 inhibitor camrelizumab significantly improves both PFS and OS in patients with unresectable HCC[110]. Furthermore, a series of ongoing clinical trials are evaluating the efficacy of ICI-based combinations with other VEGFR inhibitors, such as anlotinib and cabozantinib, which may offer new combination strategies, particularly for patients with advanced HCC[41].

Other targeted drugs

In addition to anti-angiogenic agents, the combination of ICIs with other targeted drugs has also demonstrated improved efficacy in patients with HCC. For instance, the TGF-β receptor I inhibitor galunisertib significantly enhances the antitumor activity of PD-L1 inhibitors by suppressing TGF-β signaling[111]. Similarly, a peroxisome proliferator-activated receptor (PPAR) γ antagonist resensitizes tumors to anti-PD-L1 therapy in HCC models by inhibiting PPARγ/vascular endothelial growth factor A (VEGF-A)-mediated immunosuppression[112]. Furthermore, DNA damage repair (DDR) inhibitors are demonstrated to enhance the clinical efficacy of ICIs in HCC through mechanisms such as promoting neoantigen release, increasing TMB, and upregulating PD-L1 expression[113]. Currently, more than 80 clinical trials are investigating the therapeutic potential of combining DDR-targeted agents with ICIs, with several studies reporting favorable tolerability and significant antitumor activity[114,115]. Additionally, the combination of PD-1 blockade with the phosphatidylserine-targeting agent bavituximab has been shown to prolong the PFS and OS of HCC patients[116]. Preclinical studies also indicate that PEGylated IFN-α enhances the efficacy of PD-1 inhibitors by promoting the infiltration and activation of $CD8^+$ T cells[117]. Epigenetic inhibitors targeting HDACs or DNMTs are reported to improve ICI response by modulating the infiltration of $CD8^+$ T cells and IL-17-producing Th cells, as well as PD-1/PD-L1 expression, providing a rationale for combining epigenetic therapies with ICIs[118,119]. Recently, inhibitors of carbonic anhydrase XII, a novel class of anticancer agents, have shown the ability to remodel the immunosuppressive TME, suggesting another promising combination strategy for HCC treatment[120].

In conclusion, therapeutic approaches that combine targeted agents with ICIs to overcome ICI resistance are strongly supported by robust clinical trial evidence. In particular, the combination of ICIs with anti-angiogenic agents has gained approval in multiple countries as first-line and second-line treatments for HCC, demonstrating considerable promise for clinical application.

DUAL-ICIS COMBINATION THERAPY

The combination of PD-1/PD-L1 inhibitors and CTLA-4 inhibitors represents one of the most frequently evaluated strategies in clinical trials, as it enhances antitumor immunity through complementary mechanisms of T-cell activation. For example, the combination of nivolumab (PD-1 inhibitor) and ipilimumab (CTLA-4 inhibitor) significantly improves ORR and OS in patients with HCC[121]. This regimen received Food and Drug Administration (FDA) approval as a second-line treatment for advanced HCC previously treated with sorafenib. Similarly, clinical trials evaluating durvalumab (PD-L1 inhibitor) in combination with tremelimumab (CTLA-4 inhibitor) demonstrate promising therapeutic outcomes[122], and this combination was approved in 2022 for the first-line treatment of unresectable HCC. Recent findings from a phase III trial indicate that tremelimumab plus durvalumab significantly extends OS of HCC patients compared to monotherapy[123]. Additionally, the combination of sintilimab (PD-1 inhibitor) and IBI310 (CTLA-4 inhibitor) has shown encouraging efficacy in clinical trials. Based on these findings, this combination was approved in 2021 as a first-line treatment for unresectable or metastatic HCC[90].

In addition to CTLA-4 inhibitors, combinations of PD-1/PD-L1 inhibitors and other ICIs are also under investigation. For instance, clinical evidence suggests that PD-L1 inhibitors sometimes induce complete tumor eradication in HCC patients who have developed acquired resistance to PD-1 inhibitors[124]. The combination of the TIM-3 inhibitor cobolimab with dostarlimab has demonstrated acceptable safety and encouraging clinical activity as a first-line treatment for advanced HCC[125]. Furthermore, the LAG-3 inhibitor relatlimab and IDO1 inhibitor abrine significantly enhance antitumor efficacy when combined with anti-PD-1 therapy[8]. Recently, several clinical trials evaluating inhibitors targeting TIM-3, LAG-3, or TIGIT in combination with other ICIs for HCC treatment are currently underway[126,127]. Positive results from preclinical HCC models may offer promising new dual-ICI therapeutic options for HCC.

As one of the well-established combination strategies, dual-ICI therapy is backed by substantial clinical trial evidence, laying a solid foundation for the clinical application of PD-1/PD-L1 inhibitors combination with CTLA-4 inhibitors in HCC treatment. In the future, with the discovery of novel ICIs and corresponding inhibitors, an increasing number of ICI-based combinations are expected to enter clinical practice for HCC management.

ICIS COMBINED WITH LOCOREGIONAL THERAPY

Locoregional therapies constitute a group of liver-directed interventions that augment the immunogenicity of residual tumor cells and potentiate their susceptibility to immunotherapy. Accumulating evidence indicates that locoregional modalities enhance responsiveness to ICIs through mechanisms including the upregulation of tumor-associated antigens (TAAs) and enhanced immunogenicity, promotion of DC and $CD8^+$ T-cell infiltration, and release of pro-inflammatory cytokines (e.g., TNF-α, IL-12)[128]. In addition, locoregional therapies mitigate the activities of immunosuppressive cells and elicit immunogenic cell death (ICD)[129]. Together, these mechanisms underpin the rational combination of locoregional therapy with ICIs as a promising strategy to amplify antitumor immunity[130].

In recent years, multiple studies have evaluated the efficacy and safety of combining ICIs with transarterial chemoembolization (TACE) for the treatment of HCC. A phase III trial demonstrates that combining lenvatinib, pembrolizumab, and TACE significantly prolongs PFS of patients with unresectable HCC, with

some achieving complete tumor regression[131]. Another study shows that adding anti-PD-L1 and targeted drugs to TACE improves both OS and ORR of HCC patients[132]. A clinical trial conducted by Roche showed that atezolizumab plus bevacizumab combined with TACE led to significant benefits in unresectable HCC, highlighting the synergy between ICIs, anti-angiogenic therapy, and locoregional treatment[97]. Studies on hepatic arterial infusion chemotherapy (HAIC) combined with ICIs have also shown promise in improving the antitumor efficacy. A retrospective analysis finds that HAIC combined with ICIs and TKIs offers a favorable safety profile and better liver function preservation in intermediate and advanced HCC[133]. Furthermore, an ongoing trial is investigating concurrent hepatic arterial delivery of a PD-L1 antibody with FOLFOX chemotherapy to enhance local immune blockade and suppress tumor immune evasion[134]. Radiotherapy eliminates tumor cells within the irradiated field through high-energy radiation and elicits systemic immune effects, making it a promising partner for immunotherapy[135]. Supported by positive outcomes from phase I/II trials, the combination of radiotherapy [e.g., Yttrium-90 (Y90)] with ICIs is being increasingly explored in advanced HCC[136]. Similarly, thermal ablation techniques including microwave ablation (MWA) and radiofrequency ablation (RFA) can also mitigate local immunosuppression and synergize effectively with ICIs[130].

In summary, locoregional therapies represented by TACE combined with ICIs have accumulated abundant clinical trial evidence in the management of HCC. Approved for clinical application in HCC treatment, this combination regimen has emerged as an effective therapeutic strategy to overcome ICI resistance.

ICIS COMBINED WITH TCM

The combination of PD-1/PD-L1 inhibitors with TCM has shown superior efficacy compared to ICI monotherapy in the treatment of HCC. For instance, ginseng-derived nanoparticles (GDNPs) and Huaier enhance the antitumor effect of PD-1/PD-L1 inhibitors by promoting M1 macrophage polarization and recruiting $CD8^+$ T cells into the TME[137,138]. Similarly, natural compounds such as bufalin and curcumin (isolated from turmeric) potentiate the activity of anti-PD-L1 antibodies by shifting TAMs from M2 to M1 phenotype and downregulating PD-1 expression on $CD8^+$ T cells[139,140]. Other TCM-derived agents, including icaritin, luteolin, baicalein and baicalin, and osthole have been shown to induce antitumor immune responses through regulating immune cell function[141-143]. Additionally, YIV-906, a standardized formulation derived from Huang Qin Tang, reduces M2 polarization and enhances antitumor activity when combined with PD-1 inhibition[144]. Besides, a clinical trial is currently underway to evaluate the efficacy of Huaier granules in combination with atezolizumab (anti-PD-L1) and bevacizumab in patients with HCC[145].

Furthermore, the emergence of new immune checkpoints opens new avenues for combining TCM with ICIs in the treatment of HCC. For example, network pharmacological analyses suggest that the herbal medicine Yinchen may activate immune cells by suppressing CTLA-4 and LAG-3[146]. Abrine, a compound derived from Abrus cantoniensis, is identified as a specific inhibitor of IDO1. Studies also demonstrate that abrine synergizes with PD-1 inhibitors to suppress HCC progression by downregulating IDO1 and PD-L1 expression while enhancing $CD8^+$ T-cell infiltration within tumors[8]. Moreover, the YANGYIN Fuzheng Jiedu prescription has been shown to inhibit tumor growth in HCC models by alleviating exhausted T cells and reducing the expression of immune checkpoints, including PD-1, TIM-3, and TIGIT[147,148]. These mechanisms highlight the potential of TCM agents as promising candidates for combination strategies with ICIs in HCC therapy.

Although TCM has exhibited considerable application potential in enhancing the efficacy of ICI therapy, most relevant studies are still in the preclinical exploration stage. In the future, more mechanistic research and clinical trials are needed to clarify its prospects for clinical application in HCC treatment.

ICIS COMBINED WITH INTESTINAL MICROBIOTA

Increasing evidence indicates a strong correlation between the composition of the gut microbiome and clinical outcomes following ICI treatment in patients with HCC. For instance, a higher abundance of *Lachnospiraceae bacterium-GAM79*, *Alistipes sp. Marseille-P5997*, *Bifidobacterium*, *Coprococcus*, *Akkermansia*, and *Acidaminococcus* is associated with improved responses to ICIs in HCC[149-152]. Studies indicate that specific intestinal bacteria, including *Bifidobacterium* and *Bacteroides fragilis,* restore response to ICIs by promoting the activation of DCs, $CD8^+$ T cells, and Th1 cells[153,154]. Additionally, *Akkermansia muciniphila*, whose abundance decreases during metabolic dysfunction-associated fatty liver disease (MAFLD)-promoted hepatocarcinogenesis, has been shown to exert maximal tumor-suppressive effects in HCC models when combined with PD-1 inhibition[149]. Moreover, metabolites such as ursodeoxycholic acid and ursocholic acid, which correlate with the abundance of *Lachnoclostridium*, are significantly enriched in fecal samples from HCC patients who respond to ICI therapy[155]. These findings underscore the potential of combining ICIs with microbiota-targeted interventions to enhance the efficacy of immunotherapy in HCC.

Probiotic supplementation, fecal microbiota transplantation (FMT), and antibiotic modulation have emerged as promising strategies to enhance the efficacy of ICIs in HCC. For instance, probiotic strains such as *Lactobacillus rhamnosus Probio-M9*, *Lachnospiraceae bacterium-GAM79*, *Ruminococcus*, and *Bifidobacterium bifidum* are associated with improved ICI efficacy[153,156,157]. Moreover, several studies showed that FMT enhances antitumor efficacy of ICIs, and combining FMT with atezolizumab or bevacizumab prolonged both PFS and OS of HCC patients[158,159]. The use of antibiotics in combination with ICIs represents another emerging approach, and vancomycin has been shown to improve the efficacy of nivolumab in patients with refractory primary or metastatic HCC[160]. However, the impact of antibiotics on ICI therapy remains controversial and warrants further investigation.

In summary, combining ICIs with gut microbiota modulation has demonstrated promising efficacy in overcoming ICI resistance in HCC. Nevertheless, its clinical translation is hampered by insufficient understanding of the underlying mechanisms and a lack of robust clinical evidence. Further well-designed trials are essential to advance this therapeutic strategy toward clinical application.

CONCLUSION AND FUTURE PERSPECTIVES

ICIs have ushered in a paradigm shift in the treatment of HCC, offering the potential for durable antitumor responses. However, the emergence of drug resistance remains a formidable challenge, undermining clinical outcomes and restricting the broader application of ICIs in HCC. In recent years, combination therapies have emerged as the most promising and widely adopted approaches to counteract ICI resistance. ICIs plus targeted therapy and dual-ICI blockade currently represent the most well-established and clinically validated approaches, with robust evidence from clinical trials supporting their approval as first- or second-line treatments. Moreover, the combination of ICIs with locoregional therapies has gained relatively broad application in HCC management. Notably, the combination of TACE with ICIs has demonstrated significant efficacy in delaying HCC progression in clinical trials, and the regimen of TACE plus lenvatinib and pembrolizumab was approved in China in 2025 for the treatment of unresectable non-metastatic HCC. In contrast, combinations of ICIs with TCM or microbiota modulators currently rely mainly on exploratory evidence and have not yet been substantiated for routine clinical use.

However, these combination strategies still face distinct challenges in clinical practice. For ICI-based targeted therapy combinations, key issues include the need for high-quality clinical data to support diverse regimens, lengthy trial and approval cycles, increased risks of combined drug toxicity, and the imperative for precise patient stratification. Safety profiles for dual-ICI blockade regimens are still being accumulated, and the

absence of unified guidelines for managing immune-related adverse events (irAEs) complicates monitoring. Furthermore, the lack of reliable predictive biomarkers hinders the accurate identification of patients most likely to benefit. The diversity of locoregional treatment modalities further complicates the standardization of combination regimens with ICIs and the accurate assessment of therapeutic outcomes. For example, there is no consensus on the sequence, interval, or dose adjustment between locoregional and systemic therapies, which affects the consistency of treatment efficacy. The complex composition of TCM preparations poses significant challenges for quality control, making it difficult to establish unified clinical trial protocols and evaluation systems. As for microbiota-based combination therapies, major limitations currently include the lack of HCC-specific guidelines for probiotic strain selection, dosage, and treatment duration, as well as the absence of a dedicated regulatory pathway for FMT combined with ICIs.

Moreover, incompletely elucidated mechanisms of ICI resistance are another important reason for the limited therapeutic efficacy and clinical application of combination strategies. For example, while ICIs and targeted drugs have well-defined targets such as PD-1/PD-L1 and VEGF, the mechanisms underlying the production of these drug resistance-related factors remain incompletely understood. Consequently, combination therapy cannot completely overcome ICI resistance. According to the two categories of factors inducing ICI resistance displayed in this review, tumor cell intrinsic factors appear to play a dominant role. In addition to altering their own functions, tumor cells can secrete factors including cytokines and metabolites that influence TME, thereby coordinating multiple processes to evade immune surveillance. Therefore, elucidating the molecular mechanisms in tumor cells that drive ICI resistance is critical to the optimization of HCC immunotherapy. Besides, considering the importance of heterogeneity in influencing the functions of tumor cells, systematic evaluation of intra- and inter-patient heterogeneity may clarify the specific causes of ICI resistance. Moreover, intestinal microbiota has emerged as a key regulator of antitumor immunity[161]. Whether tumor cells escape immunotherapy through the intestinal microbiota requires further investigation, which will expand our understanding of ICI resistance mechanisms.

Given the complex and variable mechanisms that induce ICI resistance, staged combination therapy represents a promising direction for HCC. Beyond currently established regimens, innovative approaches such as cellular immunotherapies [e.g., chimeric antigen receptor T (CAR-T) cells, chimeric antigen receptor (CAR)-NK, and CAR-macrophage (CAR-M)][162-164], inhibitors targeting immunosuppressive metabolites (e.g., adenosine and lactate)[165,166], and oncolytic viruses [e.g., talimogene laherparepvec and pexastimogene devacirepvec (JX-594)] show promise in augmenting ICI efficacy[167,168]. Additionally, tumor vaccines represent a promising modality for enhancing tumor-specific immune responses through targeted delivery of tumor antigens, offering a new avenue to combat ICI resistance[169]. For instance, DC vaccines and peptide vaccines targeting alpha-fetoprotein (AFP) or glypican-3 (GPC-3) have demonstrated acceptable safety profiles in HCC patients[170]. Furthermore, precision or personalized immunotherapy, in which regimens are tailored to individual tumor biology and host characteristics, offers a compelling avenue to maximize therapeutic impact.

Predictive biomarkers are indispensable for guiding ICI therapy, enabling identification of likely responders, monitoring therapeutic efficacy, assessing recurrence risk, and anticipating irAEs, thereby advancing precision immunotherapy for HCC. Currently, biomarkers under investigation encompass host-related factors (e.g., AFP, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, gut microbiota), and tumor-derived markers [e.g., PD-L1 expression, TMB, microsatellite instability, circulating tumor DNA (ctDNA), tumor-infiltrating lymphocytes, and driver gene mutations [e.g., tumor protein p53 (TP53)][171-173]. Although most remain in the investigational phase and are not yet embedded in routine clinical workflows, these markers have considerably deepened our understanding of ICI responsiveness and hold substantial potential for future integration into clinical practice.

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Conception, design, and editing of the manuscript: Zhao Q
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