Feng *et al*. *Hepatoma Res* 2021;7:3
DOI: 10.20517/2394-5079.2020.107

Hepatoma Research

Review

Open Access

The immune microenvironment and progression of immunotherapy and combination therapeutic strategies for hepatocellular carcinoma

Zun-Yong Feng[1,2,#], Fang-Gui Xu[3,#], Yu Liu[3], Hao-Jun Xu[1], Fu-Bing Wu[3], Xiao-Bing Chen[4], Hong-Ping Xia[1,2,3]

[1]Department of Pathology, School of Basic Medical Sciences & State Key Laboratory of Reproductive Medicine & Key Laboratory of Antibody Technique of National Health Commission, Nanjing Medical University, Nanjing 211166, China.
[2]Interdisciplinary Innovation Institute for Medicine and Engineering, Southeast University-Nanjing Medical University, Nanjing 211189, China.
[3]Sir Run Run Hospital, Nanjing Medical University, Nanjing 21116, China.
[4]Department of Medical Oncology, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou 450008, China.
[#]Contribute equally to this work.

Correspondence to: Dr. Hong-Ping Xia, Department of Pathology, School of Basic Medical Sciences & State Key Laboratory of Reproductive Medicine & Key Laboratory of Antibody Technique of National Health Commission, Nanjing Medical University, Nanjing 211166, China. E-mail: xiahongping@njmu.edu.cn; Dr. Xiao-Bing Chen, Department of Medical Oncology, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou 450008, China. E-mail: xiaobingchen01@163.com; Dr. Fu-Bing Wu, Sir Run Run Hospital, Nanjing Medical University, Nanjing 21116, China. E-mail: wfbwkx@163.com

How to cite this article: Feng ZY, Xu FG, Liu Y, Xu HJ, Wu FB, Chen XB, Xia HP. The immune microenvironment and progression of immunotherapy and combination therapeutic strategies for hepatocellular carcinoma. *Hepatoma Res* 2021;7:3. http://dx.doi.org/10.20517/2394-5079.2020.107

Received: 14 Sep 2020 **First Decision**: 22 Sep 2020 **Revised**: 9 Oct 2020 **Accepted**: 20 Oct 2020 **Published**: 7 Jan 2021

Academic Editor: Sandi Alexander Kwee **Copy Editor**: Cai-Hong Wang **Production Editor**: Jing Yu

Abstract

Hepatocellular carcinoma (HCC) accounts for 75%-85% of all primary liver cancers and is the leading cause of cancer-related deaths. China accounts for almost half of the global incidence and deaths of HCC. The poor response of chemotherapeutics and targeted drugs may be due to the drug resistance, heterogeneity of HCC, severe chronic liver damage and cirrhosis. Restoration of the liver microenvironment changes caused by chronic injury is crucial. Immunotherapy recently seems to show promise for the treatment of HCC induced by inflammatory injury. However, the unique liver immune system and resident immune tolerance state also pose a challenge for HCC immunotherapy. Different combinations of strategies have been developed for enhancement of HCC treatment. Here, we will discuss the immune microenvironment and progression of immunotherapy and combination therapeutic strategies for HCC.

Keywords: Immune microenvironment, immunotherapy, immune checkpoint inhibitors, Chimeric antigen receptor T, hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for 75%-85% of all primary liver cancers. Due to the rapid progression of HCC, the lack of effective treatment programs, and poor prognosis makes it the fourth leading cause of cancer-related deaths[1]. Due to regional differences in medical diagnosis and treatment, more than half of the new cases and deaths of HCC each year occur in the Asia-pacific region. Patients with early HCC in Europe and United States can be diagnosed and effectively treated in time[2]. More than 70% of HCC patients do not benefit from medical therapy. The vast majority of HCC patients present with an advanced stage at diagnosis, and the most effective surgical programs are often challenging to implement. In the past ten years, dozens of promising chemotherapeutics have failed the phase III trial, with only sorafenib demonstrating a low objective response rate and a slight increase in survival[3]. Research on targeted drugs for cell proliferation, metastasis and angiogenesis are encouraging, such as regorafenib and lenvatinib, although the overall survival rate remains dissatisfactory[4]. The ineffectiveness of chemotherapeutics and targeted drugs may be due to drug resistance and heterogeneity of HCC. HCC is usually accompanied by severe chronic liver damage and cirrhosis. Hence, anti-HCC drugs require a good balance of therapeutic response and drug toxicity and this often limits the application of highly active compounds with high toxicity[5,6]. Therefore, restoration of the liver microenvironment caused by chronic injury should be incorporated in the holistic management of HCC. In recent years, immunotherapy has been used in the treatment of various solid tumors. This was observed through the checkpoint inhibition of programmed cell death 1/programmed cell death ligand 1 (PD-1/PD-L1) and cell toxic T lymphocyte-associated protein 4 (CTLA-4) while improving the tumor immune microenvironment which seems to be particularly relevant for the treatment of HCC. However, the unique liver immune system and resident immune tolerance state make it different from other organs. Besides, continuous matrix remodeling of the malignant hepatocyte transformation caused by chronic inflammation and scars has created an immunosuppressive microenvironment that promotes the development of HCC, posing a challenge for HCC immunotherapy[7].

THE IMMUNE MICROENVIRONMENT OF THE LIVER AND HCC

The liver has a unique immune regulation and balance mechanism. On one hand, the portal vein system is directly exposed to gastrointestinal pathogens and requires an effective immune response. On the other hand, it needs to deal with a large number of harmless blood antigens and maintain the immune tolerance of the liver[8]. In most cases, the liver is in a physiological immune tolerance state[9]. Most non-parenchymal cells, such as live sinusoidal endothelial cells (LSEC), Kupffer cells (KCs) and hepatic dendritic cells (HDC), gather in the liver sinusoids. It constitutes the physiological basis of the liver's immunosuppressive microenvironment[10,11]. LSEC has the dual functions of immune surveillance and immune tolerance. It acts as an antigen-presenting cell (APC) to present pathogens or tumor antigens[12]. At the same time, it inhibits the excessive responses of DC and T lymphocytes to bacterial antigens from the portal system[13-15]. KCs maintain immune tolerance by engulfing pathogenic microorganisms derived from the intestine, secreting inhibitory factors (such as IL-10 and prostaglandins) and activating the proliferation of regulatory T cells (Tregs)[16-19]. Besides, HDC is also a component of the liver immune tolerance by reducing the expression of MHC II and co-stimulatory molecules[20]. In summary, this immune-tolerant physiological environment creates a huge obstacle to the host's anti-tumor immunity.

The pathogenesis of HCC is characterized by destruction of the sinusoidal structure by a viral infection and inflammatory injury, impairment of immune surveillance and immune tolerance functions leading to liver cirrhosis and liver cancer[6,10]. The high-risk factors of HCC (hepatitis virus, alcohol, aflatoxin, *etc.*) drive hepatocyte DNA damage, endoplasmic reticulum stress and necrosis, which in turn leads to the formation of regenerative nodules, proliferative nodules and ultimately HCC[21]. HCC has abundant immune cell infiltration, which is the immune response of the host trying to clear the tumor. Unfortunately, this immune

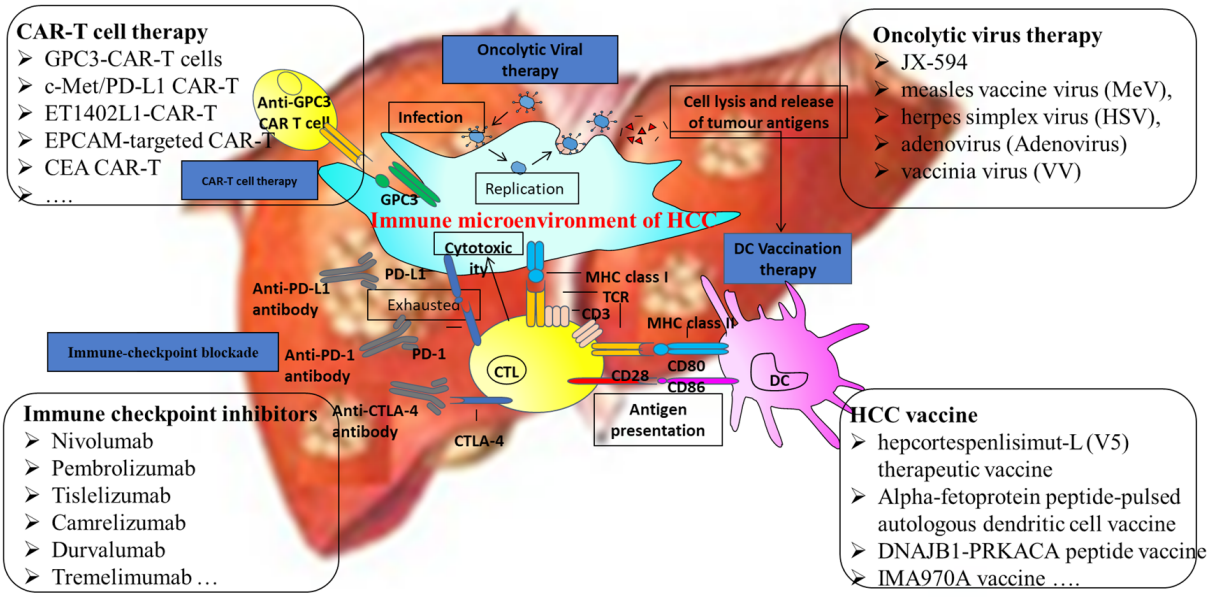

Figure 1. The immune microenvironment and immunotherapy for HCC. We divide current HCC immunotherapy into four categories, namely immune checkpoint inhibitors, oncolytic virus therapy, HCC vaccine and chimeric antigen receptor T (CAR-T) cell therapy. HCC: hepatocellular carcinoma

response is often dysregulated[22]. Tumor-infiltrating lymphocytes (TILs) account for a high proportion of HCC[23,24], but these ineffective TILs often prove to be insufficient to control tumor growth[25]. The increased FoxP3+ Treg may impair the effector function of CD8+ T cells, which exacerbates the immunosuppressive microenvironment in HCC and is associated with a poor prognosis[26]. In addition, adaptive immune cells (such as CD8 + T cells, Th17 cells and B cells) can also stimulate the development of HCC[27,28]. There are a large number of bone marrow-derived suppressor cells (MDSCs) and Tregs in the microenvironment of HCC, which evade immune surveillance through a variety of mechanisms, such as the expressing high-levels of SOCS3 and IL-10 to limit immune cell activation[29] and secretion fibrosis factor TGF-β. This is used to build an environment of immunosuppression and drug resistance[30], and directly down-regulates the expression of T cells or NK cell activation ligands (MHC class I and NKG2D, *etc.*)[31,32]. Therefore, the immunosuppressive environment of HCC is an arduous challenge to the host's immune system, which makes immunotherapy a promising approach for HCC treatment in the future.

THE STRATEGIES OF HCC IMMUNOTHERAPY

According to the immunological basis of HCC, we divide current HCC immunotherapy into four categories, including immune checkpoint inhibitors, oncolytic virus therapy, HCC vaccine and chimeric antigen receptor T (CAR-T) cell therapy [Figure 1]. Due to the destruction of the HCC sinusoidal structure, it is difficult for LSEC and HDC to complete the antigen presentation process. Therefore, the specific DC vaccine is obtained by impinging tumor-associated antigen (TAA) or tumor lysate into DC *in vitro*. It activates cytotoxic T lymphocytes (CTLs) through major histocompatibility complex (MHC) class II-TCR antigen presentation and CD40/CD80/CD86-CD28 interaction. CTLs recognize and destroy tumor cells containing HCC-related antigens on MHC class I molecules. In addition to blocking the antigen presentation process, cancer cells will evade CTLs by upregulating immune checkpoint ligands, such as PD-L1 binding to the PD-1 receptor on the surface of CTLs to exhaust it, and CTLA-4 blocking the interaction between CD40/CD80/CD86 and CD28. Therefore, antibodies against PD-L1/PD-1 and CTLA-4 are used for immune checkpoint inhibitor therapy of HCC. The other alternative is more direct, by cloning *in vitro* chimeric antigen receptor T cells that can target specific antigen genes [such as Glypican 3 (GPC3) or alpha-fetoprotein (AFP)] related antigens to directly kill tumor cells. Finally, genetically engineered

oncolytic virus therapy can also selectively replicated in tumors, killing cancer cells while stimulating antigen presentation and adaptive anti-tumor immune responses.

IMMUNE CHECKPOINT INHIBITORS

Tumor cells express a variety of immunosuppressive ligands on their surface, which bind to the indicated inhibitory receptors of activated T cells involved in the anti-tumor response. This process in turn reduces the intensity of the anti-tumor immune response, thereby evading immune surveillance[33]. Drugs that block these immunosuppressive targets to eliminate tumor immune escape are called immune checkpoint inhibitors (ICI). PD-1 is a member of the CD28 superfamily and is expressed on the surface of T cells and B cells. Its activation will lead to the phosphorylation of ITSM (Immunoreceptor tyrosine-based switch motif) in the cytoplasm of the cell, inhibiting energy metabolism in T cells, thereby hindering cell cloning proliferation and secretion of cytokines. In order to avoid the killing of T cells, tumor cells highly express PD-L1 and release the PD-L1 into the peripheral blood, which causes the exhaustion of T cells and the loss of tumor antigen presentation ability of myeloid immune cells[34]. Therefore, targeted inhibition of the interaction of PD-1 and PD-L1 is of great significance for the treatment of HCC.

Nivolumab, as the first PD-1 targeted drug to be used in clinical practice, was initially used in the treatment of melanoma, and its objective response rate and one-year survival rate were 40.0% and 72.9%, respectively[35]. Subsequently, Nivolumab was tried to treat advanced HCC. Among 144 HCC patients, 20% showed a good response to nivolumab, and 3 of them achieved complete remission (CR), highlighting the potential of Nivolumab to treat advanced HCC[36]. Another anti-PD-1 targeted drug Pembrolizumab has also shown effectiveness in the treatment of advanced HCC, with an objective response rate and a one-year survival rate of 17% and 54%[37]. In fact, a single ICI is not satisfactory for the treatment of advanced HCC. The current ICI therapy is mostly performed in a variety of combinations (for example, anti-PD-L1 antibody plus anti-CTLA-4 antibody), which is more effective than a single agent. In the absence of targetable lymphocytes in the tumor microenvironment, inhibition of PD-1/PD-L1 cannot stimulate cancer immunity, and inhibition of the CTLA-4 can cause CD8 + T cells to proliferate in the lymph nodes and infiltrate the tumor tissue, thereby enhancing the efficacy of anti-tumor. In fact, combination therapy of molecularly targeted drugs and immune checkpoint inhibitors has received considerable attention. For example, immunosuppressive cytokines that cause the immunosuppressive liver environment of patients with liver cancer, such as interleukin (IL)-10, transforming growth factor (TGF)-β and vascular endothelial growth factor (VEGF) molecular targeted drugs[38,39]. Table 1 shows the ongoing use of ICI in combination with various interventions (such as kinase inhibitors, cytokine or receptor inhibitors, and embolotherapy).

ONCOLYTIC VIRUS THERAPY

The oncolytic virus can specifically host in cancer cells, replicate and destroy the cell structure and hence was not initially classified as immunotherapy. Subsequent studies confirmed that oncolytic viruses could induce anti-cancer immune responses and immunogenic cancer cell death, making them a form of immunotherapy[47]. Compared with traditional therapies, oncolytic virus therapy is safer, has the selective specificity of host cancer cells, and continuously self-replicates to lyse cancer cells[48]. In the tumor microenvironment, pathogen-associated molecular patterns (PAMP) of oncolytic viruses can be recognized by pattern recognition receptors (PRR) of immune cells, such as through TLR or MDA5 activation of macrophages or dendritic cells[49,50]. As a secondary effect, oncolytic viruses enhance the recognition and presentation of tumor antigens, and activate the infiltration of cytotoxic T cells into tumors[51]. Therefore, oncolytic virus therapy is a very interesting method to overcome HCC immunosuppression. Currently, oncolytic virus therapies used for HCC include dsDNA or ssRNA viruses, such as measles vaccine virus (MeV), herpes simplex virus (HSV), adenovirus (Adenovirus) and vaccinia virus (VV), *etc.*, which are used to engineer infection vectors[52]. For example, inserting the overexpression sequence of granulocyte-

Table 1. Clinical trials of immune checkpoint inhibitors for HCC

Clinical trials identifier	Target	Status	Active treatment	N	Primary endpoints or outcomes	Ref.
Single immune checkpoint inhibitors						
NCT03630640	PD-1	Recruiting Phase 2	Nivolumab	50	OS, 2 years	
NCT03383458	PD-1	Recruiting, Phase 3	Nivolumab	530	Recurrence-free Surviva, 49 months; OS, 7 years; Time to recurrence, 49 months	
NCT04161911	PD-1	Completed Phase 3	Nivolumab	1,426	OS, 7.75 years	
Combination of immune checkpoint Inhibitors						
NCT03222076	CTLA-4 PD-1	Recruiting Phase 2	Ipilimumab Nivolumab	45	AEs, 5 years	
NCT03682276	CTLA-4 PD-1	Recruiting, Phase I/II	Ipilimumab Nivolumab	32	AEs, 127 Days; Delay to surgery, 89 Days	
NCT03510871	PD-1 CTLA-4	Not yet recruiting, Phase II	Nivolumab Ipilimumab	40	The percentage of subjects with tumor shrinkage, 4 years	
Combination of Immune Checkpoint Inhibitors with Tyrosine kinase inhibitor						
NCT04310709	Multikinase PD-1	Recruiting, Phase II	Regorafenib Nivolumab	42	ORR, 6 months	[40-42]
NCT04170556	Multikinase PD-1	Recruiting, Phase II	Regorafenib Nivolumab	60	AEs, 24 months	
NCT03299946	Multikinase PD-1	Active, not recruiting, Phase I	Cabozantinib Nivolumab	15	AEs, 4 years	
NCT03841201	Multikinase PD-1	Recruiting, Phase II	Lenvatinib Nivolumab	50	ORR, 6 months	
NCT03418922	Multikinase PD-1	Active, not recruiting Phase 1	Lenvatinib Nivolumab	30	DLTs, 28 days	
NCT03006926	Multikinase PD-1	Phase 1; Active, not recruiting	Lenvatinib Pembrolizumab	104	AEs, 3 years; DLT, 21 days; ORR, 3 years	
NCT02856425	Multikinase PD-1	Phase 1; Recruiting	Nintedanib Pembrolizumab	18	MTD, 24 months	
NCT02572687	PD-L1 VEGF	Phase 1; Active, not recruiting	Ramucirumab MEDI4736	114	DLTs, 28 days	
NCT02576509	Raf-1 PD-1	Active, not recruiting, Phase III	Sorafenib Nivolumab	743	OS, 41 months	
NCT02988440	Raf1 PD-1	Phase 1; Completed	PDR001 Sorafenib	20	AEs, 30 days; DLT, 8 weeks;	
NCT03893695	ALK-1 PD-1	Recruiting Phase 1 Phase 2	GT90001 Nivolumab	20	DLTs, 28 days	
NCT03059147	PI3k PD-1	Active, not recruiting, Phase II	SF1126 Nivolumab	14	DLTs, 56days	
NCT03655613	C-Met PD-1	Recruiting Phase 1 Phase 2	APL-101 Nivolumab	119	DLTs, 35 days	
NCT02795429	PD-1+cMet	Phase 1/2; Active, not recruiting	PDR001 INC280	90	DLT, 42 days; ORR, 3 years	
Combination of immune checkpoint inhibitors with Cytokine/receptor inhibitor						
NCT02423343	TGFβR1 PD-1	Active, not recruiting Phase 1 Phase 2	Galunisertib Nivolumab	75	MTD, 6 months	
NCT04123379	PD-1 CCR2/CCR5	Recruiting Phase 2	Nivolumab BMS-813160 BMS-986253	50	Primary pathologic response: 2 years; Significant tumor necrosis: 2 years	
Combination of immune checkpoint inhibitors with embolotherapy						
NCT03033446	Embolotherapy PD-1	Recruiting, Phase II	Radioembolization Nivolumab	40	ORR, 8 weeks	
NCT03380130	PD-1 Embolotherapy	Active, not recruiting Phase 2	Nivolumab SIR-Spheres	40	AEs, 2 years	[43,44]
NCT03572582	PD-1 Embolotherapy	Active, not recruiting Phase 2	Nivolumab TACE	49	ORR, 42 months	
NCT04268888	PD-1 Embolotherapy	Recruiting Phase 2 Phase 3	Nivolumab and TACE/TAE	522	OS: 2 years; TTTP	
Multiple combination therapy						
NCT01658878	PD-1 Raf-1 CTLA-4 multikinase	Active, not recruiting, Phase I/II	Nivolumab Sorafenib Ipilimumab Cabozantinib	1,097	AEs, 100 days; ORR, 6 months	[45,46]

NCT04039607	PD-1 CTLA-4 Raf-1 VEGFR/FGFR	Recruiting, Phase III	Nivolumab Ipilimumab Sorafenib lenvatinib	1,084	OS, 4 years
NCT04472767	PD-1 CTLA-4 Multikinase Embolotherapy	Not yet recruiting, Phase II	Nivolumab Ipilimumab Cabozantinib Transarterial Chemoembolization	35	Percentage of Progression-free Survival, 6 Months; Complete Response Rate, 1 year
NCT04050462	PD-1 Multikinase IL-8	Not yet recruiting, Phase II	Nivolumab Cabiralizumab BMS-986253	74	ORR, 6 years
NCT03071094	Oncolytic therapy PD-1	Active, not recruiting, Phase I/II	Pexastimogene Devacirepvec; Nivolumab	30	DLTs, 4Weeks; ORR, 6 months
NCT03897543	PD-1 INK T cells Agonist	Recruiting Phase 1 Phase 2	Nivolumab ABX196	48	AEs, 1 year

Most data were obtained from findings from www.clinicaltrials.gov using the search terms "hepatocellular carcinoma" and "Immune Checkpoint Inhibitors". AEs: rate of adverse events; ORR: objective response rate; MTD: maximum tolerated dose; DLTs: dose-limiting toxicities; OS: overall survival; TTTP: time to TACE progression

macrophage colony-stimulating factor (GM-CSF) into the oncolytic virus sequence, GM-CSF recruits myeloid cells in the periphery to enhance the immune response in the tumor microenvironment[53]. So far, preclinical studies for HCC oncolytic virus therapy have been very encouraging. We have compiled preclinical studies on HCC oncolytic therapy for the past ten years, as shown in Table 2.

Although many preclinical research attempts have been made in oncolytic therapy in recent years, there are still very few programs that have entered the clinical stage. At present, the only HCC oncolytic virus entering clinical research is JX-549, with VV as an engineered vector. VV has the stability and efficiency of intravenous administration, is widely used in the safety of live vaccines, has the advantages of immune-inducing activity and better editability, and has become a carrier of various engineered tumor-melting viruses[73-75]. The thymidine kinase gene (TK) gene of JX-594 (also known as PexaVec; Jennerex Inc.) was deleted to make it more specific for cancer cell infection. In addition, hGM-CSF and β-galactosidase were inserted to enhance its immunostimulatory activity and replication capabilities[73,76,77]. JX-594 showed complete tumor response and systemic efficacy in a phase I clinical study[78]. In the phase II trial, low-dose JX-594 has significant anti-cancer effect and immune activation ability[79], but this requires earlier interventional therapy[80]. Currently, a large-scale 600-person multicenter Phase 3 trial is still in progress (NCT02562755). More clinical studies of HCC oncovirus are shown in Table 3.

HCC VACCINE

Tumor vaccine is a treatment program to increase the specificity of tumor antigens, mainly antigen peptide vaccines and DCs vaccines, which are used to stimulate specific immune responses. The clinical trials of therapeutic vaccines for HCC are summarized in Table 4. At present, there are relatively few registered clinical trials for DCs vaccines in HCC, partly because of the unsatisfactory results of previous clinical trials of such vaccines[86]. On the other hand, the tumor heterogeneity of HCC also limits the development of a single antigen peptide or DCs vaccine. With the development of large-scale DNA sequencing technology, patient-specific multi-target peptide or DCs vaccine is still a promising strategy for the treatment of HCC. DC, as professional antigen-presenting cells (APC), recognize, process and present TAA. Allogeneic DC vaccines can provide T cells with antigens and co-stimulatory molecules needed for immune response. In short, DCs are mobilized from peripheral blood and their expansion is stimulated with GM-CSF to produce DCs for reinfusion. Prior to this, DC needs to be exposed to TAA to trigger the specificity of the vaccine[87]. DCs can be transduced with DNA or RNA encoding known TAA, or directly co-cultured with patient tumor lysate[88]. Phase I clinical studies have shown that the allogeneic DCs vaccine can produce a specific immune response in 73% of HCC patients[89].

Table 2. Representative Oncolytic therapy used in preclinical studies

Virus strain	Modification	Therapeutic gene	HCC cell lines	Animal model	Dose	Ref.
Recombinant VSV-NDV, L289A	Replaced of hemagglutinin-neuraminidase (HN)	None	HepG2 Huh7	NOD.CB17-prkdcscid/NCrCrl (NOD-SCID).	10^7 TCID50,IV	[54]
Getah-like alphavirus, M1	Insertion of valosin-containing protein (VCP) inhibitors	XBP1	Hep3B	Hep3B xenografts, Nonhuman primate *Macaca fascicularis*.	5×10^5 PFUs, IV 1×10^9 PFUs, IV	[55]
HSV, d0-GFP	Mutated in glycoprotein K and glycoprotein B	None	Huh7, SMMC7721, QGY7703, L-02, BEL7404, GSG7701, HCCLM3, MHHC97H, H22	Huh7 and Hep3B xenografts BALB/c.	1×10^7 PFU, IV	
Ad5	Insertion of Golgi protein 73 (GP73) promoter and sphingosine kinase 1 (SphK1)-short hairpin RNA (shRNA)	SphK1	Huh7, HL-7702	Huh7 xenografts BALB/c nude mice.	6×10^8 PFU, IT	
Recombinant influenza viral, PR8	deletion in *NS* and insertion of h GM-CSF	hGM-CSF	MDCK, A549, SMCC7721,HepG2	HepG2 xenografts BALB/c nude mice.	2×10^9 PFU, IT	[56]
MeV, MV-Edm	None	None	CC-LM3, MHCC-97H	LM3 xenografts BALB/c nude mice.	5×10^6 PFU, IT	[57]
Ad, Ad-sp	Insertion of Vestigial-Like Family Member 4 (VGLL4)	VGLL4	Hep3B, Huh-7	Huh-7 xenografts BALB/c nude mice.	5×10^8 PFU, IT	[58]
HSV, HSV T-01	α47 and γ34.5 loci are deleted and the LacZ gene replaces the ICP6 gene	None	HuH-7, Li-7 JHH-1, JHH2, JHH5, JHH6, JHH7, HLE, HLF, PLC/PRF/5, huH-1	Hepa1-6 xenografts BALB/c nude mice.	2×10^6 PFU, IT	[59]
Ad, Ad-ΔB	Insertion of ING4 and TRAIL	ING4 and TRAIL	Hep3B	Hep3B xenografts BALB/c nude mice.	1×10^{10} PFU, IV	[60]
Ad, Ad-wnt-E1A(Δ24bp)-TSLC1	Insertion of TSLC1	Wnt and Rb pathway	MHCC-97H, PLC/PRF/5	PLC/PRF/5 xenografts BALB/c nude mice.	6×10^8 PFU, IT	[61]
Ad, OAV SG655-mGMP	Insertion of 11R-P53 and GM-CSF	11R-P53 and GM-CSF	Hep3B-C, ECCG5	ECCG5 xenografts BALB/c mice	Unknow	[62]
Ad, Ad-ΔB/TRAIL and Ad-ΔB/IL-12	Mutated in *E1A* and deleted in *E1B* regions. Insertion of *hTRAIL* or *hIL-12*	*hTRAIL* or *hIL-12*	Hep3B and HuH7	Athymic nude mice, orthotopic model	2×10^8 PFU, IV 1 $\times 10^{10}$ PFU, IV	[63]
MeV, (Res + MeV)	Encoding of *GFP* as a marker gene and *SCD* as suicide gene	None	HepG2 and Hep3B	No animal model used	Various MOIs	[64]
VV, GLV-2b-372	Deletion of *TK* and insertion of *TurboFP635* gene	None	Huh-7, Hep G2, SNU-449, and SNU-739	Athymic nude mice Huh-7 xenograft	1×10^5 PFU, IT	[65]
VV, GLV-1h68	Deletion of TK and insertion of Renilla luciferasegreen	None	Huh-7, Hep 3B, SNU-449 and SNU-739	No animal model used	Various MOIs	[66]
Ad, Telomelysin	*hTERT* inserted upstream of *hTERT* the *E1*gene	*hTERT*	Human: Huh-7, Hep3B, PLC5, HA22T, HCC36, and HepG2 Mouse: Hepa-1c1c7 and Hepa 1-6	HBx transgenic mice, orthotopic model	1.25×10^8 PFU, IT 6.25×10^8 PFU, IT 3.0×10^8 PFU, IT	[67]
HSV, G47Δ	ICP47 and γ34.5-deletion	None	HepG2, HepB, SMMC-7721, BEL-7404, and BEL-7405	Balb/c nude mice SMMC-7721, BEL-7404 xenograft	2×10^7 PFU, IT	[68]
HSV, LCSOV	Viral glycoprotein H gene linked with liver-specific apolipoprotein E (apoE)-AAT promoter. miR-122a complimentary sequence to the 3′ untranslated region (3′UTR). miR-124a and let-7 also inserted at 3′ UTR	*miR122*, *miR-124a* and *let-7*	HuH-7, HepG2, and Hep3B	Hsd: athymic (nu/nu) mice, Hep3B xenograft	5×10^6 PFU, IT	[69]
VV, GLV-1h68	Deletion of *TK* and insertion of Renilla luciferasegreen fluorescent protein (*Ruc-GFP*), *β-galactosidase, β-glucuronidase*	None	HuH7 and PLC/PRF/5	Athymic Nude-*Foxn1*nu HuH7 and PLC xenografts	5×10^6 PFU, IV	[70]

Ad, SG7011^{let7T}	Insertion of eight copies of let-7 target sites (let7T) into the 39 untranslated region of *E1A*	miRNA, */et-7*	HepG2, Hep3B, PLC/PRF/5, and Huh7	Hep3B and SMMC-7721 xenografts BALB/c nude mice.	5×10^8 PFU, IT	[71]
VV, JX-963	Deletion of *TK* and *VGF*, insertion of *h GM-CSF*	*hGM-CSF*	None	Immunocompetent, orthotopic, NZW rabbits VX2 tumor model	Various PFU, IV	[72]

MeV: measles vaccine virus; HSV: herpes simplex virus; Ad: Adenovirus; VV: vaccinia virus; NDV: newcastle disease virus; VSV: vesicular stomatitis virus; IV: intravenous; IT: intratumoral; MOI: multiplicity of infection; PFU: plaque-forming units

Table 3. Clinical trials of oncolytic viral therapy for HCC

Clinical trials identifier	Status	Active treatment	*n*	Primary end points or outcomes	Ref.
NCT03071094	Active, not recruiting. Phase 1 and 2 trials	JX-594; Nivolumab	30	DLTs, 4 weeks; ORR, 6 months	
NCT02562755	Active, not recruiting. Phase 3 trials	JX-594; Sorafenib	600	OS, 53 months	
NCT00554372	Completed. Phase 2 trials	JX-594	30	mRECIST v1.0 criterion; Choi criterion. 4 weeks	[81]
NCT01387555	Completed. Phase 2b trials	JX-594;	129	OS, 21 months	[82]
NCT00629759	Completed. Phase 1 trials	JX-594	14	MTD, Safety evaluation throughout study participation	[83]

Most data were obtained from findings from www.clinicaltrials.gov using the search terms "hepatocellular carcinoma" and "oncolytic". JX-594: Recombinant vaccinia virus [Thymidine Kinase (TK)-deletion plus granulocyte-macrophage colony-stimulating factor (GM-CSF)]. DLTs: dose limiting toxicities; ORR: overall response rate; OS: overall survival; MTD: maximum tolerable dose; mRECIST: modified response evaluation criteria in solid tumors

Table 4. Clinical trials of therapeutic vaccines for HCC

Clinical trials identifier	Status	Active treatment	*n*	Primary endpoints or outcomes	Ref.
NCT04248569	Recruiting, Phase I	DNAJB1-PRKACA peptide vaccine, Nivolumab, Ipilimumab.	12	DLTs, 4 weeks; Fold change in interferon-producing DNAJB1-PRKACA-specific CD8+ and CD4+ T cells, 12 weeks;	
NCT03674073	Recruiting, Phase I	Neoantigen Vaccines; Microwave Ablation	24	CTCAE v4.0, 1 year	
NCT02409524	Completed, Phase II	Individualized anti-cancer vaccine (CRCL-AlloVax)	15	OS, 12 weeks	
NCT01974661	Completed, Phase I	COMBIG-DC vaccine (ilixadencel).	18	Registration of adverse events. 0.5 years	[84]
NCT03203005	Completed, Phase I/II	IMA970A vaccine; CV8102 adjuvant; Cyclophosphamide.	22	Registration of adverse events, 2 years; Immunogenicity, 2 years	
NCT00005629	Completed, Phase I/II	Alpha-fetoprotein peptide-pulsed autologous dendritic cell vaccine	6	Safety, 1 month	
NCT00022334	Completed, Phase I/II	Alpha-fetoprotein peptide-pulsed autologous dendritic cell vaccine	33	DLT and MTD, 1 year	
NCT04147078	Recruiting, Phase I	Neoantigen-primed dendritic cell (DC) cell vaccine	80	DFS, 5 years	
NCT04251117	Recruiting, Phase, I/IIa	Personalized neoantigen DNA vaccine (GNOS-PV02) and plasmid-encoded IL-12 (INO-9012) in combination with pembrolizumab (MK-3475)	12	CTCAE v5.0, 2 years Immunogenicity, 2 years	
NCT02089919	Completed, Phase I/II	Cancer stem cell vaccine	40	Adverse events. 3 months	[85]
NCT00028496	Completed, Phase I	Recombinant fowlpox-CEA(6D)/TRICOM vaccine	48	DLT and MTD, 56 days.	
NCT03942328	Recruiting, Phase I	Autologous dendritic cells and Prevnar vaccine	26	Adverse events. 1 year	
NCT02232490	Recruiting, Phase III	Hepcortespenlisimut-L (V5) therapeutic vaccine	120	Changes in plasma AFP, 3 months	

Most data were obtained from findings from www.clinicaltrials.gov using the search terms "hepatocellular carcinoma" and "vaccines". DLTs: dose limiting toxicities; CTCAE: common terminology criteria for adverse events; OS: overall survival; MTD: maximum tolerable dose; DFS: disease-free survival

CHIMERIC ANTIGEN RECEPTOR T CELL THERAPY

In addition to immune checkpoint inhibitors, oncolytic viruses and vaccines, adoptive therapy using genetically modified T cells have also become one of the potential immunotherapy options for HCC. T cells can be engineered to express a chimeric antigen receptor (CAR), which is composed of a T cell receptor CD3ζ chain and co-stimulatory receptors (e.g., CD28 and TNFRSF9) to form an antigen recognition domain[90]. The antigen recognition domain endows CAR-T cells with specificity for tumor-associated antigens, which shows promise in the treatment of HCC. Besides, CAR-T cells have a strong adaptive immunity and can recognize antigens that are not present in MHC molecules. CAR-T cell therapy has been used in the preclinical treatment of a variety of solid tumors, but there are few clinical studies on HCC, and more are still in the preclinical research stage. Like the HCC vaccine, the technical difficulty lies in the choice of tumor-specific antigens[91]. CD133 is expressed by cancer stem cells derived from various epithelial cells and is an attractive cancer treatment target. CAR-T cells targeting CD133 have shown the feasibility of treating advanced HCC, with controllable toxicity and effective activity[92]. Glypican-3 (GPC3) is a member of the heparan sulfate glycoprotein family and belongs to a transmembrane glycoprotein. It plays an important role in cell proliferation, differentiation and metastasis. CAR-T cells targeting glypican-3 can inhibit the growth of HCC[93,94]. Besides, there are HCC recognition antigens such as NKG2D[95] and CD147[96] for CAR-T cell transformation. In addition, the CAR of CAR-T cells can be inserted into the expression of a variety of cytokine genes to overcome the immunosuppressive effects of the HCC microenvironment[97,98]. The clinical trials of CAR-T cell therapy for liver cancer are summarized in Table 5.

THE CURRENT COMBINATION OF THERAPEUTIC STRATEGIES FOR HCC

Currently, there are many immunotherapy and other target therapy drugs approved by the Food and Drug Administration (FDA) of The United States of America (USA) for liver cancer treatment, including Atezolizumab, Avastin (Bevacizumab), Bevacizumab, Cabometyx (Cabozantinib-S-Malate), Cyramza (Ramucirumab), Keytruda (Pembrolizumab), Lenvatinib Mesylate, Lenvima (Lenvatinib Mesylate), Nexavar (Sorafenib Tosylate), Nivolumab, Opdivo (Nivolumab), Pemazyre (Pemigatinib), Pembrolizumab, Pemigatinib, Ramucirumab, Regorafenib, Sorafenib Tosylate, Stivarga (Regorafenib), Tecentriq (Atezolizumab). Single agent therapy has historically shown poor results in HCC, leading to trials of combination therapy for a more efficacious outcome. For example, the FDA has approved Opdivo (nivolumab) + Yervoy (ipilimumab) based on the CheckMate 040 trial, atezolizumab + bevacizumab for patients with advanced HCC based on the IMbrave150 (NCT03434379) study. The CheckMate 040 is a multicentered, open-labelled, multicohort, phase 1/2 study. The result showed that nivolumab + ipilimumab had manageable safety, promising objective response rate, and durable responses. The arm A regimen (4 doses nivolumab 1 mg/kg + ipilimumab 3 mg/kg every 3 weeks then nivolumab 240 mg every 2 weeks) received accelerated approval in the US based on this study[99]. The IMbrave150a study is a global, open-labelled, phase 3 trial for patients with unresectable HCC who had not previously received systemic treatment. The study included 336 patients in the atezolizumab + bevacizumab group and 165 patients in the sorafenib group. The result showed that atezolizumab + bevacizumab resulted in better overall (overall survival at 12 months was 67.2% *vs.* 54.6%) and progression-free survival (6.8 months *vs.* 4.3 months) outcomes than sorafenib[100]. There are many different combinations of immune checkpoint inhibitors with other different therapeutic strategies under investigation. Some of the combination clinical trials are concluded in the Table 1.

CONCLUSION AND PROSPECT

Immunotherapy is a revolution in HCC treatment. Significant responses have been observed in various tumor types with immunotherapy, especially immune checkpoint inhibitors and CAR-T cells. However, it is clear that not all HCC patients are sensitive to current immunotherapy, and even in those who do respond, the effect is difficult to last. Lots of data indicate that most HCCs are immunosuppressive

Table 5. Clinical trials of Chimeric antigen receptor T cell therapy for liver cancer

No.	Title	Status	Conditions	Interventions	URL
1	Study evaluating the efficacy and safety With CAR-T for liver cancer	Unknown status	Liver neoplasms	Biological: EPCAM-targeted CAR-T cells	https://ClinicalTrials.gov/show/NCT02729493
2	Clinical study of ET1402L1-CAR T cells in AFP expressing hepatocellular carcinoma	Terminated	Hepatocellular carcinoma| liver cancer	Biological: autologous ET1402L1-CART cells	https://ClinicalTrials.gov/show/NCT03349255
3	T cells co- expressing a second generation glypican 3-specific chimeric antigen receptor with cytokines interleukin-21 and 15 as immunotherapy for patients with liver cancer (TEGAR)	Withdrawn	Hepatocellular carcinoma| hepatoblastoma	Genetic: TEGAR T cells|drug: cytoxan|drug: fludarabine	https://ClinicalTrials.gov/show/NCT04093648
4	Glypican 3-specific chimeric antigen receptor expressed in t cells for patients with pediatric solid tumors (GAP)	Recruiting	Liver Cancer	Genetic: GAP T cells| drug: cytoxan|drug: fludara	https://ClinicalTrials.gov/show/NCT02932956
5	Safety and Efficacy of CEA-targeted CAR-T therapy for relapsed/refractory CEA+ cancer	Recruiting	Solid Tumor|Lung Cancer	Biological: CEA CAR-T cells	https://ClinicalTrials.gov/show/NCT04348643
6	Autologous CAR-T/TCR-T cell immunotherapy for solid malignancies	Recruiting	Esophagus cancer| hepatoma|glioma| gastric cancer	Biological: CAR-T/TCR-T cells immunotherapy	https://ClinicalTrials.gov/show/NCT03941626
7	A Study of MG7 redirected autologous T cells for advanced MG7 positive liver metastases (MG7-CART)	Unknown status	Liver Metastases	Biological: MG7-CART	https://ClinicalTrials.gov/show/NCT02862704
8	A Study of CD147-targeted CAR-T by hepatic artery infusions for very advanced hepatocellular carcinoma	Recruiting	Advanced hepatocellular carcinoma	Biological: CD147-CART	https://ClinicalTrials.gov/show/NCT03993743
9	CAR-T hepatic artery infusions and Sir-Spheres for liver metastases	Completed	Liver Metastases	Biological: anti-CEA CAR-T cells|Device: Sir-Spheres	https://ClinicalTrials.gov/show/NCT02416466
10	CAR-T hepatic artery infusions or pancreatic venous infusions for CEA-expressing liver metastases or pancreas cancer	Active, not recruiting	Liver Metastases	Biological: anti-CEA CAR-T cells	https://ClinicalTrials.gov/show/NCT02850536
11	Hepatic transarterial administrations of NKR-2 in patients with unresectable liver metastases from colorectal cancer	Active, not recruiting	Colon Cancer Liver Metastasis	Biological: NKR-2 cells	https://ClinicalTrials.gov/show/NCT03370198
12	Dose escalation and dose expansion phase I study to assess the safety and clinical activity of multiple doses of NKR-2 administered concurrently with FOLFOX in colorectal cancer with potentially resectable liver metastases	Active, not recruiting	Colon Cancer Liver Metastasis	Biological: NKR-2 cells	https://ClinicalTrials.gov/show/NCT03310008
13	Interleukin-15 armored Glypican 3-specific chimeric antigen receptor expressed in T cells for pediatric solid tumors	Not yet recruiting	Liver Cancer| Rhabdomyosarcoma, *et al.*	Genetic: AGAR T cells|drug: cytoxan|drug: fludara	https://ClinicalTrials.gov/show/NCT04377932
14	Treatment of relapsed and/or chemotherapy refractory advanced malignancies by CART133	Completed	Liver Cancer|Pancreatic Cancer, *et al.*	Biological: anti-CD133-CAR vector-transduced T cells	https://ClinicalTrials.gov/show/NCT02541370
15	Autologous CAR-T/TCR-T cell immunotherapy for malignancies	Recruiting	Solid tumors	Biological: CAR-T cell immunotherapy	https://ClinicalTrials.gov/show/NCT03638206
16	A study of chimeric antigen receptor T cells combined with interventional therapy in advanced liver malignancy	Unknown status	Carcinoma, Hepatocellular| Pancreatic Cancer, *et al.*	Drug: CAR-T cell	https://ClinicalTrials.gov/show/NCT02959151
17	A clinical research of CAR T cells targeting EpCAM positive cancer	Recruiting	Hepatic Carcinoma, *et al.*	Biological: CAR-T cell immunotherapy	https://ClinicalTrials.gov/show/NCT03013712
18	NKG2D-based CAR T-cells immunotherapy for patient with r/r NKG2DL+ solid tumors	Not yet recruiting	Hepatocellular Carcinoma|Glioblastoma, *et al.*	Biological: NKG2D-based CAR T-cells	https://ClinicalTrials.gov/show/NCT04270461

19	GPC3-T2-CAR-T cells for immunotherapy of cancer with GPC3 expression	Recruiting	Hepatocellular Carcinoma, *et al.*	Biological: GPC3 and/or TGF-beta targeting CAR-T cells	https://ClinicalTrials.gov/show/NCT03198546
20	NKG2D CAR-T(KD-025) in the treatment of relapsed or refractory NKG2DL+ tumors	Not yet recruiting	Solid Tumor|Hepatocellular Carcinoma, *et al.*	Drug: KD-025 CAR-T cells	https://ClinicalTrials.gov/show/NCT04550663
21	GPC3-CAR-T Cells for the hepatocellular carcinoma	Not yet recruiting	Hepatocellular Carcinoma	Biological: GPC3-CAR-T cells	https://ClinicalTrials.gov/show/NCT04506983
22	CAR-T cell immunotherapy for HCC targeting GPC3	Withdrawn	GPC3 Positive Hepatocellular Carcinoma	Biological: CAR-T cell immunotherapy	https://ClinicalTrials.gov/show/NCT02723942
23	Clinical Study on the efficacy and safety of c-Met/PD-L1 CAR-T cell injection in the treatment of HCC	Unknown status	Primary Hepatocellular Carcinoma	Biological: c-Met/PD-L1 CAR-T cell injection	https://ClinicalTrials.gov/show/NCT03672305
24	A study of GPC3 redirected autologous T cells for advanced HCC	Unknown status	Carcinoma, Hepatocellular	Drug: TAI-GPC3-CART cells	https://ClinicalTrials.gov/show/NCT02715362
25	GPC3-targeted CAR-T cell for treating GPC3 positive advanced HCC	Recruiting	Hepatocellular Carcinoma	Biological: CAR-T cell immunotherapy	https://ClinicalTrials.gov/show/NCT04121273
26	A Study of GPC3-targeted T cells by intratumor injection for advanced HCC (GPC3-CART)	Unknown status	Carcinoma, Hepatocellular	Drug: GPC3-CART cells	https://ClinicalTrials.gov/show/NCT03130712
27	Phase I/II study of anti-Mucin1 (MUC1) CAR T cells for patients with MUC1+ advanced refractory solid tumor	Unknown status	Hepatocellular Carcinoma, *et al.*	Biological: anti-MUC1 CAR T cells	https://ClinicalTrials.gov/show/NCT02587689
28	Anti-GPC3 CAR T for treating patients with advanced HCC	Completed	Hepatocellular Carcinoma	Biological: anti-GPC3 CAR T	https://ClinicalTrials.gov/show/NCT02395250
29	Anti-GPC3 CAR-T for treating GPC3-positive advanced hepatocellular carcinoma (HCC)	Unknown status	Hepatocellular Carcinoma	Biological: retroviral vector-transduced autologous T cells to express anti-GPC3 CARs|drug: fludarabine|drug: cyclophosphamide	https://ClinicalTrials.gov/show/NCT03084380
30	Clinical study of redirected autologous T cells with a chimeric antigen receptor in patients with malignant tumors	Active, not recruiting	Hepatocellular Carcinoma, *et al.*	Genetic: CAR-CD19 T cell|genetic: CAR-BCMA T cell|genetic: CAR-GPC3 T cell|genetic: CAR-CLD18 T cell|drug: fludarabine|drug: cyclophosphamide	https://ClinicalTrials.gov/show/NCT03302403
31	A clinical research of CAR T cells targeting CEA positive colorectal cancer (CRC)	Not yet recruiting	Stage III Colorectal Cancer|Colorectal Cancer Liver Metastasis	Biological: Anti-CEA-CAR T	https://ClinicalTrials.gov/show/NCT04513431
32	Study of anti-CEA CAR-T + chemotherapy *vs*. chemotherapy alone in patients with CEA+ pancreatic cancer & liver metastases	Not yet recruiting	Malignant tumor of pancreas metastatic to liver	Biological: anti-CEA CAR-T cells|drug: gemcitabine/nab paclitaxel|drug: NLIR+FU/FA|drug: capecitabine	https://ClinicalTrials.gov/show/NCT04037241
33	Glypican 3-specific chimeric antigen receptor expressing T cells for hepatocellular carcinoma (GLYCAR)	Recruiting	Hepatocellular Carcinoma	Genetic: GLYCAR T cells|drug: cytoxan|drug: fludarabine	https://ClinicalTrials.gov/show/NCT02905188
34	4th generation chimeric antigen receptor T cells targeting glypican-3	Recruiting	Advanced Hepatocellular Carcinoma	Drug: CAR-GPC3 T cells	https://ClinicalTrials.gov/show/NCT03980288
35	PD-1 antibody expressing CAR-T cells for EGFR family member positive advanced solid tumor (lung, liver and stomach)	Unknown status	PD-1 Antibody|CAR-T cells|advanced solid tumor	Biological: HerinCAR-PD1 cells	https://ClinicalTrials.gov/show/NCT02862028
36	Chimeric antigen receptor T cells targeting glypican-3	Recruiting	Hepatocellular carcinoma	Biological: CAR-GPC3 T cells	https://ClinicalTrials.gov/show/NCT03884751
37	A clinical study in patients with high-risk recurrent primary hepatocellular carcinoma using autologous TILs	Active, not recruiting	Hepatic Carcinoma	Drug: tumor infiltrating lymphocyte	https://ClinicalTrials.gov/show/NCT04538313
38	CAR-GPC3 T cells in patients with refractory hepatocellular carcinoma	Completed	Hepatocellular Carcinoma	Genetic: CAR-GPC3 T cells	https://ClinicalTrials.gov/show/NCT03146234

tumors. Therefore, ongoing research using a multifaceted approach to enhance the activity of the immune environment remain underway to enhance current immunotherapy strategies.

DECLARATIONS

Authors' contributions

Drafted the outline of this review: Feng ZY, Xia HP
Drafted the manuscript: Feng ZY, Xu FG, Liu Y, Xu HJ, Wu FB, Chen XB, Xia HP
Finalized the manuscript: Chen XB, Xia HP

Availability of data and materials

Not applicable.

Financial support and sponsorship

This work was supported by grants from the National Natural Science Foundation of China (82072739), The Recruitment Program of Overseas High-Level Young Talents, "Innovative and Entrepreneurial Team" [No.(2018) 2015], Science and Technology Development Fund of Nanjing Medical University and Chinese Foundation for Hepatitis Prevention and Control-TianQing Liver Disease Research Fund (TQGB20190164, TQGB20200139).

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
2. Liu X, Qin S. Immune Checkpoint Inhibitors in Hepatocellular Carcinoma: Opportunities and Challenges. *Oncologist* 2019;24:S3-10.
3. Montella L, Palmieri G, Addeo R, Del Prete S. Hepatocellular carcinoma: Will novel targeted drugs really impact the next future? *World J Gastroenterol* 2016;22:6114-26.
4. Kudo M. A new era of systemic therapy for hepatocellular carcinoma with Regorafenib and Lenvatinib. *Liver Cancer* 2017;6:177-84.
5. Wörns MA, Galle PR. HCC therapies--lessons learned. *Nat Rev Gastroenterol Hepatol* 2014;11:447-52.
6. Hernandez-Gea V, Toffanin S, Friedman SL, Llovet JM. Role of the microenvironment in the pathogenesis and treatment of hepatocellular carcinoma. *Gastroenterology* 2013;144:512-27.
7. Karin M. Nuclear factor-kappaB in cancer development and progression. *Nature* 2006;441:431-6.
8. Horst AK, Neumann K, Diehl L, Tiegs G. Modulation of liver tolerance by conventional and nonconventional antigen-presenting cells and regulatory immune cells. *Cell Mol Immunol* 2016;13:277-92.
9. Buonaguro L, Mauriello A, Cavalluzzo B, Petrizzo A, Tagliamonte M. Immunotherapy in hepatocellular carcinoma. *Ann Hepatol* 2019;18:291-7.
10. Jenne CN, Kubes P. Immune surveillance by the liver. *Nat Immunol* 2013;14:996-1006.
11. Racanelli V, Rehermann B. The liver as an immunological organ. *Hepatology* 2006;43:S54-62.
12. Shetty S, Lalor PF, Adams DH. Liver sinusoidal endothelial cells - gatekeepers of hepatic immunity. *Nat Rev Gastroenterol Hepatol* 2018;15:555-67.
13. Carambia A, Frenzel C, Bruns OT, et al. Inhibition of inflammatory CD4 T cell activity by murine liver sinusoidal endothelial cells. *J Hepatol* 2013;58:112-8.

14. Diehl L, Schurich A, Grochtmann R, Hegenbarth S, Chen L, Knolle PA. Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8+ T cell tolerance. *Hepatology* 2008;47:296-305.
15. Schildberg FA, Hegenbarth SI, Schumak B, Scholz K, Limmer A, Knolle PA. Liver sinusoidal endothelial cells veto CD8 T cell activation by antigen-presenting dendritic cells. *Eur J Immunol* 2008;38:957-67.
16. Dixon LJ, Barnes M, Tang H, Pritchard MT, Nagy LE. Kupffer cells in the liver. In: Terjung R, editor. Comprehensive physiology. Hoboken: John Wiley & Sons, Inc.; 2013.
17. Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. *Nat Rev Immunol* 2010;10:753-66.
18. You Q, Cheng L, Kedl RM, Ju C. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. *Hepatology* 2008;48:978-90.
19. Ormandy LA, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res* 2005;65:2457-64.
20. Dou L, Ono Y, Chen YF, Thomson AW, Chen XP. Hepatic dendritic cells, the tolerogenic liver environment, and liver disease. *Semin Liver Dis* 2018;38:170-80.
21. Severi T, van Malenstein H, Verslype C, van Pelt JF. Tumor initiation and progression in hepatocellular carcinoma: risk factors, classification, and therapeutic targets. *Acta Pharmacol Sin* 2010;31:1409-20.
22. Qin LX. Inflammatory immune responses in tumor microenvironment and metastasis of hepatocellular carcinoma. *Cancer Microenviron* 2012;5:203-9.
23. Cancer Genome Atlas Research Network. Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell* 2017;169:1327-41.e23.
24. Sia D, Jiao Y, Martinez-Quetglas I, et al. Identification of an immune-specific class of hepatocellular carcinoma, based on molecular features. *Gastroenterology* 2017;153:812-26.
25. Behboudi S, Boswell S, Williams R. Cell-mediated immune responses to alpha-fetoprotein and other antigens in hepatocellular carcinoma. *Liver Int* 2010;30:521-6.
26. Fu J, Xu D, Liu Z, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology* 2007;132:2328-39.
27. Yang YM, Kim SY, Seki E. Inflammation and liver cancer: molecular mechanisms and therapeutic targets. *Semin Liver Dis* 2019;39:26-42.
28. Liu CY, Chen KF, Chen PJ. Treatment of liver cancer. *Cold Spring Harb Perspect Med* 2015;5:a021535.
29. Jadid FZ, Chihab H, Alj HS, et al. Control of progression towards liver fibrosis and hepatocellular carcinoma by SOCS3 polymorphisms in chronic HCV-infected patients. *Infect Genet Evol* 2018;66:1-8.
30. Kudo M. Immuno-oncology in hepatocellular carcinoma: 2017 Update. *Oncology* 2017;93 Suppl 1:147-59.
31. Chuang W, Liu H, Chang W. Natural killer cell activity in patients with hepatocellular carcinoma relative to early development and tumor invasion. *Cancer* 1990;65:926-30.
32. Wu Y, Kuang DM, Pan WD, et al. Monocyte/macrophage-elicited natural killer cell dysfunction in hepatocellular carcinoma is mediated by CD48/2B4 interactions. *Hepatology* 2013;57:1107-16.
33. Abril-Rodriguez G, Ribas A. SnapShot: immune checkpoint inhibitors. *Cancer Cell* 2017;31:848.e1.
34. Chen G, Huang AC, Zhang W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature* 2018;560:382-6.
35. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015;372:320-30.
36. El-khoueiry AB, Sangro B, Yau T, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
37. Zhu AX, Finn RS, Edeline J, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol 2018;19:940-52.
38. Kudo M. Molecular targeted therapy for hepatocellular carcinoma: where are we now? *Liver Cancer* 2015;4:I-VII.
39. Zhang B, Finn RS. Personalized clinical trials in hepatocellular carcinoma based on biomarker selection. *Liver Cancer* 2016;5:221-32.
40. Bruix J, Qin S, Merle P, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;389:56-66.
41. Yoo C, Park JW, Kim YJ, et al. Multicenter retrospective analysis of the safety and efficacy of regorafenib after progression on sorafenib in Korean patients with hepatocellular carcinoma. *Invest New Drugs* 2019;37;567-72.
42. Yoo C, Ryu YM, Kim SY, et al. Association between the exposure to anti-angiogenic agents and tumour immune microenvironment in advanced gastrointestinal stromal tumours. *Br J Cancer* 2019;121;819-26.
43. Titano J, Noor A, Kim E. Transarterial chemoembolization and radioembolization across barcelona clinic liver cancer stages. *Semin Intervent Radiol* 2017;34;109-15.
44. Bolondi L, Burroughs A, Dufour JF, et al. Heterogeneity of patients with intermediate (BCLC B) hepatocellular carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis* 2012;32;348-59.
45. El-Khoueiry AB, Sangro B, Yau T, et al. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017;389:2492-502.
46. Yau T, Hsu C, Kim TY, et al. Nivolumab in advanced hepatocellular carcinoma: sorafenib-experienced Asian cohort analysis. *J Hepatol* 2019;71;543-52.
47. Lichty BD, Breitbach CJ, Stojdl DF, Bell JC. Going viral with cancer immunotherapy. *Nat Rev Cancer* 2014;14:559-67.
48. Guo ZS, Thorne SH, Bartlett DL. Oncolytic virotherapy: molecular targets in tumor-selective replication and carrier cell-mediated

delivery of oncolytic viruses. *Biochim Biophys Acta* 2008;1785:217-31.
49. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;2:675-80.
50. Kato H, Takeuchi O, Sato S, et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 2006;441:101-5.
51. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. *Nat Rev Drug Discov* 2015;14:642-62.
52. Yoo SY, Badrinath N, Woo HY, Heo J. Oncolytic virus-based immunotherapies for hepatocellular carcinoma. Mediators Inflamm 2017;2017:5198798.
53. Kanerva A, Nokisalmi P, Diaconu I, et al. Antiviral and antitumor T-cell immunity in patients treated with GM-CSF-coding oncolytic adenovirus. *Clin Cancer Res* 2013;19:2734-44.
54. Kirn DH, Thorne SH. Targeted and armed oncolytic poxviruses: a novel multi-mechanistic therapeutic class for cancer. *Nat Rev Cancer* 2009;9:64-71.
55. Abdullahi S, Jäkel M, Behrend SJ, et al. A novel chimeric oncolytic virus vector for improved safety and efficacy as a platform for the treatment of hepatocellular carcinoma. *J Virol* 2018;92:e01386-18.
56. Zhang H, Li K, Lin Y, et al. Targeting VCP enhances anticancer activity of oncolytic virus M1 in hepatocellular carcinoma. *Sci Transl Med* 2017;9:eaam7996.
57. Luo Y, Lin C, Ren W, et al. Intravenous injections of a rationally selected oncolytic herpes virus as a potent virotherapy for hepatocellular carcinoma. *Mol Ther Oncolytics* 2019;15;153-65.
58. Chen A, Zhang Y, Meng G, et al. Oncolytic measles virus enhances antitumour responses of adoptive CD8(+)NKG2D(+) cells in hepatocellular carcinoma treatment. *Sci Rep* 2017;7:5170.
59. Xie W, Hao J, Zhang K, et al. Adenovirus armed with VGLL4 selectively kills hepatocellular carcinoma with G2/M phase arrest and apoptosis promotion. *Biochem Biophys Res Commun* 2018;503:2758-63.
60. Nakatake R, Kaibori M, Nakamura Y, et al. Third-generation oncolytic herpes simplex virus inhibits the growth of liver tumors in mice. *Cancer Sci* 2018;109:600-10.
61. El-Shemi AG, Ashshi AM, Oh E, et al. Efficacy of combining ING4 and TRAIL genes in cancer-targeting gene virotherapy strategy: first evidence in preclinical hepatocellular carcinoma. *Gene Ther* 2018;25;54-65.
62. Zhang J, Lai W, Li Q, et al. A novel oncolytic adenovirus targeting Wnt signaling effectively inhibits cancer-stem like cell growth via metastasis, apoptosis and autophagy in HCC models. *Biochem Biophys Res Commun* 2017;491;469-77.
63. Lv SQ, Ye ZL, Liu PY, et al. 11R-P53 and GM-CSF expressing oncolytic adenovirus target cancer stem cells with enhanced synergistic activity. *J Cancer* 2017;8:199-206.
64. El-Shemi AG, Ashshi AM, Na Y, et al. Combined therapy with oncolytic adenoviruses encoding TRAIL and IL-12 genes markedly suppressed human hepatocellular carcinoma both in vitro and in an orthotopic transplanted mouse model. *J Exp Clin Cancer Res* 2016;35:74.
65. Ruf B, Berchtold S, Venturelli S, et al. Combination of the oral histone deacetylase inhibitor resminostat with oncolytic measles vaccine virus as a new option for epi-virotherapeutic treatment of hepatocellular carcinoma. *Mol Ther Oncolytics* 2015;2:15019.
66. Ady JW, Johnsen C, Mojica K, Heffner J, Love D, et al. Oncolytic gene therapy with recombinant vaccinia strain GLV-2b372 efficiently kills hepatocellular carcinoma. *Surgery* 2015;158;331-8.
67. Ady JW, Heffner J, Mojica K, et al. Oncolytic immunotherapy using recombinant vaccinia virus GLV-1h68 kills sorafenib-resistant hepatocellular carcinoma efficiently. *Surgery* 2014;156;263-9.
68. Lin WH, Yeh SH, Yang WJ, et al. Telomerase-specific oncolytic adenoviral therapy for orthotopic hepatocellular carcinoma in HBx transgenic mice. *Int J Cancer* 2013;132:1451-62.
69. Wang J, Xu L, Zeng W, et al. Treatment of human hepatocellular carcinoma by the oncolytic herpes simplex virus G47delta. *Cancer Cell Int* 2014;14:83.
70. Fu X, Rivera A, Tao L, et al. Construction of an oncolytic herpes simplex virus that precisely targets hepatocellular carcinoma cells. *Mol Ther* 2012;20:339-46.
71. Gentschev I, Müller M, Adelfinger M, et al. Efficient colonization and therapy of human hepatocellular carcinoma (HCC) using the oncolytic vaccinia virus strain GLV-1h68. *PLoS One* 2011;6:e22069.
72. Jin H, Lv S, Yang J, et al. Use of microRNA Let-7 to control the replication specificity of oncolytic adenovirus in hepatocellular carcinoma cells. *PLoS One* 2011;6:e21307.
73. Lee JH, Roh MS, Lee YK, et al. Oncolytic and immunostimulatory efficacy of a targeted oncolytic poxvirus expressing human GM-CSF following intravenous administration in a rabbit tumor model. *Cancer Gene Ther* 2010;17:73-9.
74. Wein LM, Wu JT, Kirn DH. Validation and analysis of a mathematical model of a replication-competent oncolytic virus for cancer treatment: implications for virus design and delivery. Cancer Res 2003;63:1317-24.
75. Thorne SH, Hwang TH, O'Gorman WE, et al. Rational strain selection and engineering creates a broad-spectrum, systemically effective oncolytic poxvirus, JX-963. *J Clin Invest* 2007;117:3350-8.
76. Kim JH, Oh JY, Park BH, et al. Systemic armed oncolytic and immunologic therapy for cancer with JX-594, a targeted poxvirus expressing GM-CSF. Mol Ther 2006;14:361-70.
77. Parato KA, Breitbach CJ, Le Boeuf F, et al. The oncolytic poxvirus JX-594 selectively replicates in and destroys cancer cells driven by genetic pathways commonly activated in cancers. *Mol Ther* 2012;20:749-58.
78. Park B, Hwang T, Liu T, et al. Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial. *Lancet Oncology* 2008;9:533-42.

79. Heo J, Reid T, Ruo L, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nat Med* 2013;19:329-36.
80. Moehler M, Heo J, Lee HC, et al. Vaccinia-based oncolytic immunotherapy pexastimogene devacirepvec in patients with advanced hepatocellular carcinoma after sorafenib failure: a randomized multicenter Phase IIb trial (TRAVERSE). *Oncoimmunology* 2019;8:1615817.
81. Heo J, Reid T, Ruo L, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nat Med* 2013;19;329-36.
82. Moehler M, Heo J, Lee HC, et al. Vaccinia-based oncolytic immunotherapy Pexastimogene Devacirepvec in patients with advanced hepatocellular carcinoma after sorafenib failure: a randomized multicenter Phase IIb trial (TRAVERSE). *Oncoimmunology* 2019;8:1615817.
83. Park BH, Hwang T, Liu TC, et al. Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial. *Lancet Oncol* 2008;9:533-42.
84. Rizell M, Eilard MS, Andersson M, et al. Phase 1 trial with the cell-based immune primer ilixadencel, alone, and combined with sorafenib, in advanced hepatocellular carcinoma. *Front Oncol* 2019;9:19.
85. Ning N, Pan Q, Zheng F, et al. Cancer stem cell vaccination confers significant antitumor immunity. *Cancer Res* 2012;7:1853-64.
86. Johnston MP, Khakoo SI. Immunotherapy for hepatocellular carcinoma: current and future. *World J Gastroenterol* 2019;25:2977-89.
87. Gustafsson K, Ingelsten M, Bergqvist L, Nyström J, Andersson B, Karlsson-Parra A. Recruitment and activation of natural killer cells in vitro by a human dendritic cell vaccine. *Cancer Res* 2008;68:5965-71.
88. Shang N, Figini M, Shangguan J, et al. Dendritic cells based immunotherapy. Am J Cancer Res 2017;7:2091-102.
89. Rizell M, Sternby Eilard M, Andersson M, Andersson B, Karlsson-Parra A, Suenaert P. Phase 1 trial with the cell-based immune primer ilixadencel, alone, and combined with sorafenib, in advanced hepatocellular carcinoma. *Front Oncol* 2019;9:19.
90. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. *Blood* 2010;116:1035-44.
91. Jiang Z, Jiang X, Chen S, et al. Anti-GPC3-CAR T cells suppress the growth of tumor cells in patient-derived xenografts of hepatocellular carcinoma. *Front Immunol* 2016;7:690.
92. Wang Y, Chen M, Wu Z, et al. CD133-directed CAR T cells for advanced metastasis malignancies: a phase I trial. *Oncoimmunology* 2018;7:e1440169.
93. Li D, Li N, Zhang YF, et al. Persistent polyfunctional chimeric antigen receptor T cells that target glypican 3 eliminate orthotopic hepatocellular carcinomas in mice. *Gastroenterology* 2020;158:2250-65.e20.
94. Liu X, Wen J, Yi H, et al. Split chimeric antigen receptor-modified T cells targeting glypican-3 suppress hepatocellular carcinoma growth with reduced cytokine release. *Ther Adv Med Oncol* 2020;12:1758835920910347.
95. Sun B, Yang D, Dai H, et al. Eradication of hepatocellular carcinoma by NKG2D-based CAR-T cells. *Cancer Immunol Res* 2019;7:1813-23.
96. Zhang RY, Wei D, Liu ZK, et al. Doxycycline inducible chimeric antigen receptor T cells targeting CD147 for hepatocellular carcinoma therapy. *Front Cell Dev Biol* 2019;7:233.
97. Morgan RA, Johnson LA, Davis JL, et al. Recognition of glioma stem cells by genetically modified T cells targeting EGFRvIII and development of adoptive cell therapy for glioma. *Hum Gene Ther* 2012;23:1043-53.
98. Finney HM, Akbar AN, Lawson AD. Activation of resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. *J Immunol* 2004;172:104-13.
99. Yau T, Kang YK, Kim TY, et al. Efficacy and safety of Nivolumab plus Ipilimumab in patients with advanced hepatocellular carcinoma previously treated with Sorafenib: the CheckMate 040 randomized clinical trial. *JAMA Oncol* 2020;e204564.
100. Finn RS, Qin S, Ikeda M, et al; IMbrave150 Investigators. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med* 2020;382:1894-905.