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Immunotherapy for hepatocellular carcinoma

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Abstract
Hepatocellular carcinoma (HCC) is a leading cause of cancer-related deaths worldwide. Its high recurrence rate and lack of effective control drugs result in a 5-year survival rate of only about 10%. HCC is a tumor regulated by the immune system. Significant breakthroughs have occurred in treating solid tumors with immunotherapy in recent years. Various immunotherapies, such as immune checkpoint inhibitors (ICIs), including combination therapies, have demonstrated promising therapeutic effects in both clinical applications and research. Other immunotherapies, such as adoptive cell therapies and oncolytic viruses, are also emerging, offering hope for addressing long-term survival issues in HCC. This article reviews current commonly used immunotherapy strategies and the latest research findings for reference.
Keywords: Immunotherapy; Immune checkpoint inhibitors; Adoptive cell therapies; Hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the most common and leading cause of cancer-related deaths.[1] Over the past decade, there have been significant advances in HCC treatment, including non-pharmacological and pharmacological approaches. Treatment choices depend on clinical staging, primarily based on tumor burden, patient performance status (PS), and liver function impairment. HCC has low survival rates due to high recurrence rates with surgical and interventional treatments and limited efficacy of chemotherapy and targeted drugs. Only around 10% of patients survive for five years.[2]

The liver is crucial for metabolism, nutrient absorption, detoxification, and immune functions. HCC treatment aims to remove tumors while preserving liver function to prolong survival, considering the liver's functional status.[3]

Most HCCs develop on a background of liver fibrosis/cirrhosis, progressing at an annual rate of 1.82%, leading to a "multicenter, multinodular" growth pattern.[4] Many HCC patients with cirrhosis have poor liver reserve function, even though they may be classified as Child-Pugh A–B, which can result in liver dysfunction or failure during HCC treatment. The primary driver genes of liver cancer have not yet been identified. Therefore, targeted therapies for liver cancer often use multi-kinase inhibitors like sorafenib, lenvatinib, and regorafenib, or anti-angiogenic agents. These targeted drugs can further cause liver function damage during the anti-tumor process. Advanced cases with poor liver function may be forced to reduce the drug dosage or complications, which, in turn, leads to the development of drug resistance and treatment failure.

Immunotherapy regulates the body's innate and adaptive immune systems to control cancer while monitoring and preventing immune evasion to prevent cancer recurrence and metastasis. Since 2018 when effectively removing immune checkpoint brakes was confirmed to clear tumor cells, immune checkpoint inhibitors (ICIs) have been rapidly developed and applied. The earliest ICIs used in HCC treatment were programmed cell death-1 (PD-1) monoclonal antibodies,[5,6] followed by programmed cell death-ligand-1 (PD-L1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors.[7,8] Compared to chemotherapy and targeted drugs, immunotherapy has milder side effects. Therefore, combination strategies involving immune suppressors, targeted drugs, and local treatment have quickly entered clinical trials, becoming highly promising approaches for HCC treatment. In addition to ICIs, adoptive cell therapies (ACTs), tumor vaccines, oncolytic viruses (OVs), and cytokine therapy have also entered clinical treatment for HCC. This article provides a concise summary of ICIs and their combination

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approaches that have been applied in clinical practice, as well as ACTs and other immunotherapies.

Mechanisms of HCC Immunotherapy

The immune system plays a crucial role in the development of tumors, with interactions between innate and adaptive immune systems enabling effective immune surveillance of tumors.[9] Recognition of antigens is impaired in a tumor microenvironment (TME) characterized by immune suppression, leading to a dysregulation of the body's immune response and resulting in immune evasion by the tumor.[10] Epigenetic changes, post-transcriptional silencing, alterations in antigen presentation, or peptide processing mechanisms can all reduce immune cell recognition of tumor-associated antigens (TAAs).[11] The appearance of an immune-suppressive TME may result from the upregulation of immune checkpoint ligands and receptors that inhibit lymphocyte signals, the accumulation of cells with immune-suppressive activity, the presence of metabolites detrimental to immune cells, increased tolerance enzyme levels, and reduced immune-mediated phagocytosis, among other factors.[12] Any attempts to effectively kill tumor cells by overcoming these obstacles represent promising avenues for immunotherapy.

Immune checkpoints include co-inhibitory receptor molecules expressed by effector lymphocytes, which prevent excessive activation of these lymphocytes. HCC exploits this physiological mechanism to evade anti-tumor immune responses by expressing corresponding ligands in tumor or stromal cells.[13] Co-inhibitory receptors include CTLA-4, PD-1, T-cell immunoglobulin and mucin domain 3 (TIM-3), and lymphocyte activation gene 3 (LAG-3).[14] CTLA-4 is typically highly expressed on the surface of activated T cells. It functions as a co-stimulatory molecule that downregulates T-cell activity, thereby dampening the body's anti-tumor immune response.[15] PD-1 is expressed by activated T cells, natural killer (NK) cells, regulatory T (Treg) cells, myeloid-derived suppressor cells (MDSCs), monocytes, and dendritic cells (DCs). In contrast, its ligand PD-L1 is expressed by many stromal cells, tumor cells, and bone marrow cells, including DCs. PD-1 inhibits effector cell function and leads to the exhaustion or dysfunction of effector T cells. Monoclonal antibodies targeting immune checkpoints can block the interaction between checkpoint proteins and their ligands, thereby preventing T-cell inactivation [Figure 1]. ICIs have effectively eliminated tumor cells in various cancers, revolutionizing cancer treatment.[5,6]

The liver receives blood from both the systemic circulation and the intestines, exposing it regularly to antigens and microbial products from intestinal bacteria. This establishes a unique immune microenvironment in the liver, promoting tolerance to harmless foreign substances such as food antigens.[16] The TME in HCC is intricate, and patients often demonstrate compromised immune function within the TME. While a certain number of lymphocytes are present in HCC tumors, the functionality of these infiltrating lymphocytes is impaired mainly or in a state of dysfunction. T-cell quantity and activation status are important factors.[17] Decreased T lymphocyte numbers directly reduce their anti-tumor capability, inhibit the body's tumor-specific immune response, and promote tumor immune evasion.[18] Researchers are working to control the T-cell infiltration level in HCC using various immunotherapies such as T-cell receptor T (TCR-T) cells, chimeric antigen receptor T (CAR-T) cells, vaccines, viruses, and cytokine therapy. However, the process of tumorigenesis is complex and often involves immune suppression in the TME.[19] Therefore, combining different immunotherapy approaches can yield a more durable anti-tumor effect.

Clinical Immunotherapy Strategies—ICIs

In a normal immune response, the activation of T cells requires two signals: first, antigen-presenting cells (APCs) recognize specific antigen–major histocompatibility complex (MHC) molecule complexes and bind to T-cell surface TCR-CD3 molecules, providing the initial activation signal. Second, the interaction of the co-stimulatory molecule receptor CD28 on the surface of resting T cells with B7 on the surface of APCs provides the second signal for T-cell activation. However, during persistent inflammation or stimulation by tumor cells, various immune checkpoint molecules, such as PD-1, PD-L1, CTLA-4, TIM-3, and LAG-3, inhibit T-cell activation in the normal T-cell immune activation process, leading to apoptosis and allowing tumor cells to evade immune surveillance, leading to tumor formation. ICIs can relieve their inhibitory effects on T cells, upregulate T-cell activation and proliferation, and induce cytotoxic T cells (CTLs) to restore their

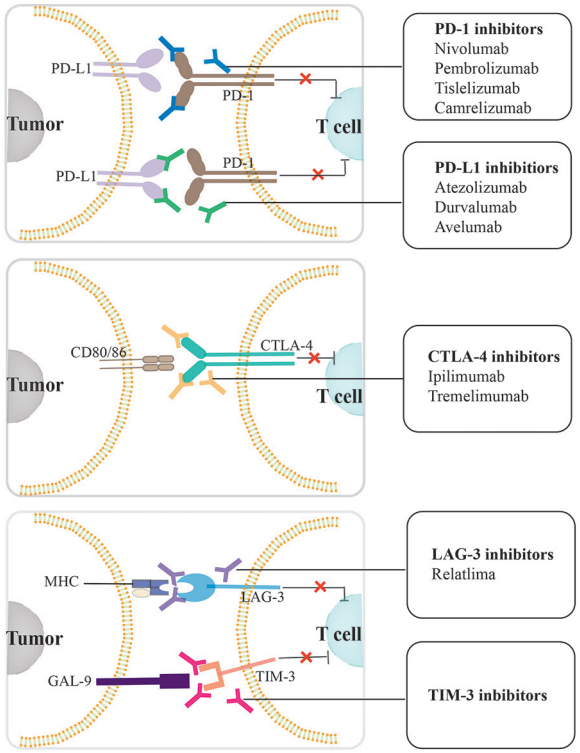

Figure 1: The mechanisms of HCC immunotherapy. PD-1: Programmed cell death protein-1; PD-L1: Programmed cell death ligand-1; CTLA-4: Cytotoxic T lymphocyte associated antigen-4; LAG-3: Lymphocyte activation gene-3; TIM-3: T cell immunoglobulin domain and mucin domain-3; GAL-9: Galectin-9

ability to kill tumor cells, achieving effective anti-tumor effects. The most mature and clinically applied ICIs are PD-1/PD-L1 inhibitors and CTLA-4 inhibitors. In recent years, new immune checkpoints such as TIM-3 and LAG-3 have been continuously discovered, and ICIs developed target these immune checkpoints, which also have anti-tumor potential.

PD-1 inhibitor

Nivolumab was approved for immunotherapy for advanced HCC in 2017. Studies showed a 15–20% objective response rate (ORR) in patients with advanced HCC. It was approved as a second-line treatment following sorafenib failure.[20] In a Phase-III trial, nivolumab showed a higher response rate than sorafenib, making it a potential treatment option for patients who cannot take other drugs.[21]

Combining nivolumab with anti-angiogenic drugs can boost the anti-tumor effect. Lenvatinib and nivolumab significantly extend the ORR, progression-free survival (PFS), and overall survival (OS) compared to lenvatinib alone.[22] This combination is now widely used for HCC treatment.

The Food and Drug Administration (FDA) approved pembrolizumab, a PD-1 monoclonal antibody, for second-line treatment in advanced HCC. It showed a higher ORR than the placebo, and ongoing studies explore its use in adjuvant therapy.

Camrelizumab, a PD-1 inhibitor approved by the National Medical Products Administration (NMPA), has shown benefits with an ORR of 14.7% and median OS of 14.2 months in HCC patients.[23] It works well when combined with apatinib as a first- or second-line treatment. Clinical trials are comparing it with sorafenib.

Tislelizumab is safe and beneficial for HCC patients. In a Phase-III trial, it had a longer median OS (15.9 months) than sorafenib (14.1 months) with fewer adverse events. Tislelizumab is also being studied in combination therapies for unresectable HCC.[24]

The current clinical trials of these ICIs in HCC are summarized in Table 1.

PD-L1 inhibitor

Atezolizumab is a humanized IgG1 monoclonal antibody that primarily works by binding to PD-L1 on the surface of tumor cells, blocking its interaction with PD-1 and B7.1 on T cells, thereby exerting anti-tumor effects.[8] Combining ICIs with molecularly targeted agents blocking PD-1 and vascular endothelial growth factor (VEGF) pathways can enhance anti-tumor activity. The safety and potential efficacy of the atezolizumab and bevacizumab combination (T + A regimen) in unresectable HCC patients were confirmed in the GO30140 clinical trial.[25] Subsequently, the renowned IMbrave150 clinical trial (501 previously untreated unresectable HCC patients) demonstrated that T + A treatment significantly extended the PFS (6.8 months *vs*. 4.3 months) and one-year survival rate (67.2% *vs*. 54.6%) compared to the first-line drug sorafenib.[26] Moreover, the quality-of-life assessments using European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) and EORTC QLQ-HCC18 scales showed that the T + A treatment group significantly improved the quality of life for HCC patients, reducing the deterioration of symptoms like fatigue, diarrhea, and pain, indicating a favorable benefit-to-risk ratio for late-stage unresectable HCC patients.[27] Consequently, the FDA formally approved it for first-line treatment in unresectable HCC patients. Besides late-stage HCC patients, several ongoing studies are evaluating the anti-tumor effects of the T + A treatment regimen in early- and intermediate-stage HCC patients, expanding its application.[28,29] However, since bevacizumab is an angiogenesis inhibitor, and transcatheter arterial chemoembolization (TACE) therapy also targets the vasculature within liver cancer tumors, the safety of this strategy needs further evaluation or precautions for individuals with liver cancer due to cirrhosis or those with compromised liver function or severe esophageal varices. Furthermore, a Phase-III clinical trial is underway for atezolizumab in combination with the second-line drug cabozantinib for advanced HCC (COSMIC-312).[30]

Durvalumab and avelumab are similar PD-L1 inhibitors,[31] showing better therapeutic effects for HCC patients with high PD-L1 expression in biopsies or baseline alpha-fetoprotein (AFP) levels >400 mg/dL (OS 16.5 months *vs*. 5.7 months).[32] Durvalumab is combined with other adjuvant therapies [Table 1]. Avelumab, another PD-L1 monoclonal antibody, has fewer ongoing clinical trials in HCC.

CTLA-4 inhibitor

CTLA-4 is also an immune checkpoint, primarily expressed on Treg and activated T cells. CTLA-4 on Treg cells conveys direct immunosuppressive signals to effector T cells, exerting immunosuppressive effects. CTLA-4 on activated T cells, on the other hand, can bind to CD80/CD86 on the surface of tumor cells, transmitting immunosuppressive signals leading to impaired immune function in T cells. CTLA-4 inhibitors competitively bind to CTLA-4 on Treg cells and activated T cells, blocking subsequent immunosuppressive effects, thus exhibiting anti-tumor activity. The currently most studied CTLA-4 inhibitors are ipilimumab and tremelimumab.

Ipilimumab was initially approved for melanoma treatment,[33] which executes anti-tumor effects in combination with PD-1 inhibitors in HCC patients previously treated with ICIs.[34] Here, HCC patients received ipilimumab (1 mg/kg) in combination with nivolumab (3 mg/kg) or pembrolizumab (2 mg/kg) every three weeks. Among the treated group, an ORR of 16% was observed, with a median OS of 10.9 months and one-year, two-year, and three-year survival rates of 42.4%, 32.3%, and 21.6%, respectively. Regarding safety, 12% of patients experienced grade 3 or higher adverse events, indicating that ipilimumab in combination with PD-1 inhibitors

Table 1: Clinical trials of various ICIs in HCC treatment.

Trial name	Identifier	Phase	Treatment arms	Primary outcome measures
1. PD-1 inhibitors				
1.1 Nivolumab				
CheckMate 040	NCT01658878	I/II	Nivolumab single arm	Safety and tolerability, ORR
CheckMate 459	NCT02576509	III	Nivolumab *vs*. sorafenib	OS
CheckMate 9DX	NCT03383458	III	Nivolumab + surgery/ablation	RFS
GOING	NCT04170556	1/II	Regorafenib + nivolumab	Safety
NASIR-HCC	NCT03380130	II	Nivolumab + SIR-spheres	Safety
RENOBATE	NCT04310709	II	Nivolumab + regorafenib	ORR
NIVOLEP	NCT03630640	II	Nivolumab + electroporation	Local RFS
N/A	NCT03418922	I	Nivolumab + lenvatinib	DLTs, safety
1.2 Pembrolizumab				
KEYNOTE 224	NCT02702414	II	Pembrolizumab single arm	ORR
KEYNOTE-937	NCT03867084	III	Pembrolizumab *vs*. placebo	RFS, OS
KEYNOTE-240	NCT02702401	III	Pembrolizumab *vs*. placebo	PFS, OS
N/A	NCT03163992	II	Pembrolizumab single arm	ORR
PLENTY202001	NCT04425226	II	Pembrolizumab + lenvatinib	RFS
LEAP-012	NCT04246177	III	Lenvatinib + pembrolizumab + TACE *vs*. TACE + placebo	PFS, OS
BAYER19497	NCT03347292	Ib/II	Regorafenib + pembrolizumab	Safety, DLTs
LEAP-002	NCT03713593	III	Lenvatinib + pembrolizumab *vs*. lenvatinib + placebo	PFS, OS
RENOTACE	NCT04777851	III	Regorafenib + pembrolizumab *vs*. TACE	PFS
N/A	NCT04696055	II	Regorafenib + pembrolizumab	ORR
N/A	NCT03006926	I	Lenvatinib + pembrolizumab	Safety, DOR, ORR, DLTs
N/A	NCT03337841	II	Pembrolizumab + surgery/ablation	One-year RFS
N/A	NCT04442581	II	Cabozantinib + pembrolizumab	ORR
N/A	NCT03397654	Ib	Pembrolizumab + TACE	Safety and tolerability
1.3 Tislelizumab				
RATIONALE-301	NCT03412773	III	Tislelizumab *vs*. sorafenib	OS, safety
N/A	NCT03941873	I/II	Tislelizumab + sitravatinib *vs*. sitravatinib	Safety, ORR
N/A	NCT04183088	II	Tislelizumab + regorafenib	ORR, PFS, safety
N/A	ChiCTR2200063003	II	Tislelizumab + donafenib + TACE	RFS
N/A	NCT05185531	Ib	Tislelizumab + SBRT	Delay to surgery, ORR, pathologic response rate, safety and tolerability
RATIONALE-208	NCT03419897	II	Tislelizumab *vs*. sorafenib	ORR
1.4 Camrelizumab				
N/A	NCT02989922	II	Camrelizumab	ORR, OS
N/A	NCT03092895	Ib/II	Camrelizumab + apatinib Camrelizumab + FOLFOX4 or GEMOX	Safety and tolerability
N/A	NCT03463876	II	Camrelizumab + apatinib	ORR
N/A	NCT03764293	III	Camrelizumab + apatinib *vs*. sorafenib	OS, PFS
2. PD-L1 inhibitors				
2.1 Atezolizumab				
GO30140	NCT02715531	Ib	Group A: atezolizumab plus bevacizumab Group F1: atezolizumab plus bevacizumab Group F2: atezolizumab	ORR, PFS
DEMAND	NCT04224636	II	Up-front Atezo/Bev, then TACE Atezo/Bev combined with TACE	24-months survival rate
IMbrave150	NCT03434379	III	Atezolizumab + bevacizumab *vs*. sorafenib	OS, PFS
COSMIC-312	NCT03755791	III	Cabozantinib + atezolizumab *vs*. sorafenib *vs*. cabozantinib	PFS, OS
ABC-HCC	NCT04803994	III	Atezolizumab + bevacizumab *vs*. TACE	Time to failure of treatment strategy
IMbrave050	NCT04102098	III	Atezolizumab + bevacizumab Active surveillance	RFS
IMbrave251	NCT04770896	III	Atezolizumab + lenvatinib or sorafenib *vs*. lenvatinib or sorafenib	OS
AMETHISTA	NCT04487067	IIIB	Atezolizumab + bevacizumab	Number of participants with grade 3–5
N/A	ChiCTR2200061793	N/A	Atezolizumab + bevacizumab + IMRT	ORR
2.2 Durvalumab				
N/A	NCT03970616	I/II	Tivozanib + durvalumab	Safety
EMERALD-2	NCT03847428	III	Durvalumab + bevacizumab *vs*. durvalumab	RFS
EMERALD-1	NCT03778957	III	Durvalumab + TACE *vs*. durvalumab + bevacizumab + TACE *vs*. TACE	PFS

(*continued*)

Table 1
(Continued)

Trial name	Identifier	Phase	Treatment arms	Primary outcome measures
N/A	NCT04945720	II	HAIC + durvalumab	One-year OS
N/A	NCT04961918	II	HAIC + durvalumab + lenvatinib	PFS
N/A	NCT04517227	NA	TACE + ablation + durvalumab	Safety
N/A	NCT04124991	I/II	Durvalumab + radioembolization	TTP
N/A	NCT04443322	NA	Durvalumab + lenvatinib	PFS, RFS
N/A	NCT04913480	II	Durvalumab + SBRT	PFS
2.3 Avelumab				
N/A	NCT03389126	II	Avelumab	Response rate
N/A	NCT03289533	Ib	Avelumab + axitinib	Safety
3. CTLA-4 inhibitors				
3.1 Ipilimumab				
PRIME-HCC	NCT03682276	Ib	Ipilimumab + nivolumab	Safety, tolerability
TRIPLET-HCC	NCT05665348	II-III	Atezolizumab + bevacizumab *vs*. atezolizumab + bevacizumab + ipilimumab	OR (II), OS (III)
3.2 Tremelimumab				
N/A	NCT01008358	II	Tremelimumab	Tumor response by RECIST
N/A	NCT01853618	I/II	Tremelimumab + RFA or TACE	Safety
N/A	NCT02519348	I/II	Tremelimumab *vs*. tremelimumab + durvalumab *vs*. durvalumab *vs*. durvalumab + bevacizumab	DLTs, TEAEs
4. Dual therapies with ICIs				
4.1 PD-1/PD-L1 + CTLA-4				
CheckMate040	NCT01658878	I/II	Nivolumab *vs*. nivolumab + ipilimumab *vs*. nivolumab + cabozantinib *vs*. nivolumab + ipilimumab + cabozantinib *vs*. sorafenib	Safety, tolerability, ORR
CheckMate9DW	NCT04039607	III	Nivolumab + ipilimumab *vs*. sorafenib/lenvatinib	OS
CheckMate 74W	NCT04340193	III	Nivolumab + ipilimumab + TACE *vs*. Nivolumab + TACE *vs*. TACE	Safety
HIMALAYA	NCT03298451	III	Durvalumab + tremelimumab *vs*. durvalumab *vs*. sorafenib	OS
PRIME-HCC	NCT03682276	Ib	Nivolumab + ipilimumab	Delay to surgery, safety tolerability
N/A	NCT05665348	II-III	Ipilimumab + atezolizumab + bevacizumab *vs*. atezolizumab + bevacizumab	OR (II), OS (III)
N/A	NCT02519348	I/II	Tremelimumab + durvalumab *vs*. durvalumab *vs*. tremelimumab *vs*. durvalumab + bevacizumab	DLTs, TEAEs, ECG
N/A	NCT03482102	II	Tremelimumab + durvalumab + radiation	ORR
N/A	NCT04430452	II	Tremelimumab + durvalumab + hypofractionated RT *vs*. durvalumab + hypofractionated RT	ORR
N/A	NCT03638141	II	Durvalumab + tremelimumab (cohort A dose) *vs*. durvalumab + tremelimumab (Cohort B dose)	ORR
N/A	NCT05063565	II	TheraSphere *vs*. TheraSphere + durvalumab + tremelimumab	ORR, DOR
N/A	NCT04522544	II	TACE + tremelimumab + durvalumab *vs*. Y-90 SIRT + tremelimumab + durvalumab	ORR
N/A	NCT04988945	II	Durvalumab + tremelimumab + TACE + SBRT	Downstaging for hepatectomy
N/A	NCT03937830	II	Durvalumab + bevacizumab + tremelimumab *vs*. durvalumab + bevacizumab + tremelimumab + TACE	PFS
4.2 PD-1/PD-L1 + LAG-3				
N/A	NCT04567615	–	Nivolumab + relatlimab *vs*. nivolumab	ORR
N/A	NCT04658147	–	Nivolumab + relatlimab *vs*. nivolumab	Number of patients who complete pre-op treatment and proceed to surgery

CTLA-4: Cytotoxic T-lymphocyte-associated antigen 4; DLT: Dose-limited toxicity; DOR: Duration of response; HCC: Hepatocellular carcinoma; ICIs: Immune checkpoint inhibitors; IMRT: Intensity-modulated radiotherapy; LAG-3: Lymphocyte activation gene 3; ORR: Objective response rate; OS: Overall survival; PD-1: Programmed cell death-1; PD-L1: Programmed cell death-ligand-1; PFS: Progression-free survival; RFA: Radiofrequency ablation; RFS: Relapse-free survival; SBRT: Stereotactic body radiotherapy; TACE: Transcatheter arterial chemoembolization; TEAEs: Treatment-emergent adverse events; TTP: Time to progress; Y-90 SIRT: Selective internal yttrium-90 radioembolization therapy.

has anti-tumor effects and an acceptable safety profile in HCC treatment.

Tremelimumab was the first CTLA-4 inhibitor used for HCC treatment. In 2013, Sangro *et al*[35] reported a Phase-II clinical trial of tremelimumab, including 21 HCC patients, of whom 17 completed the treatment. Among them, 58.8% of patients responded well to treatment, with a disease control rate (DCR) of 76.4%. The disease remained stable for over six months, and a decline in AFP levels was observed during treatment, suggesting favorable anti-tumor effects of tremelimumab. No treatment-related deaths were observed during the trial, with the most common adverse reaction being itching. In addition to

monotherapy, a Phase-I/II clinical trial evaluated the safety and anti-tumor efficacy of tremelimumab in combination with radiofrequency ablation (RFA) in advanced HCC patients. The trial included 32 advanced HCC patients who received tremelimumab at different doses every 4 weeks, followed by RFA on day 36. The results showed no dose-dependent toxic reactions in any HCC patients receiving combination treatment. The 6-month and 12-month PFS rates in treated HCC patients were 57.1% and 33.1%, respectively, with a median OS of 12.3 months, indicating that tremelimumab in combination with RFA is a viable treatment option for advanced HCC patients.[36]

Other ICIs

New immune checkpoints like TIM-3 and LAG-3 have been discovered as targets for cancer therapy. TIM-3 inhibits the function of effector T cells in tumor tissues. Blocking TIM-3 expression enhances immune cell proliferation and function, especially when combined with dual immunotherapy targeting different immune checkpoints.[37] LAG-3 is found on $CD4^+$, $CD8^+$ T cells, and Treg cells. It inhibits $CD4^+$ T-cell proliferation by binding to MHC class II molecules, suppresses immune responses in $CD8^+$ T cells, and activates Treg cell differentiation and function.[38] Relatlimab, the first LAG-3 inhibitor, is being tested in a clinical trial for safety and efficacy in treating HCC patients. The trial compares relatlimab with nivolumab to nivolumab alone for future use in HCC therapy.[39]

Dual therapy with ICIs

ICIs have improved anti-tumor therapy, but monotherapy can lead to tumor resistance and reduced efficacy. Koyama *et al*[40]found that mice resistant to PD-1 inhibitor treatment in a mouse lung cancer model often exhibited upregulation of other immune checkpoint markers such as CTLA-4, TIM-3, and LAG-3. Combining TIM-3 inhibitor with PD-1/PD-L1 inhibitors can boost effector T-cell proliferation and interferon γ (IFN-γ) secretion and extend median survival from 5 weeks to 11.9 weeks. The approach can overcome resistance to PD-1/PD-L1 inhibitors, resulting in lasting anti-tumor effects.

The FDA approved ipilimumab and nivolumab for treating advanced liver cancer patients who had received sorafenib therapy. A clinical trial is ongoing to observe the effectiveness of the combination in treating advanced HCC.[41] In the HIMALAYA clinical trial, tremelimumab in combination with durvalumab was approved by the FDA in 2022 for first-line treatment in advanced HCC patients. In addition to the two aforementioned ICI combination strategies, clinical trials assessing the efficacy of LAG-3 inhibitor relatlimab in combination with nivolumab for advanced HCC patients or perioperative treatment of resectable HCC patients are currently underway.[42,43]

"Live" Immunotherapy Strategies—ACT

ACT refers to expanding "live" immune cells, including both genetically unmodified and genetically modified immune cells, outside the body and reintroducing them into the patient to eliminate cancer cells.[44] Genetically unmodified autologous immune cells include tumor-infiltrating lymphocytes (TILs), NK cells, invariant natural killer T (iNKT) cells, cytokine-induced killer (CIK) cells, and other autologous immune cells. Genetically modified immune cells include TCR-T, CAR-T, and CAR-NK cells. Due to the ease of obtaining T cells and their ability to undergo significant expansion *in vitro*, current research focuses on gene modification and engineering T cells. These cells have shown promise in treating HCC.

T-cell immunotherapy

TILs

TILs are naturally infiltrating lymphocytes isolated from tumor tissues. They comprise a heterogeneous group of immune cells, primarily composed of effector T cells, alongside other immune cells such as NK cells, macrophages, and B lymphocytes.[45] TIL adoptive cell therapy involves isolating, screening, and amplifying TIL cells from a patient's tumor tissue *ex vivo* and reintroducing them into the patient's body to induce specific tumor cell killing.[46] TILs isolated from various human tumor tissues exhibit strong anti-tumor characteristics after *in vitro* sensitization and expansion.[47] TILs are non-genetically modified, resulting in fewer adverse reactions. However, they are not universally applicable due to MHC restrictions, requiring personalized treatment. This makes them particularly promising for personalized therapy in solid tumors.

Research indicates that in HCC patients, TILs primarily consist of $CD8^+$ T lymphocytes. Patients with a high proportion of TIL infiltration have lower recurrence rates and higher survival rates following surgical resection.[48,49] This suggests a significant anti-tumor role in infiltrating TILs in HCC tissues. In 2015, Jiang *et al*[50] observed the clinical safety of autologous TILs in HCC patients, with 15 out of 17 patients undergoing TIL expansion and subsequent treatment after tumor resection. A median follow-up of 14 months showed 100% survival, 80% without recurrence, and three cases of recurrence. The time from treatment to diagnosis of tumor recurrence ranged from 105 days to 261 days. Only one case exhibited mild flu-like symptoms post-treatment. Subsequently, in 2020, further evaluation of the tolerability and efficacy of autologous TIL treatment for high-risk recurrent HCC patients was initiated [Table 2]. Currently, there are no clinical randomized controlled studies related to TIL therapy for HCC, possibly due to the low lymphocyte content in the liver, making TIL isolation and expansion challenging, time-consuming, and inefficient. This is why optimizing TIL preparation and enhancing efficacy will likely be the direction for TIL therapy in HCC patients.

TCR-T

T-cell receptor-engineered T (TCR-T)-cell therapy, involves identifying specific TCR sequences capable of recognizing TAAs and then using genetic engineering to

Table 2: Clinical trials of ACTs in HCC treatment.

Identifier	Phase	Research object	Treatment arms	Primary outcome measures
1. TILs				
NCT01462903	I	HCC	TILs + IL-2	Safety and tolerability
NCT04538313	I/II	HCC patients with high-risk recurrent	High dose (10^{10}) *vs*. low dose (10^{9})	DLT, PFS
2. TCR-T				
NCT03899415	I	HBV-related HCC	HBV antigen specific TCR-T	Safety
NCT03132792	I	Advanced HCC	$AFP^{c332}T$ cells	DLT, safety
NCT03971747	I	HLA-A02$^+$ AFP$^+$ HCC unresectable HCC	AFP specific TCR-T	Safety
NCT02869217	I	HLA-A*02:01$^+$ or HLA-A*02:06$^+$ HCC	NY-ESO-1 specific TCR-T	Safety
NCT04368182	I	HLA-A02$^+$ AFP$^+$ unresectable HCC	C-TCR055 (AFP TCR)	Safety
NCT03965546	I	HLA-A02$^+$ AFP$^+$ HCC	ET140202 AFP TCR-T + sorafenib *vs*. ET140202 AFP TCR-T + TAE *vs*. ET140202 AFP TCR-T	Safety
NCT01967823	II	HLA-A*0201 Basket (including HCC)	Anti-NY-ESO-1 mTCR	Clinical response
NCT03441100	I	Advanced HCC	TCR-T cells targeting MAGE-1	Safety
3. CAR-T				
NCT02395250, NCT03146234	I	Advanced HCC	GPC3 CAR T (2nd generation)	Safety
NCT02715362	I/II	Advanced HCC	GPC3-CART (2nd generation)	Safety
NCT03980288	I	Advanced HCC	GPC3-CART (4th generation)	Safety, tolerability
NCT02587689	I	MUC1$^+$ advanced HCC	MUC1-CART	Safety
NCT03013712	I/II	EpCAM$^+$ HCC	EpCAM-CART	Safety
NCT04121273	I	Advanced HCC	GPC3 CAR T (2nd generation)	DLT
NCT02905188	I	Previously treated HCC	GPC3 CAR T (2nd generation)	DLT
NCT03198546	I	Advanced HCC	GPC3 and/or TGFβ-CAR-T	DLT
NCT03884751	I	Advanced HCC	GPC3 CAR T (2nd generation)	Safety, tolerability
NCT02723942	I/II	Previously treated HCC	GPC3 CAR T	Safety, tolerability
NCT02395250	I	Previously treated HCC	GPC3 CAR T (2nd generation)	Safety
NCT04506983	I	Advanced HCC	GPC3 CAR T (2nd generation)	Safety
NCT03993743	I	Advanced HCC	CD147-CART	Safety
NCT04550663	I	HCC	NKG2D-025 CAR-T cells	Safety, tolerability
NCT04270461	I	NKG2DL$^+$ HCC	NKG2D-CAR T	Safety, tolerability
4. NK				
NCT02725996	II	HCC after curative therapy	Curative therapy + NK *vs*. curative therapy	RFS, OS
NCT01147380	I	HCC after curative therapy	Liver NK cell inoculation	Safety
NCT02008929	II		NK cells	DFS
NCT04162158 NCT03228667	I/II	HCC after curative liver resection	Targeted drug + NK *vs*. targeted drug	Safety, OS
5. iNKT				
NCT04011033	II/III	Advanced HCC	TACE + iNKT *vs*. TACE	OS, PFS, DCR

ACTs: Adoptive cell therapies; AFP: Alpha-fetoprotein; CAR-T: Chimeric antigen receptor T; DCR: Disease control rate; DLT: Dose-limited toxicity; EpCAM: Epithelial cell adhesion molecule; GPC3: Glypican-3; HCC: Hepatocellular carcinoma; iNKT: Invariant natural killer T; MAGE-1: Melanoma-associated antigen 1; MUC1: Mucin1; NK: Natural killer; NKG2D: Natural killer group 2, member D; NKG2DL: Natural killer group 2, member D ligands; NY-ESO-1: New York esophageal squamous cell carcinoma-1; OS: Overall survival; PFS: Progression-free survival; RFS: Relapse-free survival; TACE: Transcatheter arterial chemoembolization; TCR-T: T-cell receptor T cell; TILs: Tumor-infiltrating lymphocytes.

introduce these sequences into T cells. These modified T cells are subsequently infused into cancer patients, enabling them to identify and eliminate tumor cells.[51] Therefore, a critical aspect of TCR-T-cell therapy is the identification of specific tumor target antigens. Currently, the main target antigens for TCR-T-cell therapy in HCC include AFP, glypican-3 (GPC-3), and hepatitis B surface antigen (HBsAg). In 2018, Zhu *et al*[52] identified a TCR capable of recognizing human leukocyte antigen (HLA)-A2-AFP_{158} specificity within transgenic mice via lentiviral vectors. They successfully transduced this TCR into CD8$^+$T cells, generating AFP_{158}-specific CD8$^+$ T cells. The adoptive transfer of AFP_{158}-specific CD8$^+$T cells killed AFP$^+$HepG2 cells and suppressed tumor growth. When

this TCR gene was introduced into primary human T cells, AFP_{158}-specific TCR-T cells demonstrated specific recognition of HLA-A2-AFP$^+$HepG2 cells and produced effector cytokines such as IFN-γ and interleukin 2 (IL-2). A clinical trial for autologous AFP-specific TCR-T-cell therapy in HCC is ongoing (NCT03132792). Additionally, TCR-T cells targeting GPC-3 can recognize and eliminate HLA-A2-GPC3$^+$ tumor cells and inhibit tumor growth.[53]

TCR-T-cell therapy that targets hepatitis viruses in Hepatitis B (HBV)/ Hepatitis C (HCV)-related HCC has shown promise. A Phase-I clinical trial targeting HBV with TCR-T-cell therapy in HBV-related HCC saw no adverse effects and resulted in a sustained decrease in HBsAg and HBV DNA levels. This therapy has anti-tumor efficacy and can clear the HBV virus while demonstrating sound safety and tolerance.[54] Furthermore, there are several clinical trials for autologous TCR-T-cell therapy targeting AFP in HCC [Table 2].

CAR-T

CAR-T-cell therapy involves genetic engineering to create a CAR by combining an antibody's antigen-binding region (scFv) with intracellular signaling domains. This CAR is then introduced to the surface of T cells, enabling them to recognize specific TAAs and exert anti-tumor effects.[55] CAR-T cells target tumor cells independently of MHC molecules and are categorized into four generations based on the following intracellular signaling domain structures: First-generation CAR-T cells contain only the CD3ζ chain in their intracellular domain, which has limited proliferative capacity and shorter *in vivo* survival. Second-generation CAR-T cells, built upon the first generation, incorporate a single co-stimulatory domain (CD28 or 4-1BB), enhancing T-cell proliferation and cytotoxicity compared to the first generation. Third-generation CAR-T cells further enhance proliferation and survival rates by incorporating two co-stimulatory domains (CD28 and 4-1BB). Fourth-generation CAR-T cells include an additional intracellular co-stimulatory domain, the nuclear transcription factor of activated T cells (NFAT), which controls the secretion of IL-12 upon CAR-T-cell activation, thereby modulating the immune response.[56,57]

The anti-tumor effects of CAR-T cells have been confirmed in hematological malignancies. However, due to the diversity of solid tumor types, their complex composition, and immunosuppression in the TME, CAR-T-cell therapy for solid tumors is mainly in the preclinical research stage.[44] AFP is a well-known specific marker for HCC, with overexpression detected in some HCC patients. Liu *et al*[58] demonstrated *in vitro* that CAR-T cells targeting AFP when co-cultured with AFP-positive liver cancer cells, secreted higher levels of cytokines such as tumor necrosis factor-α (TNF-α) and IFN-γ, and exhibited cytolytic killing effects on AFP-positive liver cancer cells. However, clinical validation is still required.

GPC-3 is a heparan sulfate proteoglycan not expressed in normal liver tissue but is significantly upregulated in the serum and tumor tissue of HCC patients.[59] Shi *et al*[60] conducted a Phase-I clinical trial of GPC-3-targeted CAR-T-cell therapy for advanced HCC patients in 2015 to assess safety and efficacy. CAR-T-cell therapy has exhibited promising safety results for HCC patients as no grade 3–4 neurotoxic reactions across 13 patients were observed. Notably, survival rates at the 6-month, 1-year, and 3-year marks were 50.3%, 42%, and 10.5%, respectively. Ongoing studies are currently assessing the safety and tolerability of fourth-generation GPC-3-targeted CAR-T cells.[61]

In addition to the mentioned specific markers, clinical trials for CAR-T cells targeting various liver cancer-related tumor antigens such as c-Met, natural killer group 2, member D ligands (NKG2DL), CD133, and CD147 are underway [Table 2].

Other immune cell therapy

NK cells

NK cells are essential in the immune system and can kill virus-infected or tumor cells without antigen presentation. They use receptors on their surface to regulate their activation and function.[62] Therefore, NK cells have strong immunocytotoxic effects on tumor cells with reduced MHC class molecule expression.

Adoptive NK cell therapy involves extracting NK cells from peripheral blood mononuclear cells (PBMCs) and expanding them *in vitro* through cytokine stimulation before reintroducing them into the patient's body.[63] Studies have found that *in vitro*-developed NK cells increase the expression of natural killer group 2, member D (NKG2D) receptor, leading to greater cytotoxicity against liver cancer cells. This enhances anti-tumor effects and improves NK cell function in HCC patients. Combining NK cell therapy with sorafenib treatment significantly boosts anti-tumor efficacy.[64,65] Lin *et al*[66] conducted a clinical study to observe the efficacy of cryoablation combined with allogeneic NK cells in advanced HCC patients. Combination therapy with adoptive transfer of NK cells and cryoablation resulted in a longer median PFS of 9.1 months compared to cryoablation alone (7.6 months). The combination therapy group also had a higher ORR and DCR, showing specific anti-tumor efficacy in HCC patients. NK cell therapy was given to 17 HCC patients after liver transplantation, with no significant side effects observed. Four patients died during a 2-year follow-up, but HCC did not recur in the remaining patients.[67] Although many studies have indicated the high potential of allogeneic NK cells in anti-tumor therapy, current clinical research on NK cell therapy for liver cancer is primarily in the early stages of safety exploration, and further data accumulation is needed.

iNKT cells

iNKT cells are a unique subset of T cells activated by glycolipid antigens presented by CD1d. They can produce IFN-γ and IL-4, directly killing tumor cells through various pathways.[68] iNKT cells can also promote DC maturation and activate NK cells, indirectly contributing

to tumor cell killing. As early as 2006, Motohashi *et al*[69] observed the safety of autologous iNKT-cell infusion in six non-small-cell lung cancer patients. Subsequently, in a study involving 17 non-small-cell lung cancer patients, they used α-GalCer-pulsed APCs to activate iNKT cells *in vivo*. They found that the therapeutic response of iNKT cells was closely associated with patient prognosis.[70] In 2021, Gao *et al*[71] demonstrated the safety of autologous iNKT-cell infusion in liver cancer patients. In 2023, the same research group compared the efficacy of iNKT-cell therapy in combination with TACE to TACE alone in treating BCLC-stage B HCC patients. They discovered that combination therapy significantly improved the PFS, ORR, DCR, and quality of life (QoL) of HCC patients.[72] Research has revealed that following the activation of iNKT cells and the release of cytokines, there is an increase in the expression of PD-1 on iNKT cells. Consequently, in the future, combined therapies involving iNKT cells and either DC or PD-1/PD-L1 may garner more attention.

Other immunotherapy strategies

Tumor vaccines

Tumor vaccines enhance the body's immune response to TAAs to exert anti-tumor effects. Compared to ICIs and ACT, tumor vaccine research began earlier in cancer treatment. However, due to challenges such as immune evasion by tumors and difficulties in finding suitable tumor antigens, the application of tumor vaccines in anti-tumor therapy has been limited. In treating HCC, the most extensively studied tumor vaccines are DC and peptide vaccines composed of amino acid sequences derived from TAAs.

DCs are the body's most potent APCs, effectively presenting antigens to T lymphocytes and activating the body's immune response. DC vaccines are based on the strong antigen-presenting ability of DCs. They involve pulsing DCs with proteins or nucleic acids derived from TAAs or tumor lysates and then infusing them into the patient's body. This process activates a specific anti-tumor immune response, killing tumor cells.[73] Lee *et al*[74] used DC vaccines from autologous tumor lysates to treat 31 advanced HCC patients. DC vaccine treatment showed promising results with a 54.8% tumor stabilization and a 40.6% one-year OS rate. Combination therapy with later-stage intensified treatment had a significantly higher one-year survival rate of 63.6%. No treatment-related adverse events or autoimmune diseases were observed, confirming the safety of DC vaccines. In advanced HCC patients, DC vaccines increased the median time to tumor progression to 36.6 months compared to 11.8 months in the control group. DC vaccines also prevented recurrence in some patients. DC vaccines for cancer treatment activate effector T lymphocytes, but immune checkpoints in the TME may limit their effectiveness.[73] Therefore, combining DC vaccines with ICIs for anti-tumor therapy may yield better results, but this requires further clinical research for confirmation.

One specific HCC-related TAA is GPC-3. Research has shown that GPC-3 vaccines are safe for treating advanced HCC and can activate tumor-specific immune responses in HCC patients, improving prognosis.[75] Sawada *et al*[76] investigated the impact of tumor vaccines on tumor recurrence rates in HCC patients who had undergone surgery or RFA. They found that compared to HCC patients who only received surgery or ablation treatment, 41 patients who received surgery or ablation in combination with GPC-3 vaccines had significantly lower one-year and two-year tumor recurrence rates (24.4% *vs*. 53.7% and 42.5% *vs*. 66.2%, respectively).

OVs

OVs are genetically modified to replicate within tumor cells and cause cell lysis, sparing normal cells. This can trigger the immune response in the TME by releasing TAAs and pathogen-associated molecular patterns (PAMPs), promoting the release of inflammatory cytokines and factors. OVs can also disrupt the tumor's blood supply system, leading to tumor cell death.[77]

In 2003, Park *et al*[78] assessed the safety of JX-594 in treating primary HCC or metastatic liver cancer patients. Fourteen patients were treated; all experienced flu-like symptoms, and 4 had transient thrombocytopenia. The maximum tolerated dose was 1×10^9 pfu. Out of 10 evaluable patients, 6 had stable disease, 3 had partial responses, and 1 had disease progression. HCC patients were divided into high- and low-dose groups. Adverse events were similar between the two groups, and survival was significantly associated with dose. The median survival time for high-dose patients was 14.1 months, while low-dose patients survived only 6.7 months. Vaccine dose correlated with survival benefits.[79] Various viruses, including JX-594, herpes simplex, adenovirus, and measles, have been used as oncolytic agents. However, the TME's immunosuppressive effects and virus entry mode can impact their functionality. Combining OVs with ICIs or encoding pro-inflammatory cytokines and T-cell co-stimulatory molecules can enhance OVs' anti-tumor efficacy.

Cytokine therapy

Cytokines play a role in developing HCC, similar to signaling molecules. Immune cells secrete different types of cytokines to activate or inhibit downstream immune responses, either suppressing or promoting the proliferation and survival of tumor cells.

In recent years, several cytokines have been closely associated with the progression of HCC. Targeted drugs against VEGF, such as bevacizumab and ramucirumab, have been widely used in treating HCC to inhibit angiogenesis. However, drugs targeting hepatocyte growth factor (HGF) and Ang-2 are still developing.

IL-6 is an inflammatory cytokine with pro-angiogenic properties. Its expression levels tend to increase during the early stages of HCC formation and are an independent predictor of poor prognosis for HCC patients.[80] Shao *et al*[81] analyzed serum IL-6 levels and prognosis

in HCC patients with sorafenib. Using a cutoff value of 4.28 pg/mL, patients were divided into high-level IL-6 and low-level IL-6 groups. They found that patients in the high-level IL-6 group had shorter OS than the low-level group (8 months *vs*. 13.9 months) and that high IL-6 levels were an independent predictor of OS (hazard ratio (HR) = 2.594, $P = 0.005$). In addition, IL-6 is also involved in recruiting and polarizing neutrophils within the tumor, and it upregulates the expression of cell surface PD-L1 through the signal transducer and activator of transcription 3 (STAT3) signaling pathway. Consequently, simultaneously reducing serum IL-6 levels in HCC patients while blocking PD-L1 can synergistically exert anti-tumor effects, leading to an improved prognosis.[82,83]

Targeting cytokine expression in HCC patients can enhance anti-tumor effects and provide a monitoring indicator for disease progression and prognosis. IL-10 inhibits inflammatory mediator production and antigen presentation, making it a potential intervention option.

Combination of Different Immunotherapy Strategies

Combining various immunotherapies can have stronger anti-tumor effects than single treatments as tumors often involve multiple immune inhibitory pathways and impairments in various immune cells. Clinical trials are currently testing combination therapies involving different types of ICIs. nivolumab and ipilimumab combination therapy has been approved by the FDA for the treatment of HCC that has previously failed sorafenib treatment,[20] and tremelimumab in combination with durvalumab has been approved for first-line therapy in advanced HCC patients.[84] This dual-immunotherapy benefits advanced HCC patients more and addresses, to some extent, the resistance to PD-1/PD-L1 in some patients.[40]

Research is exploring combination therapy involving ICIs and ACT for HCC treatment. Combining these therapies can enhance immune-killing function against tumor cells. Studies show that CAR-T targeting GPC-3 has a killing function against tumor cells expressing GPC-3, and combining ACT therapy with PD-1 inhibitors is feasible. Clinical studies also explore the efficacy of combining different ICIs with NK cell therapy for various solid tumors (NCT03228667).[85] Therefore, it is reasonable to speculate that once mixing ACT with ICIs gains traction, various combination immunotherapy strategies will become the future direction of anti-tumor treatment development.

Conclusion

Immunotherapy has shown promise in treating HCC, with some patients achieving complete or long-lasting remission. Combining different approaches has proven more effective, but there are risks of autoimmune diseases and adverse reactions. These issues can hinder or even interrupt anti-tumor treatment, reducing its effectiveness. Therefore, the rational application of immunotherapies with different mechanisms is critical in the future of liver cancer immunotherapy. Future liver cancer immunotherapy will target inhibitory factors, reshape the TME, identify specific efficacy markers, and use immune checkpoint modulation and cellular immunotherapy.

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Conflicts of interest

None.

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