

Original Article

Current statuses of molecular targeted and immune checkpoint therapies in hepatocellular carcinoma

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Abstract: Treatment of advanced hepatocellular carcinoma (HCC) still confronts great challenges due to high rate of therapeutic resistance. The emergence of systemic treatment with molecular targeted and immune checkpoint therapies has brought novel approaches towards patients with advanced HCC. However, sorafenib, as the first approved systemic treatment in 2007, only increased overall survival by three months in advanced HCC patients. Afterwards, little progress has been made for molecular targeted agents. Only four molecular drugs are empirically used in clinical practice. Lenvatinib acts as a first-line drug, whereas regorafenib, ramucirumab, and cabozantinib are defined as second-line drugs. Nevertheless, clinical findings reveal that overall survival remains unchanged. Albeit immunotherapy-based approaches are currently considered promising therapeutic strategies for advanced HCC, a minority of patients could benefit from them. These beneficiaries are to be accordingly identified. Combined immunotherapies with matched molecular targeted treatments would be a novel breakthrough. Herein, we summarize the current statuses of immunotherapies and molecular targeted drug therapies, and mainly identify clinically feasible chemoimmunotherapeutic strategies.

Keywords: Hepatocellular carcinoma, molecular targeted therapy, immunotherapy, chemoimmunotherapy

Introduction

Hepatocellular carcinoma (HCC) as the second most frequent cause of cancer-related death accounts for approximately 75% of primary liver cancer cases [1]. From an etiological perspective, alcohol abuse, autoimmunity, chronic infection with hepatitis C virus or hepatitis B virus, several metabolic diseases, and nonalcoholic steatohepatitis are the main risk factors for the occurrence of HCC. However, there are considerable differences between the Euro-American region and Asia-Pacific area [2]. Since HCC is frequently detected at a late stage, only a small number of patients are eligible for transplant and surgery. Furthermore, high rate of recurrence is found after surgery. Most patients with advanced-stage HCC could not benefit from traditional medications [3]. Therefore, systemic therapies might be the most promising strategy for these patients. Since sorafenib, a molecular targeted agent, was

approved for treatment of patients with advanced HCC in 2007, systemic treatment has undergone a dramatic change, expanding the therapeutic approaches towards treating extrahepatic spread and vascular invasion. The median overall survival time of advanced HCC patients extended from 8 to 11 months [4]. Due to the high incidence of toxicity and low response rate of sorafenib treatment, many attempts have been made to develop novel molecular targeted drug candidates as alternatives in clinical trials [5]. However, most agents failed to meet clinical endpoints in phase 3 trials, and only four drugs, regorafenib, cabozantinib, ramucirumab, and lenvatinib have been demonstrated to improve patients' outcomes. Their effects are incremental and modest [1]. Although it is generally recognized that immune evasion plays a significant role in the progression of HCC, the lack of effective treatment has reversed cancer-related immunosuppression in the past few years [6]. The emergence of

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Table 1. Commonly aberrant signaling pathways in liver carcinogenesis

Pathway	Related gene alternation	Abnormal Frequency (% of patients)	Potential targeted Drugs (related target)	Function
Telomere maintenance [7, 45]	TERT promoter mutation	54%-60%	BET inhibitors [46]	Telomeres maintain chromosomal stability. [47]
	TERT amplification	About 5%		
	HBV insertion in TERT promotor	10%-15%		
Wnt/ β -catenin Pathway [7, 45]	CTNNB1 mutation	11%-37%	XAV939 (tankyrase 1 and tankyrase 2) [48]	Embryo stage: Controlling hepatobiliary development, maturation, zonation Maturity: Cell renewal and/or regeneration processes [49]
	AXIN1 mutation	5%-15%		
	APC mutation	1%-2%		
P53 Cell-cycle pathway [45]	P53 mutation	12%-48%	Ribociclib (CDK4 and CDK6) Palbociclib (CDK4/6) Milciclib (CDKs) [1]	Regulator of liver homeostasis and dysfunction [50]
	CDKN2A	2%-12%		
	RB1	3%-8%		
Epigenetic modifiers [7, 45]	MLL, MLL2, MLL3, MLL4 mutation	3%-4%, 2%-3%, 3%-6%, 2%-3%, respectively	Tefinostat (HDACs) And Resminostat (HDACs) [1]	Governing maintenance of genomic integrity and DNA repair and regulation of splicing [51].
	HBV insertions in MLL4	10%		
	ARID1A, ARID2 mutation	4%-17%, 3%-18% respectively		
Oxidative stress pathway [45]	NRF2 or KEAP1 mutation	5%-15%		Inducing protein expression and DNA oxidative damage [52].
EGFR/RAS/RAF/MAPK and PI3K/AKT/mTOR pathways [7, 45]	Amplification of the FGF19/CCND1	5%-10%	SF1126 (PI3K and mTOR) Donafenib (RAF) Sapanisertib (mTOR) gefitinib, erlotinib, afatinib, dacomitinib, and osimertinib (EGFR) [1]	Regulating cellular apoptosis, metabolism, Differentiation and proliferation [53].
	PIK3CA mutation	0%-2%		
	TSC1 or TSC2 mutation	3%-8%		
	Homozygous deletion of PTEN	1%-3%		
	RP6SKA3	2%-9%		
	EGFR mutation [54]	4%-66%		
	TKI		Activation of multiple Signaling pathways controlling mainly survival, differentiation proliferation [55].	
	IL-6/JAK/STAT mutation [56]	About 9%	Napabucasin (STAT3) [1]	Controlling different cellular processes, including proliferation, cell division and cell fate decision [57].
	TGF- β [56]	About 5%	Galunisertib (TGF β R1) [1]	Regulating fibrogenesis, Immunomodulation and inflammation in the HCC microenvironment [58].
FGF pathway [7]	FGF3, FGF4 and FGF19 mutation	4%-5.6%	BLU-554 (FGFR4) INCB062079 (FGFR4) H3B-6527 (FGFR4) Erdafitinib (FGFRs) [1]	Regulating cellular differentiation, proliferation, development, embryonic and organogenesis [59].

immune checkpoint inhibitors, such as nivolumab, pembrolizumab, created a novel therapeutic approach and made promising results, with approximately 19% response rate and durable benefits in phase 1-2 trials. Currently, related phase 3 trials are in progress [7]. In recent years, oncogenic drivers of HCC involving multiple gene mutations and silencing (Table 1), have been deciphered, which has provided a potential groundwork for the use of novel molecular targeted drugs. Nevertheless, the therapeutic options based on molecular biology of HCC are still limited [8].

In this review, we report the current statuses of the development and challenges of molecular targeted drugs and immune-related drugs, and mainly focus on combination regimens,

especially combined immunotherapies and potentially matched molecular targeted treatments.

Molecular targeted agents in HCC

Angiogenesis inhibitors

Compared with other solid tumors, hepatocellular carcinoma has the most abundant blood vessels [9], in which many proangiogenic growth factors are overexpressed, including platelet-derived growth factor (PDGF), vascular endothelial growth factor A (VEGFA), transforming growth factor β (TGF- β), and basic fibroblast growth factor (bFGF). Vascular endothelial growth factor (VEGF), one of the most important pro-angiogenic factors, regulates the mito-

genic and anti-apoptotic activities of endothelial cells which promote cell migration and diffusion and vascular permeability. It effectively promotes pathological angiogenic processes in HCC by mediating these effects [10]. Overall, chemoembolization and antiangiogenic agents are valid measures for the clinical treatment of HCC [11]. Sorafenib, a multi-kinase inhibitor, is able to reduce tumor cell growth and angiogenesis by achieving targeted inhibition of many protein kinases involved in HCC, including vascular endothelial growth factor receptor (VEGFR), c-KIT, platelet-derived growth factors receptor (PDGFR), RAF, RET etc. [12]. Compared with placebo treatment, sorafenib showed a modest benefit of patients' survival, affecting median overall survival (OS), in two phase 3 trials (ASIA-PACIFIC and SHARP) that enrolled patients with preserved liver function of Child-Pugh class A and advanced HCC [12, 13]. However, the treatment-related adverse events such as fatigue, hand-foot syndrome, and diarrhea, were more frequently observed in the sorafenib groups (80% vs 52%) [13]. The ASIA-PACIFIC study, clinical trial similar to the SHRP study completed with a group of Asian patients, showed relatively short OS (6.5 versus 4.2 months), due to high incidence of adverse prognostic factors including large tumor volumes, altered ECOG PS scores and a high incidence of HBV infection [11, 14]. In addition to severe adverse events, HCC patients could develop into the resistance to sorafenib. Several molecular mechanisms for sorafenib resistance may be involved, including epithelial-mesenchymal transition, hypoxia-inducible factor activation, and JAK/STAT, and PI3K/AKT signaling pathways activations [15]. Sorafenib still acts as a first-line drug for advanced HCC treatment, due to a relatively lower rate of early HCC and higher rate of HCC recurrence after surgery [16]. Recently, lenvatinib was approved by the Food and Drug Administration (FDA) which is a targeted multi-kinase inhibitor with several targets, including VEGFR1-3, FGFR1-4, RET, PDGFR- α and c-Kit [17]. Compared with sorafenib, lenvatinib has fewer treatment-related adverse events (hypertension, appetite, fatigue, and weight loss) and was noninferior in median OS in a randomized phase 3 trial [18]. Compared with sorafenib, other first-line drugs failed to show non-inferiority such as brivanib, linifanib, and sunitinib [11].

Genomic mutation-based molecular drugs

Genomic alterations have been uncovered in HCC in recent years, which may be potential therapeutic targets. Gene mutations are the most frequent type of these alternations, such as telomerase reverse transcriptase (TERT) promoter, β -catenin (CTNNB1), TP53, AXIN1, CDKN2A, ARID1A, and CCND1 gene mutations [7]. However, drugs targeting these alterations have been limited so far (**Table 1**). The mesenchymal to epithelial transition (MET) gene plays significant roles in the initiation and progression of many solid tumors and drug resistance, and encodes a tyrosine kinase (TK), which is a type of receptor for hepatocyte growth factor (HGF) [19]. Many complex biological activities are activated and driven by activation of the HGF/MET axis, including cell proliferation, inhibition of apoptosis and cell migration [20]. In vitro preclinical data for HCC showed that in addition to HGF, several types of tyrosine kinase receptors interacting with MET had functional cross-talk, and cross-talk also occurred between members of the EGFR family and MET [20, 21]. The use of antiangiogenic agents could increase the secretion of HIF-1 α and expression of MET in HCC cells [22, 23]. The transcription of MET is induced by hypoxia-inducible factor which can promote tumorigenesis and cancer progression [24]. Overall, promoting MET activity can be considered an adverse event in antiangiogenic drug therapy. The combination of MET inhibitors and antiangiogenic agents will be a promising therapy. The combination of sorafenib and tivantinib (a small-kinase MET inhibitor) was evaluated in a clinical trial among 20 HCC patients, which might provide novel therapeutic benefit in HCC patients. The study showed a 10% overall response rate and 70% disease control rate. Among the 8 patients pretreated with antiangiogenic drugs that obtained an encouraging response, 3 exhibited stable disease, 1 exhibited a partial response, and 1 exhibited a complete response. Tivantinib, an oral small-molecule MET inhibitor with a non-ATP-dependent competitive mechanism, has shown promising therapeutic efficacy in numerous phase 1/2 clinical trials for HCC patients with high MET expression. However, compared with a placebo, tivantinib failed to improve overall survival of HCC patients with high MET expression and pretreated with sorafenib in a double-blinded

and randomized phase 3 study [25]. The clinical study did not display a significant difference in the median OS between tivantinib and the placebo (9.1 months VS 8.4 month). However, compared with the placebo group, the tivantinib group showed worse treatment-related adverse events and more grade 3 events, including anemia, neutropenia, abdominal pain, and ascites.

Tivantinib and cabozantinib can only be used as second-line therapies for HCC, but phase 3 studies have not achieved satisfactory indications of efficacy. Cabozantinib, a multi-receptor tyrosine kinases (RTKs) inhibitor, suppresses several pathological processes, including angiogenesis, tumor growth, oncogenesis, and metastasis [26]. A phase 2 study of cabozantinib for 41 advanced HCC patients showed promising therapeutic efficacy, with an overall disease control rate of 68%. Compared with that of MET inhibitors such as tivantinib, the safety profile of cabozantinib is more similar to that of anti-VEGFR inhibitors. Palmar-plantar erythrodysesthesia (15%), thrombocytopenia (10%) and diarrhea (17%) were the most common grade 3/4 treatment-related adverse events for cabozantinib in the clinical study. Based on promising data from a phase 2 study of cabozantinib, a phase 3 trial of cabozantinib versus a placebo is ongoing in HCC patients with Child-Pugh A liver function and progression following treatment with one or more systemic therapies [27]. The therapeutic effects of other MET inhibitors as first-line therapies for HCC are being studied in phase 1b/2 clinical trials.

Molecular targeted inhibitors of HCC can be divided into two categories, inhibitors of RTKs and inhibitors of intracellular kinases. Most clinical trials have focused on molecular inhibitors of RTKs and have achieved promising therapeutic results, such as sorafenib and lenvatinib as first-line treatment drugs for HCC patients. However, intracellular molecular signaling pathways also play pivotal roles in HCC carcinogenesis. mTOR is an important central regulator of many molecular signaling pathways. Their alterations can lead to tumorigenesis. Rapamycin and its analogs such as everolimus are inhibitors of mTOR and were originally used as immunosuppressants in organ transplantation [28]. The ability of everolimus to inhibit proliferation was observed in

preclinical studies, including studies of animal models and cancer cell lines. Based on the data, everolimus was approved by the FDA as an anticancer drug for various cancers [29]. To assess the efficacy of everolimus in advanced HCC patients who failed sorafenib treatment, EVOLVE-1, a, double-blinded, randomized phase 3 trial, was implemented with patients in 17 countries. Overall survival was not significantly different between the everolimus group and the placebo group. The mortality rate of the everolimus group was similar to that of the placebo group, 83.7% and 82.1%, respectively. The most frequent grade 3/4 treatment-related adverse events were decreased, appetite (6.1%), anemia (7.8%) and asthenia (7.8%) [30]. Compared with a phase 3 study of tivantinib (a MET inhibitor), the phase 3 trial of everolimus may have failed because of the selection of a specific group of advanced HCC patients. In regard to targeting mTOR, more drugs have been developed and are currently being studied in clinical trials, and these drugs can also inhibit mTOR-PI3K and mTORC2, to better inhibit the PI3K-AKT signaling pathway [1].

Immune checkpoint inhibitors in HCC

HCC is a representative inflammation-related cancer, thereby regulating the immune micro-environment which contains many components, such as multiple types of immune cells, cytokines. Immunostimulatory molecules play essential roles in the treatment of HCC.

Programmed death protein 1 (PD-1), a negative stimulatory molecule, is expressed on the surface of activated T cells and can play biological roles in immune escape from tumor-specific T cells through binding to its ligands, programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2). They are expressed on immune cells and tumor cells [31]. Blockade of the PD-1/PD-L pathway can activate the cytotoxic T cell response, which specifically kills tumor cells [32]. Since nivolumab and pembrolizumab, anti-PD-1/PD-L1 blocking monoclonal antibodies, have been approved by the FDA for patients with HCC. Further clinical investigations on this topic is ongoing [33, 34].

Nivolumab, a monoclonal antibody specific for PD-L1, was evaluated in a phase 1/2, non-comparative, open-label trial that was registered

with clinical Trials.gov, with (NCT01658878) [33]. The trial was enrolled 262 patients and they are separated into two phases: 214 patients in the dose-expansion phase and 48 patients in the dose-escalation phase. In the dose-escalation phase, nivolumab was provided with a manageable safety profile, including acceptable tolerability. The dose expansion of nivolumab was 3 mg/kg. The objective response rates of the two phases were 15% (95% CI 6-28) in the dose-escalation phase and 20% (95% CI 15-26) in the dose-expansion phase. Based on durable objective responses, nivolumab is a potential treatment option for patients with advanced HCC.

The KEYNOTE-224, a multi-center, open-label, and non-randomised phase 2 trial, demonstrated that pembrolizumab obtained a promising treatment effect for advanced HCC patients. Two randomised, phase 3 trials, are also ongoing for further assessment as a second-line treatment for HCC patients [34]. The clinical trials enrolled 104 HCC patients and they developed disease progression after sorafenib treatment. The objective response was documented in 18 of 104 patients (17%; 95% CI 11-26), including 17 (16%) partial response and 1 (1%) complete responses. At the same time, 34 (33%) patients had progressive disease, 46 (44%) had stable disease, and 6 (6%) patients were not assessed owing to partial reasons. The severe treatment-related adverse events occurred in 76 (73%) of 104 patients. The grade 3 treatment-related events were frequently observed, which mainly included the increase of alanine aminotransferase concentration in 4 (4%) patients, aspartate aminotransferase concentration in 7 (7%) patients, and fatigue in 4 (4%) patients.

Combination treatment of molecular targeted agents and immune checkpoint inhibitors

Combination treatment with pembrolizumab plus lenvatinib was performed in a phase 1b trial which enrolled 23 patients with selected solid tumors, including endometrial cancer, renal cell carcinoma, urothelial cancer, melanoma, non-small cell lung cancer and head and neck squamous cell carcinoma [35]. The trial achieved promising treatment efficacy, and the response rate reached 65%. The combination therapy showed significant synergistic treat-

ment efficacy. Considering the treatment efficacy of pembrolizumab in combined with lenvatinib, combination treatment with TKIs and immune checkpoint inhibitors has become a highlight in recent clinical studies of HCC.

Compared with tyrosine kinase inhibitors (TKIs) monotherapy, combination treatment showed a survival benefit in a series of phase 1/2 trials with advanced HCC patients. The tolerability and toxicity of combination therapy were also assessed in the trials. Combination treatment versus sorafenib is being evaluated in an ongoing, randomized, controlled, phase 3 trial. The synergistic effect can be explained by its underlying biological mechanism. Overexpressed proangiogenic factors cause abnormal vasculature in tumors, which is disorganized and tortuous, and redundantly branched leaky vessels [36]. However, due to improvement in vascular permeability and high interstitial fluid pressure, blood oxygenation and perfusion decrease. Normalized tumor vasculature may be achieved with a reasonable dose of antiangiogenic treatment, which enhances tumor blood flow and perfusion reduces vascular permeability and produces a synergistic treatment effect with immunotherapy. Excessive inhibition of angiogenesis by antiangiogenics induces hypoxia and immunosuppression, including increased expression of PD-L1 in a dose and time-dependent manner. In addition, different combination therapies composed of anti-PD-1 antibodies and other molecular targeted drugs are being evaluated in HCC, including drugs targeting TKIs, c-Met, TGF- β , etc. (**Table 2**).

Other combination strategies with immune checkpoint inhibitors

Passive immunotherapy for HCC has been actively studied during the past two decades, such as oncolytic viruses, adoptive cellular therapies, and vaccines. Oncolytic virus injection can lead to tumor cell lysis by activating both cellular immunity and the complement cascade. Preclinical trials have demonstrated that local oncolytic virus injection could boost the sensitivity of the tumor response to checkpoint blockade by infiltration of many inflammatory immune cells outside the tumor. A related combination trial evaluating immune checkpoint inhibitors and an oncolytic virus, nivolum-

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Table 2. Clinical trials with immune checkpoint inhibitors in HCC

Combination type	Target	Clinical stage	ClinicalTrials.gov reference
Durvalumab + tremelimumab	PD-L1 and CTLA-4	Phase 3	NCT03298451
Atezolizumab + bevacizumab	PD-L1 and VEGFA	Phase 3	NCT03434379
Durvalumab + Tremelimumab	PD-L1 and CTLA-4	Phase 2	NCT02519348
Durvalumab, Tremelimumab + LRT	PD-L1, CTLA4 and locoregional therapy	Phase 1/2	NCT02821754
Nivolumab (anti PD-1 Ab), Nivolumab + Ipilimumab, Nivolumab + cabozantinib, Nivolumab + Ipilimumab + cabozantinib	PD-1 and CTLA4, PD-1 and c-Met, PD-1 and CTLA4 and c-Met	Phase 1/2	NCT01658878
Nivolumab (anti PD-1 Ab) + CC-122 (immunostimulatory pathway modifier)	PD-1 and CC-122	Phase 1/2	NCT02859324
Pembrolizumab + dendritic cells, cytokine-induced killer cells	PD-1 and dendritic cells, killer cells	Phase 1/2	NCT02886897
PDR001 (anti PD-1 Ab) + INC280 (c-Met inhibitor)	PD-1 and C-Met	Phase 1/2	NCT02795429
Nivolumab + ipilimumab	PD-1 and CTLA-4	Phase 1/2	NCT01658878
Nivolumab + galunisertib	PD-L1 and TGF- β	Phase 1/2	NCT02423343
Apatinib + SHR-1210	VEGFR2 and PD-1	Phase 1/2	NCT02942329
Capmatinib \pm Spartalizumab	MET \pm PD-L1	Phase 1/2	NCT02795429
Spartalizumab \pm FGF401	PD-1 and FGFR4	Phase 1/2	NCT02325739
XL888 + pembrolizumab	HSP90 and PD-1	Phase 1	NCT03095781
Ramucirumab + durvalumab	VEGFR2 and PD-L1	Phase 1	NCT02572687
Avelumab + axitinib	PD-L1 plus VEGFRs, KIT, and PDGFRs	Phase 1	NCT03289533
Cabozantinib + nivolumab	MET and VEGFRs plus PD-1	Phase 1	NCT03299946
Regorafenib + pembrolizumab	FGFRs, VEGFRs, KIT, PDGFRs, RAF and PD-1	Phase 1	NCT03347292
Pembrolizumab + lenvatinib	PD-1 plus VEGFR2 and VEGFR3	Phase 1	NCT03006926
Ipilimumab (anti CTLA-4 Ab) + stereotactic body radiation	CTLA-4 and stereotactic body radiation	Phase 1	NCT02239900
Durvalumab + ramucirumab	PD-L1 and VEGF-R2	Phase 1	NCT02572687
Durvalumab (anti PD-1 L Ab) + AZD4635	PD-1 and A2AR	Phase 1	NCT02740985
PDR001 (anti PD-1 Ab) + NIS793 (anti TGF- β Ab)	PD-1 and TGF- β	Phase 1	NCT02947165
Nivolumab + LRT (Yttrium 90Y glass microspheres)	PD-1 and locoregional therapy	Phase 1	NCT02837029
Durvalumab + Guadecitabine	PD-1 and epigenetic modifiers	Phase 1	NCT03257761

ab and oncolytic virus derived from vaccinia, is ongoing. (NCT03071094) [37, 38].

Vaccines can strengthen the killing effect of the immune system on tumor cells by generating of antigen-specific CD8⁺ T cells. However, a prospective outcome is not shown in clinical trials, these vaccines rarely cause durable disease control or tumor shrinkage. In addition, vaccines can lead to the progression of HCC, by improving the activity of immune checkpoint molecules [39].

Multiple clinical trials on adoptive cell therapies are ongoing. The combination strategies involving immune checkpoint inhibitors will be further explained [40].

Preclinical studies with a combination of immune checkpoint inhibitors and matched molecular targeted drugs

The tumor microenvironment is complex, large and composed of extracellular components (growth factors, hormones, cytokines, extracel-

lular matrix, etc.) and various cell types (endothelial cells, immune cells, fibroblasts, etc.) [41]. Many factors can drive tumor immune escape, including the expression of PD-L1, enrichment of tumor associated macrophages and tumor cell molecular oncogenic activity [42]. Approaches targeting these pathways may be considered novel therapeutic options. Recently, most combination treatments have been focused on immune checkpoint inhibitors and other molecular targeted drugs. There are inextricable links between the immune and nonimmune responses in the tumor microenvironment of HCC. The antitumor activity enhancement mechanism of combination therapy can be roughly separated into three categories (**Table 3**). Some molecular targeted drugs can inhibit the expression of PD-L1 by regulating the tumor immune microenvironment. Combination therapy of the molecular targeted drugs and anti-PD-L1 therapy produces a synergistic effect that enhances immune-checkpoint blockade efficacy. Another class of molecular drugs promotes the expression of

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Table 3. Potential therapeutic combinations with anti-PD-L1 in pre-clinical studies

Modle types	Target Or agent	Pathway	The change of Tumor-infiltrating immune cells by blocking target or agent therapy	The change Immune checkpoint by blocking target or agent therapy	Potential combination agents	reference
Chemotoxic agent HCC models	OPN	OPN/CSF1/CSF1R	M2 macrophage cells↑ helper cells↑ CD8+ T cells↑	PD-L1↓	Anti-PD-L1 plus CSF1R inhibitor	[42]
Orthotopic HCC models	CDK20/CCRK	CCRK/EZH2/NF-κB/IL-6	(PMN) MDSC↓ CD8+ T cells↑	PD-L1↑	Anti-PD-L1plus CCRK inhibitor	[60]
Fibrotic-HCC mouse model	P38 MAPK signaling pathway	HSC/monocyte-intrinsic p38 MAPK signaling pathway/enhance reprogramming for M-MDSC	M-MDSC↓		Anti-PD-L1 Plus iBET762	[61]
Orthotopic HCC models	TREM-1	TREM-1 + TAM/ERK/NF-κβ pathway/CCL20 (hypoxic tumor enviroment)/CCR6 + Foxp3 + Treg	CCR6 + Foxp3 + Treg↓		Anti-PD-L1 Plus TREM-1 inhibitor	[62]
Human HCC samples	PFKFB3	TDSF/PFKFB3 (TAM)/NF-κβ pathway/PD-L1	CD8+ T cells↑	PD-L1↓	Anti-PD-L1 Plus PFKFB inhibitor	[63]
HCC patients-Derived xenograft mouse model	TOX	TOX binds to PD-1 increasing Endocytic recycling of PD-1	CD8+ T cells↑	PD-1↓	Anti-PD-L1 Plus down-regulation of TOX	[64]
Fibrotic-HCC mouse model	Tyrosine kinase	Tregs can be suppressed by TKI inhibitor.	Tregs↓ CD8+ T cells↑		Anti-PD-L1 Plus Sunitinib	[44]
Human HCC samples	TIM3 and LAG3	TIL functions was increased by combination therapy of against PD-L1 and TIM3 or LAG3.	CD4+ T cells↑ CD8+ T cells↑		Anti-PD-L1 Plus Anti-TIM3 Or Anti-LAG3	[65]
Human HCC samples	EZH2	EZH2/H3K27me3/1RF1	CD8+ T cells↑	PD-L1↓	Anti-PD-L1 Plus EZH2 inhibitor	[66]
Human HCC samples	MEF2D	(Exposed to IFNγ) p300/acetylation of MEF2D/promoter region of CD274 OR (Not exposed to) SIRT7/acetylation of MEF2D/promoter region of CD274	CD8+ T cells↑	PD-L1↓	Anti-PD-L1 Plus MEF2D inhibitor	[67]
Genetically engineered mouse models	polyIC		CD8+ T cells↑ Macrophage cells↑ NK cells↑ Treg↑	PD-L1↑	Anti-PD-L1 Plus polyIC	[43]

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PD-L1 in HCC cells or non-parenchymal cells. The drugs increase the sensitivity of the hepatic response to PD-L1 blockade. The remaining agents and monoclonal antibodies against PD-L1 can collaboratively reverse the exhaustion of CD8⁺ T cells through two independent mechanisms.

(1) Tumor cell-intrinsic osteopontin (OPN) is a tumor-sustaining inflammatory mediator, that regulates tumor biological activity, including tumor immunosuppression, progression and metastasis [42]. Compared with liver tumors in wild-type mice induced by chemical methods, liver tumors in OPN-knockout mice induced by the same method showed a critically decreased number of macrophages in the tumor-infiltrating immune cell population. OPN-knockout mice exhibited markedly decreased expression levels of PD-L1 and M2 macrophage markers, but the levels of cytokines secreted by T-helper cells were increased. This trial demonstrated that OPN can activate the CSF1-CSF1R signaling pathway of macrophages, which promotes expression levels of PD-L1. The survival of OPN-high tumor-bearing mice was prolonged and antitumor activity was improved by combination treatment with a CSF1R inhibitor and an anti-PD-L1 antibody. Combination therapy with CSF1/CSF1R signaling pathway inhibitors and anti-PD-L1 antibodies will be a promising therapeutic option for HCC patients.

(2) Polyinosinic-polycytidylic acid (polyIC), a synthetic double-stranded RNA, induced the expression of PD-L1 in liver sinusoid endothelial cells of mouse HCC models, induced by hydrodynamic tail vein injection of carcinogenic plasmids [43]. PolyIC alone did not show any therapeutic effect on mouse HCC. However, combination treatment with polyIC and anti-PD-L1 therapy robustly inhibited the progression of liver tumors and showed a survival improvement in mice. The sensitivity of the hepatic response to PD-L1 blockade was strengthened and several innate and adaptive immune functions were maximally activated by the use of polyIC.

(3) The accumulation of regulatory T cells and PD-1 overexpression on the surface of CD8⁺ T cells are two independent mechanisms that contribute to the exhaustion of tumor-antigen-specific CD8⁺ T cells. Sunitinib-mediated Re-

gulatory T-cells (Tregs) suppression and blockade of PD-1 produced a synergistic effect to powerfully activate antitumor immunity and suppress tumor growth in mouse HCC models [44].

Conclusive remarks

The path to the treatment of advanced HCC is still long. However, as the depth of the understanding of malignant tumors increases, more treatments will be found. Although systemic treatment has not made a huge change in the overall survival of patients with advanced HCC since the advent of sorafenib, the emergence of genomic mutation-based therapies and immunotherapies brings new approaches. Liver tumorigenesis involves many genomic alterations. As oncogenic drivers have been decoded, pharmacologically approved agents on these abnormalities will be more and more, which will create more therapeutic options for HCC patients. Of note, immune checkpoint inhibitors show promising clinical results, with response rates of approximately 19% after anti-PD-1 treatment observed in phase 1/2 trials. Ongoing phase 3 clinical trials are expected to achieve more promising outcomes with the agents applied as a first-line treatment.

Drug treatment for advanced HCC mainly involves two approaches: molecular targeted drugs and immune-related agents. No monotherapy has achieved a satisfactory therapeutic effect. Given the lack of any new major discoveries, combination utilization may be the most promising treatment option. As new therapeutic targets and drugs continue to be approved, the combinations will become more diverse. Based on multiple combinations, we focus on finding a better therapeutic combination with acceptable toxicity. Due to the unique status of the HCC immune system and a multi-aspect integrated effector system, prospects for combination treatments including immune checkpoint inhibitors are arising. How to optimize the response of the HCC immune microenvironment, still faces substantial challenges. Combination regimes including immune checkpoint inhibitors and matched molecular targeted agents or some passive immunotherapies are the optimal solution.

Disclosure of conflict of interest

None.

Abbreviations

HCC, hepatocellular carcinoma; PDGF, platelet-derived growth factor; VEGFA, vascular endothelial growth factor A; TGF- β , Transforming growth factor β ; bFGF, Basic fibroblast growth factor; VEGF, Vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factors receptor; TKIs, tyrosine kinase inhibitors; PD-L1, Programmed cell death; TERT, telomerase reverse transcriptase; HGF, hepatocyte growth factor; MET, mesenchymal to epithelial transition; OPN, osteopontin; Tregs, regulatory T-cells; FDA, Food and Drug Administration.

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