Emerging Immunotherapeutic Strategies for Hepatocellular Carcinoma: Current Landscape and Future Directions

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Abstract. Hepatocellular carcinoma is the sixth most common cancer worldwide. The most common treatments for now are surgery and TKIs; however, both have limitations, such as recurrence and drug resistance. Therefore, they are not sufficient. In recent years, many immunotherapies have been discovered and explored, such as ICIs, CAR-T, Bispecific antibodies, and vaccines. Most of these immunotherapies are still in the early stages of development and have not yet been fully validated. Nevertheless, tumor heterogeneity and immunosuppressive environment remain major challenges for current treatments. For the future direction, immunotherapy may become one of the most advanced and effective treatment options for HCC.

Keywords: Hepatocellular carcinoma, Immunotherapy, Tumor Microenvironment, CAR-T Cell Therapy, Combination Strategies.

1. Introduction

Hepatocellular carcinoma (HCC), the predominant form of primary liver cancer, accounts for >90% of global liver malignancies and ranks as the fifth most diagnosed cancer worldwide. With liver cancer representing the third leading cause of cancer-related mortality, HCC exhibits a particularly dismal prognosis, reflected in its five-year survival rate of ~18%. This poor outlook stems largely from asymptomatic early-stage progression, resulting in >70% of patients presenting with advanced disease at diagnosis [1]. Conventional therapies for advanced HCC (systemic chemotherapy, tyrosine kinase inhibitors [TKIs], transarterial chemoembolization [TACE]) yield a median survival of just 6-12 months, while surgical approaches (resection/transplantation) show 70% recurrence rates in early-stage disease and minimal survival benefit in late stages [1, 9].

Immunotherapy has redefined HCC management, with immune checkpoint inhibitors (ICIs) now established as first-line therapy for advanced disease-enabling 25% of patients to achieve > 4-year survival [2]. Beyond ICIs, emerging modalities including bispecific antibodies, CAR-T cell therapy, and cancer vaccines demonstrate promising preclinical and early clinical activity [10]. Nevertheless, critical challenges persist: CAR-T applications in solid tumors face target heterogeneity, immunosuppressive microenvironments, and antigen escape, while broader immunotherapeutic efficacy is constrained by HCC's immunologically "cold" phenotype [10]. Future advances will require precision antigen targeting, rational combination strategies, and microenvironment reprogramming to unlock durable responses [2].

2. Immune Checkpoint Inhibitors (ICIs)

2.1 Mechanism of Action

PD-1, an inhibitory receptor expressed on T cells, binds to PD-L1, a ligand frequently overexpressed on tumor cells and immunosuppressive cells. This interaction suppresses T-cell activation, proliferation, and cytotoxic factor release, facilitating immune evasion, in contrast, CTLA-4 inhibits early T-cell activation by competitively binding to the co-stimulatory molecules CD80/CD86 [2].

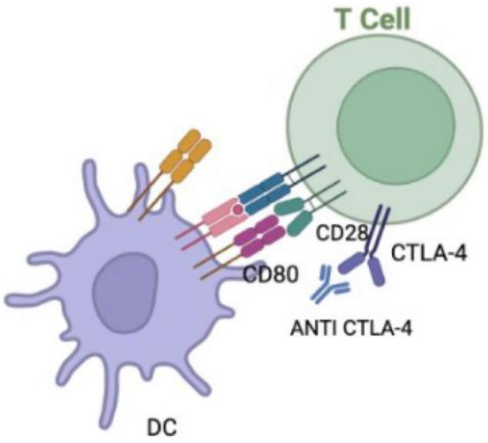

Figure 1. CTLA-4 blockade enhances T cell activation by preventing inhibitory signaling from CTLA-4/CD80 interaction during antigen presentation.

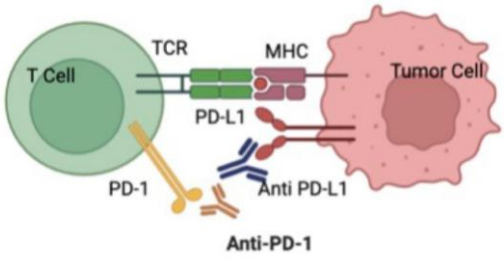

Figure 2. PD-1/PD-L1 blockade restores T cell function by disrupting tumor-mediated immune suppression in the tumor microenvironment.

2.2 Clinical Efficacy in Advanced HCC

Several landmark trials support ICI use in advanced HCC:

IMbrave 150 demonstrated superior efficacy of atezolizumab (anti-PD-L1) combined with bevacizumab (anti-VEGF) over sorafenib, establishing this regimen as first-line therapy with significant overall survival (OS) benefits [2].

HIMALAYA evaluated the STRIDE regimen (Single Tremelimumab Regular Interval Durvalumab), combining durvalumab (anti-PD-L1) with a single priming dose of tremelimumab (anti-CTLA-4) in treatment–naive advanced HCC. This regimen significantly improved OS versus sorafenib, with a 48-month OS rate of 25.2% (vs. 15.1% for sorafenib) and no new serious treatment-related adverse events.

CheckMate040 assessed nivolumab (anti-PD-1) monotherapy or combined with ipilimumab (anti-CTLA-4) in advanced HCC patients ineligible for curative therapy. Both approaches demonstrated safety and efficacy.

2.3 Expanding Applications

ICI therapy is being explored beyond advanced HCC:

Neoadjuvant setting leverages the intact tumor antigen landscape to enhance T-cell priming, reduce micrometastatic burden pre-surgery, and potentially induce durable immune memory.

Liver transplant recipients represent a challenging population, as ICI therapy carries a significant risk of triggering allograft rejection, necessitating further optimization [2].

2.4 Current Challenges

Despite significant benefits, ICI efficacy faces limitations:

An immunosuppressive tumor microenvironment (TME) enriched with regulatory cells (e.g., Tregs, MDSCs).

Loss of tumor antigen expression, impairing T-cell recognition.

T-cell exhaustion within the TME.

Low tumor mutational burden (TMB) and neoantigen deficiency, hindering de novo immune response initiation [2].

3. CAR-T Cell Therapy in Hepatocellular Carcinoma (HCC)

3.1 Structure and Generational Evolution of CAR-T Cells CAR-T cell therapy involves genetically engineering a patient's T cells to express chimeric antigen receptors (CARs). These synthetic receptors comprise four core domains:

Extracellular Domain: Contains a tumor antigen-binding single-chain variable fragment (scFv) derived from monoclonal antibodies.

Hinge Region: Provides flexibility for antigen binding (e.g., CD8α or IgG4-derived sequences).

Transmembrane Domain: Anchors and stabilizes the CAR within the cell membrane (e.g., CD3ζ, CD28).

Intracellular Domain: Mediates T-cell activation and co-stimulation.

Generational Advancements:

1st Generation: scFv + CD3ζ signaling domain → limited persistence/activation.

2nd Generation: Adds one co-stimulatory domain (CD2282 or 4-1BB) → enhanced T-cell expansion, cytotoxicity, and persistence.

3rd Generation: Incorporates two co-stimulatory domains (e.g., CD28 + 4-1BB) → further amplifies anti-tumor activity.

4th Generation ("TRUCKs"): Integrates immunomodulatory transgenes (e.g., IL-12, IL-15) → remodels the tumor microenvironment (TME) and combats immunosuppression.

5th Generation: Feathers cytokine-inducible signaling domains (e.g., JAK-STAT fusion) → augments proliferation and memory formation [1].

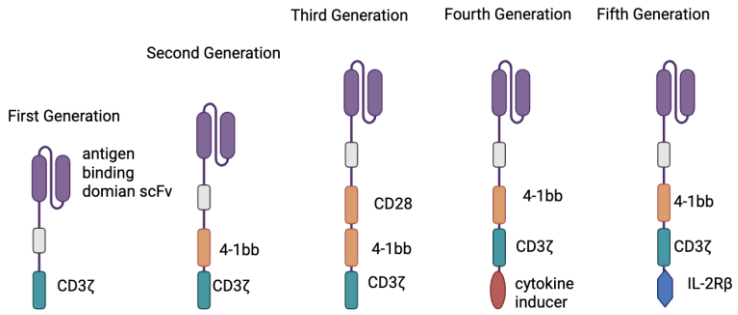

Figure 3. First to fifth-generation CAR constructs; Example: GPC3-targeted CAR-T; Co-stimulatory domains, cytokine armoring (e.g., IL-7, CCL19); Purpose: Educate on CAR evolution and relevance to HCC.

3.2 Target Antigens in HCC

Key tumor-associated antigens (TAAs) for CAR-T therapy in HCC include: GPC3 (Glypican-3): Overexpressed in >70% of HCCs with minimal expression in healthy liver tissue; correlates with poor prognosis [10].

AFP (alpha-fetoproteins): Secreted by HCC cells and expressed on cancer-associated fibroblasts (CAFs); a diagnostic/prognostic serum biomarker.

CD133 (Prominin-1): Transmembrane glycoprotein marking cancer stem cells (CSCs); drives tumor initiation, metastasis, and chemoresistance [1]. Other targets under investigation: MUC1, EpCAM, and NY-ESO-1.

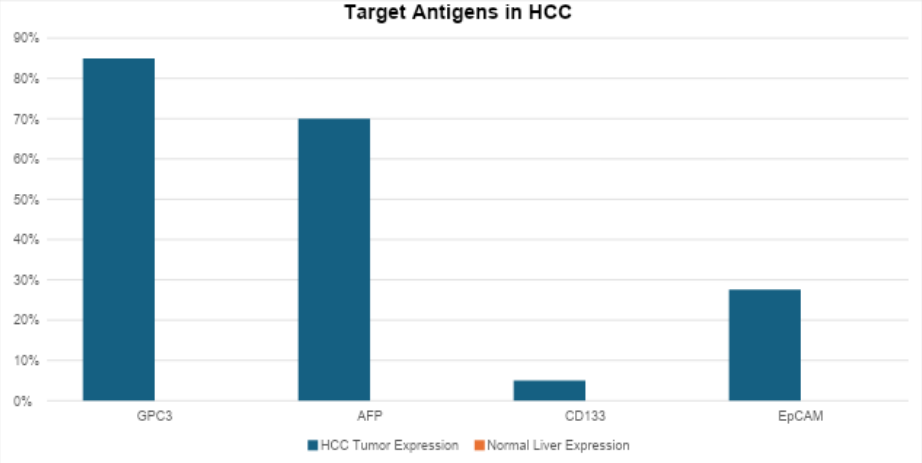

Figure 4. Tumor specificity vs. normal tissue expression [3, 4, 5, 6, 7]

3.3 Clinical Trial Progress

NCT03198546: Phase I trial of GPC3-targeted CAR-T cells in advanced HCC. Results: Disease stabilization (SD) and partial responses observed with no dose-limiting toxicities.

Emerging approaches: Dual-target CARs (e.g., GPC3 + AFP) and CSC-directed CAR-Ts (e.g., anti-CD133) to mitigate antigen escape.

3.4 Key Challenges

TAA Heterogeneity:

Ideal TAAs are scarce; GPC3/AFP/CD133 exhibit variable expression. Antigen loss or downregulation enables immune escape.

T-cell Exhaustion:

Chronic antigen exposure upregulates inhibitory receptors (PD-1, TIM-3, LAG-3). Impaired cytokine secretion and reduced cytotoxic capacity.

Immunosuppressive TME:

Metabolic barriers (hypoxia, lactate accumulation). Inhibitory cells (Tregs, MDSCs, tumor-associated macrophages). Immune checkpoint ligands (PD-L1, CTLA-4) [1].

3.5 Innovative Solutions

In response to antigen heterogeneity, current solutions include dual target CARs, affinity-tuned CAR-T cells, and CAR-T cells targeting cancer stem cells to improve tumor antigen specificity. Further optimization of the CAR framework structure and novel CAR designs are also solutions to these challenges. For example, the emergence of switchable CARs, logic-gated CARs, or armored CARs. CARs can provide greater control and flexibility in responding to evolving challenges [1].

4. Bispecific Antibodies and T Cell Engagers in HCC

4.1 Mechanism of Action & Formats

Bispecific antibodies (BsAbs) are engineered molecules capable of simultaneously binding two distinct antigens. Their primary application in oncology involves redirecting cytotoxic immune cells to tumor targets:

T cell Engagers (TCEs): The most established subclass (e.g., BiTEs - Bispecific T-cell Engagers).

These typically feature: An anti-CD3ε arm (engages and activates T cells) A tumor-associated antigen (TAA)-binding arm (e.g., GPC3, AFP, EpCAM)

Immune Checkpoint-Targeting BsAbs: Designed to block dual inhibitory pathways (e.g., PD-1 x CTLA-4), potentially enhancing T-cell function more effectively than monospecific antibodies.

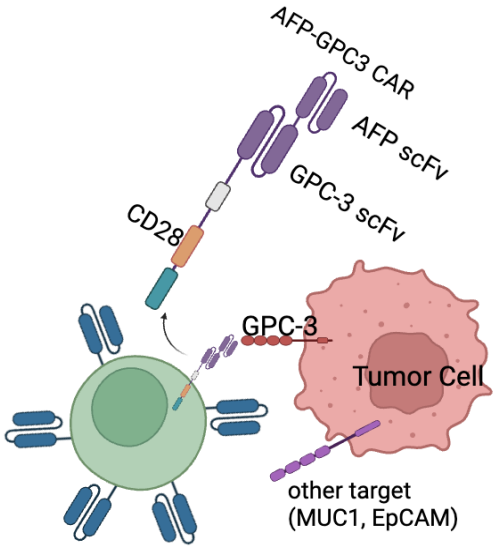

Figure 5. BiTE binding tumor antigen and CD3 on T cell; Illustration of synapse formation and T-cell activation.

4.2 Key Therapeutic Strategies

Tumor Cell Lysis via TCEs:
Upon simultaneous binding to CD3+ T cells and TAAs on HCC cells, TCEs:
Induce immunological synapse formation
Trigger potent T-cell activation and cytotoxicity
Bypass MHC restriction
Example Targets in HCC: GPC3, AFP, EpCAM, MET.
Dual Immune Checkpoint Blockade:
BsAbs targeting PD-1 x CTLA-4:
Concurrently disrupt both major T-cell exhaustion pathways
Synergistically enhance T-cell proliferation and effector function
May reduce tumor immune escape vs. monotherapy [2]

4.3 Advantages Over Monoclonal Antibodies

Feature BsAbs Target Engagement, Dual antigen binding, Single antigen T-cell recruitment, Direct physical linkage to tumor cells, Relies on endogenous priming Potency Picomolar activity (e.g., BiTEs), Nanomolar activity MHC Dependency, MHC-independent killing MHC-dependent (ICIs)

4.4 Clinical Progress & Challenges

Early Clinical Trials:

GPC3-targeted TCEs (NCT03980288): Phase I trials in advanced HCC show preliminary antitumor activity.

PD-1 × CTLA-4 BsAbs (e.g., KN046): Phase II data demonstrate manageable safety and objective responses.

Key Challenges:

Cytokine release syndrome (CRS) and neurotoxicity
On-target/off-tumor toxicity (limited TAA specificity)
Immunosuppressive TME resistance mechanisms [2]

5. Cancer Vaccines and Adoptive T Cell Therapies in HCC

5.1 Personalized Neoantigen Vaccines Mechanism:

These vaccines leverage patient-specific tumor neoantigens - unique mutations identified through genomic profiling of individual HCC tumors.

The process involves:

Tumor DNA/RNA sequencing

In silico neoantigen prediction (via MHC binding algorithms)

Vaccine formulation (peptide/RNA/DNA-based) targeting validated neoantigens

Immune Activation: Vaccines prime and expand neoantigen-specific T cells, generating a precision anti-tumor response with minimal off-target toxicity.

Combination Strategies: Synergistic use with immune checkpoint inhibitors (e.g., anti-PD-1) counteracts T-cell exhaustion, enhancing response magnitude and durability [9].

5.2 Tumor-Infiltrating Lymphocyte (TIL) Therapy Procedure

Resection: Tumor tissue harvested surgically

Isolation/Expansion: TILs extracted and expanded ex vivo with IL-2 and CD3/CD28 stimulation

Reinfusion: Activated TILs administered after lymphodepleting chemotherapy

Clinical Status:

Early-phase trials in HCC (e.g., NCT04841005) show feasibility.

Demonstrated efficacy in melanoma/ovarian cancers.

Key Challenges:

Limitation impacts low TIL reactivity

Limited expansion of tumor-specific clones in 30-50% of HCCs TME

Suppression of TGF-β, adenosine, and PGE2 inhibits TIL function post-infusion

TIL exhaustion upregulation of PD-1, TIM-3, LAG-3 during expansion

5.3 TCR-Engineered T Cell (TCR-T) Therapy

Workflow:

Antigen Identification: Screening for tumor-specific peptides (e.g., AFP158-166, NY-ESO-1)

TCR Cloning: Isolation of high-affinity TCRs from rare reactive T cells

Genetic Modification: Viral vector-mediated (e.g., Lentivirus) TCR gene transfer into patient T cells

Advantages vs. CAR-T: Recognizes intracellular antigens presented by MHC-I (broader target pool).

Critical Limitations:

Target Identification Burden: Only 1-2% of predicted neoantigens elicit immune responses

Validation requires >6 months per target [9]

TCR Mispairing: risk consequence endogenous/exogenous TCR chain pairing off-target reactivity or autoimmune toxicity

HLA Restriction: limited to patients with specific HLA alleles (e.g., HLA-A*02:01) [9]

6. Combination Strategies

Combining immunotherapy with conventional therapies represents a major advancement in HCC treatment. While single-agent immunotherapy has limited response rates, it enhances therapeutic

responses to immune checkpoint inhibitors (ICIs) by inducing tumor cell apoptosis and improving immune recognition. Key synergistic approaches include:

Transarterial Chemoembolization (TACE) + Immunotherapy

TACE activates the tumor microenvironment for immunotherapy by releasing neoantigens and inducing ischemia. The EMERALD-1 trial is evaluating atezolizumab + bevacizumab combined with TACE, providing critical clinical evidence for this strategy.

Hepatic Artery Infusion Chemotherapy (HAIC) + ICIs

HAIC delivers high local concentrations of chemotherapy, remodels the tumor microenvironment, and induces immunogenic cell death. Combined with ICIs, it enhances efficacy while reducing systemic toxicity. The TRIPLET study demonstrated the efficacy and safety of HAIC + camrelizumab + revoceranib in BCLC-C HCC patients.

Radiation Therapy + Immunotherapy

Radiation sensitizes tumors to immunotherapy through precise photon energy delivery, causing DNA damage and cancer cell death. The resulting inflammatory response and antigen release enhance tumor immunogenicity, amplifying immunotherapy efficacy. Current trials exploring Y-90 radioembolization combinations include:

Atezolizumab + bevacizumab (NCT05377034)

Crizotinib (NCT05592584, NCT06397222) [2]

7. Biomarkers and Patient Selection

The identification of predictive biomarkers is critical for optimizing immunotherapy response and enabling personalized treatment in HCC. Key biomarkers include:

PD-L1 Expression:

Predictive of ICI efficacy in certain tumors

Quantified via immunohistochemistry (IHC)

AFP (Alpha-fetoprotein): Serum/tissue biomarker associated with poor prognosis

GPC3 (Glypican-3): Overexpressed in >70% of HCCs, correlates with adverse outcomes and serves as a therapeutic target

Tumor Mutational Burden (TMB): Reflects tumor neoantigen load, associated with ICI response in select cancers [9, 10].

Clinical Implementation:

Tissue biopsy and molecular profiling enable:

Assessment of immune microenvironment status

Quantification of target antigen expression (e.g., GPC3, PD-L1)

Identification of actionable targets for therapy personalization

8. Safety and toxicity

Relatively common events in CAR-T therapy include neurotoxicity and cytokine release syndrome (CRS), characterized by excessive release of immune regulatory cytokines and factors, as well as an immune response of the host immune system to the infused CAR-T cells. T cell exhaustion can reduce the risk of an immune response to infused CAR-T cells, otherwise limiting their efficacy or leading to their premature clearance [1]. Graft-versus-host disease (GvHD) is another potential risk. When donor T cells attack the recipient's normal tissues, it can trigger a systemic inflammatory response [9]. To enhance the safety of CAR-T cells, novel systems such as the Tet-On inducible gene expression system have been introduced, which can effectively regulate the expression and activity of CAR. Compared with traditional CAR-T cells, they exhibit higher safety. For example, integrating the iCaspase-9 gene into the CAR structure can block T cell activation by inducing CAR-T cell apoptosis [1].

9. Future Directions

The future development of immunotherapy still faces many challenges. To improve the efficacy of immunotherapy, combination therapies can be used. The combination of immune checkpoint inhibitors and CAR-T cell therapy can enhance T cell activity and overcome drug resistance, including regulating the cytokine microenvironment in the TME. This may help create a more favorable immune environment for CAR-T cell function [1]. Since autologous CAR-T cell therapy requires preparation through peripheral blood mononuclear cell collection, and this preparation process is costly and may result in treatment failure in some patients. Therefore, the development of an "off-the-shelf" ACT that uses cells from healthy donors or immune cells derived from induced pluripotent stem cells (iPSCs) for large-scale production. These cells can be cryopreserved for infusion into patients as needed, thereby reducing manufacturing time and costs [9].

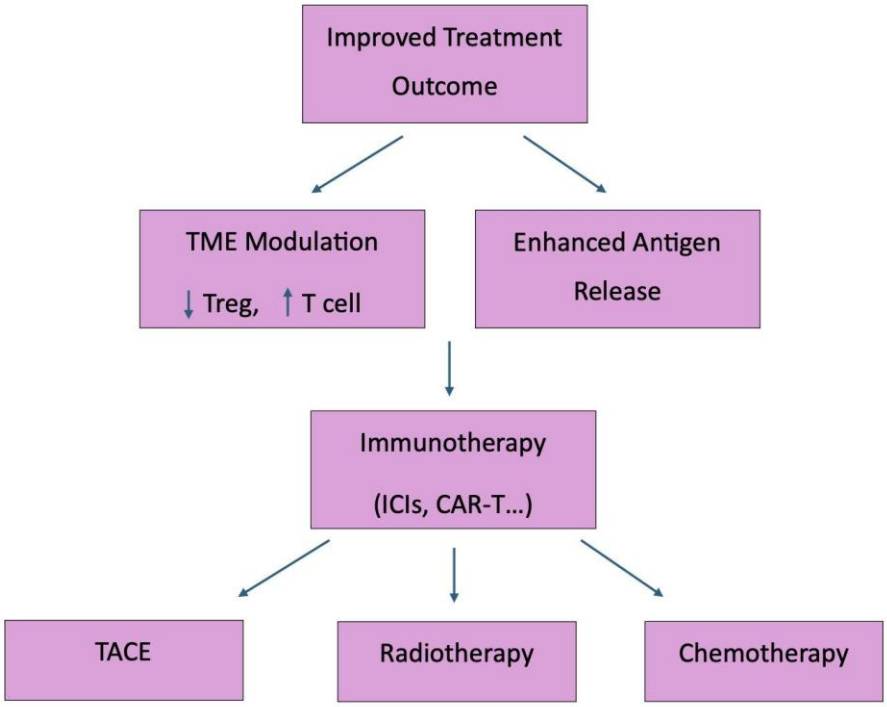

Figure 6. Immunotherapy + TACE, radiotherapy, chemotherapy; Mechanisms of synergy: improved antigen release, reduced TME suppression.

10. Conclusion

Immunotherapy is gradually changing the treatment approach for HCC. ICIs, CAR-T cells, bispecific antigens, and cancer vaccines are all frontline treatment approaches that bring hope to HCC patients. Combination therapy strategies have also improved treatment safety and diversity. Although these therapies have shown promising results in early stages, limitations still exist. Future treatment strategies need to continue to optimize treatment options and control costs. Encouraging patients to participate in clinical trials actively is also an important step in promoting the clinical translation of HCC immunotherapy.

Clinical Trial	Time	Type	Target	State	Phase	Citation
NCT03198546	26 June 2017	CAR-T	GPC-3	Recruiting	I	Pang N, Shi J, Qin L, Chen A, Tang Y, Yang H, et al. IL-7 and CCL19-secreting CAR-T cell therapy for tumors with positive glypican-3 or mesothelin. Journal of Hematology & Oncology [Internet]. 2021 Jul 29;14(1). Available from: https://jhoonline.biomedcentral.com/articles/10.1186/s13045-021-01128-9
NCT02395250	/	CAR-T	GPC-3	Completed	I	Shi D, Shi Y, Kaseb AO, Qi X, Zhang Y, Chi J, et al. Chimeric Antigen Receptor-Glypican-3 T-Cell Therapy for Advanced Hepatocellular Carcinoma: Results of Phase I trials. Clinical Cancer Research [Internet]. 2020 May 5;26(15):3979–89. Available from: https://aacrjournals.org/clincancerres/article/26/15/3979/82521/Chimeric-Antigen-Receptor-Glypican-3-T-Cell
NCT03146234	/	CAR-T	GPC-3	Completed	I	
NCT05652920	15 Dec 2022	CAR-T	GPC3	Recruiting	I/II	Shin S, Kudo M. Current landscape of adoptive cell therapy and challenge to develop off-the-shelf therapy in hepatocellular carcinoma. *J Gastroenterol Hepatol*. 2025;40(Suppl 1):49–59.
NCT04677088	29 Mar 2018	TCR-T	HBV	/	I	
NCT05339321	14 Apr 2021	TCR-T±ICI	HBV	Recruiting	I	
NCT04368182	13 Apr 2020	TCR-T	AFP	/	I	
NCT04745403	20 May 2022	TCR-T	HBV	Recruiting	I	
NCT04745403	08 Dec 2021	CAR-T	GPC-3	Recruiting	I	
NCT05120271	18 May 2022	CAR-T	GPC-3	Recruiting	I/II	

Figure 7. Timeline of Major Clinical Trials in HCC Immunotherapy [7, 8, 9]

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