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Combination of molecularly targeted therapies and immune checkpoint inhibitors in the new era of unresectable hepatocellular carcinoma treatment

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***Abstract*:** Multikinase inhibitors (MKIs) have been the only first-line treatment for advanced hepatocellular carcinoma (HCC) for more than a decade, until the approval of immune checkpoint inhibitors (ICIs). Moreover, the combination regimen of atezolizumab (anti-programmed cell death protein ligand 1 antibody) plus bevacizumab (anti-vascular endothelial growth factor monoclonal antibody) has recently been demonstrated to have superior efficacy when compared with sorafenib monotherapy. The remarkable efficacy has made this combination therapy the new standard treatment for advanced HCC. In addition to MKIs, many other molecularly targeted therapies are under investigation, some of which have shown promising results. Therefore, in the era of immuno-oncology, there is a significant rationale for testing the combinations of molecularly targeted therapies and ICIs. Indeed, numerous preclinical and clinical studies have shown the synergic antitumor efficacy of such combinations. In this review, we aim to summarize the current knowledge on the combination of molecularly targeted therapies and immune checkpoint therapies for HCC from both preclinical and clinical perspectives.

Keywords: combination therapies, hepatocellular carcinoma, immune cells, immune checkpoint inhibitors, molecularly targeted therapies

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Introduction

Liver cancer is one of the most common cancers and the fourth leading cause of cancer death globally, and its burden continues to rise steadily.[1,2] Hepatocellular carcinoma (HCC) is the most common primary liver cancer, accounting for approximately 90% of all liver cancer cases.[3] Molecularly targeted therapies, rather than chemotherapy, play crucial roles in the systematic therapy of unresectable HCC. The theory behind molecularly targeted therapies is that some molecular alterations function importantly during cancer development, growth, and metastasis, and may be potential targets for cancer treatment. For HCC, the most important molecular target is the vascular endothelial growth factor (VEGF) and its receptor (VEGFR). Despite the extensive attempts at new drug development for HCC, sorafenib [multikinase inhibitor (MKI) of VEGFR] remained the sole, effective systemic therapy for nearly a decade (2007–2016) since its approval.[4] In 2017, the situation changed due to the approval of regorafenib (MKI of VEGFR) as a second-line treatment for HCC patients who progressed on sorafenib treatment.[5] Since then, numerous clinical trials on MKIs of VEGFR have yielded positive results, leading to the approval of lenvatinib[6] as first-line treatment and cabozantinib[7] and ramucirumab[8] as subsequent-line treatments. In addition, there are many promising molecularly targeted agents currently being studied in clinical trials, such as transforming growth factor beta (TGF-β) inhibitors,[9,10] MET inhibitors,[11] fibroblast growth factor receptor 4

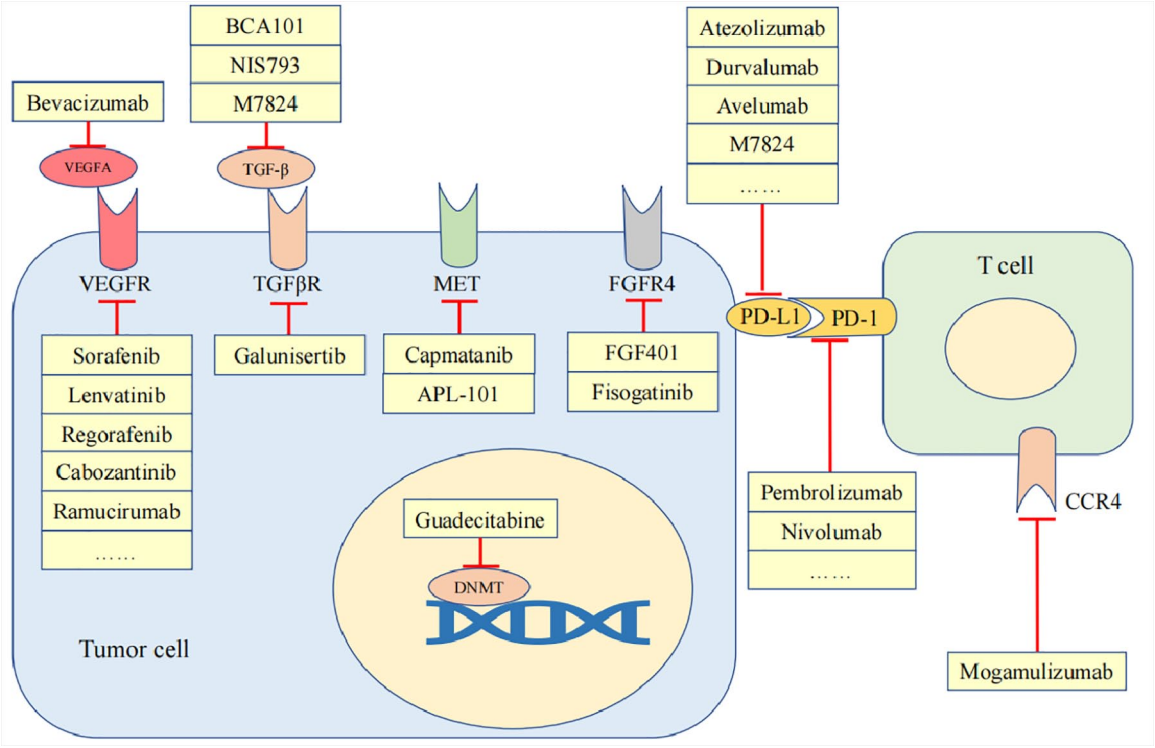

Figure 1. Molecularly targeted therapies and immune checkpoint inhibitors for hepatocellular carcinoma and their targets.

(FGFR4) inhibitors,[12] and other similar molecular inhibitors.

Over the past decade, immune checkpoint blockade therapies targeting programmed cell death protein 1 (PD-1), programmed cell death protein ligand 1 (PD-L1), and cytotoxic T lymphocyte antigen 4 (CTLA-4) have made tremendous breakthroughs that have revolutionized the treatment of various cancers. Anti-PD-1 monotherapy has been approved for the treatment of advanced HCC in both first- and second-line settings based on clinical study data.[13–18] However, in HCC, the overall response rate (ORR) to anti-PD-1 monotherapy was only 15–20% and, more importantly, the overall survival (OS) did not significantly improve.[13–18] Given this data, researchers are currently making great efforts in improving the therapeutic efficacy of immune checkpoint inhibitors (ICIs) for HCC, including combining ICIs with other molecularly targeted therapies. Recently, on the basis of promising results of the IMbrave150 study, the combination of atezolizumab (anti-PD-L1 antibody) and bevacizumab (anti-VEGF monoclonal antibody) has been approved as a novel first-line treatment for advanced HCC.[19] The final analysis of the clinical trial demonstrated that this combination resulted in significantly better OS and progression-free survival (PFS) than sorafenib monotherapy in patients with advanced HCC.[19] This exciting result also suggests the promising future of combining molecularly targeted therapies and immune checkpoint blockade therapies in the treatment of advanced HCC.

Currently, there are an increasing number of studies evaluating various combination strategies of molecularly targeted therapies and immune checkpoint therapies for HCC. Although most combination strategies lack robust evidence at present, some have shown promising efficacy in animal models or clinical studies. In this review, we focus on the current knowledge of combining molecularly targeted therapies and immune checkpoint therapies in HCC and provide an outlook on the future of such combination therapies. For molecularly targeted therapies, we will mainly focus on VEGF/VEGFR inhibitors, TGF-β inhibitors, MET inhibitors, FGFR4 inhibitors, and epigenetic drugs, which have showed promising results in clinical studies (Figure 1; Table 1).

Table 1. Clinical trials testing various combinations of molecularly targeted therapies and ICIs in HCC.

Trial identifier	Setting	Treatment	Phase	*n*	Primary endpoints	Status
ICIs + VEGF/VEGFR inhibitors						
NCT02715531	Solid tumors; first line	Atezolizumab + bevacizumab	Ib	430	PFS, ORR, safety	Recruiting
NCT04180072	Advanced HCC; first line	Atezolizumab + bevacizumab	II	48	ORR	Recruiting
NCT04563338	Unresectable HCC and NSCLC with liver metastases; first or second line	Atezolizumab + bevacizumab	II	36	PFS	Recruiting
NCT03434379	Advanced HCC; first line	Atezolizumab + bevacizumab *versus* sorafenib	III	480	OS, PFS	Completed
NCT04487067	Unresectable HCC; first line	Atezolizumab + bevacizumab	III	150	Safety	Recruiting
NCT04732286	Unresectable HCC; first line	Atezolizumab + bevacizumab	III	100	Safety	Recruiting
NCT04393220	Advanced HCC; first line	Nivolumab + bevacizumab	II	60	OS, PFS	Recruiting
NCT02519348	Advanced HCC; first or second line	Durvalumab + bevacizumab	II	433	Safety	Recruiting
NCT04605796	Advanced HCC; first line	Toripalimab + bevacizumab	II	60	ORR, safety	Recruiting
NCT04723004	Advanced HCC; first line	Toripalimab + bevacizumab *versus* sorafenib	III	280	OS, PFS	Recruiting
NCT04741165	Advanced HCC; first line	HX008 + bevacizumab/lenvatinib	II	72	ORR	Recruiting
NCT03211416	Advanced HCC; first or second line	Pembrolizumab + sorafenib	Ib/II	27	ORR	Recruiting
NCT03439891	Advanced HCC; first line	Nivolumab + sorafenib	II	40	ORR, safety	Recruiting
NCT02988440	Advanced HCC; first line	PDR001 + sorafenib	Ib	20	Safety	Completed
NCT04069949	Unresectable HCC; first line	Toripalimab + sorafenib	I/II	39	PFS, safety	Recruiting
NCT04163237	Advanced HCC; first line	PD-1 + sorafenib *versus* sorafenib	III	50	DFS	Recruiting
NCT04770896	Unresectable HCC; second line	Atezolizumab + lenvatinib/sorafenib *versus* lenvatinib/sorafenib	III	554	OS	Recruiting
NCT03006926	Advanced HCC; first line	Pembrolizumab + lenvatinib	Ib	104	ORR, DOR, safety	Recruiting
NCT04740307	Advanced HCC; first line	Pembrolizumab/quavonlimab + lenvatinib	II	110	ORR, safety	Recruiting
NCT03713593	Advanced HCC; first line	Pembrolizumab + lenvatinib *versus* lenvatinib	III	750	OS, PFS	Recruiting
NCT03418922	Advanced HCC; first or second line	Nivolumab + lenvatinib	Ib	30	Safety	Recruiting
NCT03841201	Advanced HCC; first line	Nivolumab + lenvatinib	II	50	ORR, safety	Recruiting
NCT04443309	Advanced HCC; first line	SHR-1210 + lenvatinib	I/II	53	ORR	Recruiting

(continued)

Table 1. (continued)

Trial identifier	Setting	Treatment	Phase	*n*	Primary endpoints	Status
NCT04542837	Advanced HCC; first or second line	KN046 + lenvatinib	II	30	ORR	Recruiting
NCT04401800	Unresectable HCC; first line	Tislelizumab + lenvatinib	II	66	ORR	Recruiting
NCT04444167	Advanced HCC; first line	AK104 + lenvatinib	Ib/II	30	ORR	Recruiting
NCT04728321	Advanced HCC; first line	AK104 + lenvatinib	II	75	ORR	Recruiting
NCT04368078	Advanced HCC; first or second line	Toripalimab + lenvatinib	IIb	76	ORR	Recruiting
NCT04523493	Advanced HCC; first line	Toripalimab + lenvatinib *versus* lenvatinib	III	486	OS, PFS	Recruiting
NCT04194775	Advanced HCC; first line	CS1003 + lenvatinib *versus* lenvatinib	III	525	OS, PFS	Recruiting
NCT03475953	Solid tumors; first or second line	Avelumab + regorafenib	Ib/II	362	ORR, safety	Recruiting
NCT03347292	Advanced HCC; first line	Pembrolizumab + regorafenib	Ib	57	Safety	Recruiting
NCT04696055	Advanced HCC; second line	Pembrolizumab + regorafenib	II	119	ORR	Recruiting
NCT04170556	Advanced HCC; second line	Nivolumab + regorafenib	I/IIa	60	Safety	Recruiting
NCT04310709	Advanced HCC; first line	Nivolumab + regorafenib	II	42	ORR	Recruiting
NCT04718909	Unresectable HCC; second line	Sintilimab + regorafenib	II	180	PFS	Recruiting
NCT04183088	Advanced HCC; first line	Tislelizumab + regorafenib	II	125	ORR, safety, PFS	Recruiting
NCT03170960	Solid tumors; first or second line	Atezolizumab + cabozantinib	Ib	1732	ORR, safety	Recruiting
NCT03755791	Advanced HCC; first line	Atezolizumab + cabozantinib *versus* sorafenib	III	740	OS, PFS	Recruiting
NCT03539822	GI cancers; first or second line	Durvalumab + cabozantinib	Ib	30	Safety	Recruiting
NCT04442581	Advanced HCC; first line	Pembrolizumab + cabozantinib	II	29	ORR	Recruiting
NCT04514484	Advanced solid tumors; first or second line	Nivolumab + cabozantinib	I	18	Safety	Recruiting
NCT02572687	GI or thoracic cancers; first or second line	Durvalumab + ramucirumab	I	114	Safety	Recruiting
NCT02942329	Advanced HCC or GC; first or second line	SHR-1210 + apatinib	I/II	60	OS	Completed
NCT03463876	Advanced HCC; first or second line	SHR-1210 + apatinib	II	190	ORR	Recruiting
NCT04014101	Advanced HCC; first or second line	SHR-1210 + apatinib	II	40	ORR	Recruiting

(continued)

Table 1. (continued)

Trial identifier	Setting	Treatment	Phase	*n*	Primary endpoints	Status
NCT03764293	Advanced HCC; first line	SHR-1210 + apatinib *versus* sorafenib	III	510	OS, PFS	Recruiting
NCT03825705	Advanced biliary adenocarcinoma/ HCC; first or second line	TQB2450 + anlotinib	Ib	60	ORR	Recruiting
NCT04052152	Advanced HCC; first or second line	Sintilimab + anlotinib	II	20	ORR, safety	Recruiting
NCT04172571	Advanced HCC; first line	AK 105 + anlotinib	Ib/II	30	ORR	Recruiting
NCT04344158	Advanced HCC; first line	AK 105 + anlotinib *versus* sorafenib	III	648	OS	Recruiting
NCT04612712	Advanced GI cancers; first or second line	KN046 + donafenib	I/II	42	ORR, safety	Recruiting
NCT04503902	Advanced HCC; first line	Toripalimab + donafenib	I/II	46	ORR, safety	Recruiting
NCT04472858	Advanced solid tumors; first or second line	CS1001 + donafenib	I/II	120	ORR, safety	Recruiting
NCT03289533	Advanced HCC; first line	Avelumab + axitinib	Ib	22	Safety	Completed
NCT03970616	Advanced HCC; first line	Durvalumab + tivozanib	Ib/II	42	Safety	Recruiting
NCT02856425	Solid tumors; first or second line	Pembrolizumab + nintedanib	Ib	18	Safety	Recruiting
NCT03941873	Unresectable HCC or GC; first or second line	Tislelizumab + sitravatinib	I/II	104	ORR, safety	Recruiting
NCT04601610	Advanced HCC; second line	KN046 + ningetinib	Ib/II	70	ORR, safety	Recruiting
NCT04560894	Advanced HCC; first line	SCT-I10A + SCT510 *versus* sorafenib	III	621	OS, PFS	Recruiting
NCT03973112	Advanced HCC; second line	HLX10 + HLX04	II	150	ORR	Recruiting
NCT04465734	Advanced HCC; first line	HLX10 + HLX04 *versus* sorafenib	III	477	OS, PFS	Recruiting
NCT04072679	Advanced HCC; second line	Sintilimab + IBI305	Ib	50	Safety	Completed
NCT03794440	Advanced HCC; first line	Sintilimab + IBI305 *versus* sorafenib	II/III	595	OS, PFS	Recruiting
ICIs + TGF-β inhibitors						
NCT04429542	Advanced solid tumors; first or second line	Pembrolizumab + BCA101	I	292	Safety	Recruiting
NCT02423343	Solid tumors; second line	Nivolumab + galunisertib	Ib/II	75	Safety	Completed

(continued)

Table 1. (continued)

Trial identifier	Setting	Treatment	Phase	*n*	Primary endpoints	Status
NCT02947165	Solid tumors; first or second line	PDR001 + NIS793	I	120	Safety	Recruiting
NCT02517398	Solid tumors; first or second line	M7824 (PD-L1 and TGF-β inhibitors)	I	600	ORR, Safety	Recruiting
NCT02699515	Solid tumors; first or second line	M7824 (PD-L1 and TGF-β inhibitors)	I	114	Safety	Recruiting
ICIs + MET inhibitors						
NCT03655613	Advanced HCC and RCC; first or second line	Nivolumab or APL-501 + APL-101	I/II	119	Safety	Recruiting
NCT02795429	Advanced HCC; second line	PDR-001 + capmatanib	Ib/II	90	ORR, safety	Recruiting
ICIs + FGFR4 inhibitors						
NCT02325739	Solid tumors; first or second line	PDR001 + FGF401	I/II	172	ORR, safety	Completed
NCT04194801	Advanced HCC; first or second line	CS1001 + fisogatinib	Ib/II	52	ORR, Safety	Recruiting
ICIs + epigenetic drugs						
NCT03257761	Solid tumors; second line	Durvalumab + guadecitabine	Ib	90	ORR, safety	Recruiting

DOR, duration of response; FGFR4, fibroblast growth factor receptor 4; GC, gastric cancer; GI cancers, gastrointestinal cancers; HCC, hepatocellular carcinoma; ICI, immune checkpoint inhibitor; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein ligand 1; PFS, progression-free survival; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Selection of clinical trials

To include clinical trials on the combination of molecularly targeted therapies and immune checkpoint therapies in HCC, we first searched the https://clinicaltrials.gov website by using the terms "hepatocellular carcinoma" and "drug." A total of 1367 interventional trials were identified and further screened until February 2021. Trials were included only if they met the following criteria: (1) included patients with advanced or unresectable HCC; (2) included combination therapies of molecularly targeted therapies and immune checkpoint therapies; and (3) specific classes of molecularly targeted therapies, including VEGF/VEGFR inhibitors, TGF-β inhibitors, MET inhibitors, FGFR4 inhibitors, and epigenetic drugs. Studies were excluded if they met any of the following criteria: (1) combination therapies, such as neoadjuvant or adjuvant therapy; (2) combinations of molecularly targeted therapies and immune checkpoint therapies with other therapies; (3) have already been terminated or withdrawn; and (4) contained insufficient required information. The included clinical trials are summarized in Table 1.

VEGF/VEGFR inhibitors

HCC is a hypervascularized solid tumor in which angiogenesis plays a critical role in cancer development, proliferation, and metastasis.[20] VEGF/VEGFR inhibitors that modulate angiogenesis, including sorafenib, regorafenib, lenvatinib, cabozantinib, and ramucirumab, currently dominate the approved systemic therapies for advanced HCC. As for bevacizumab, a monoclonal anti-VEGF antibody with a long history, there is also some evidence, from clinical trials, indicating its potential efficacy in HCC.[21,22] With the encouraging results of ICIs in HCC and the mounting evidence supporting the synergic effects of anti-angiogenesis and immunotherapy, the potential of combining anti-VEGF/VEGFR drugs and ICIs in the treatment of advanced HCC has been investigated by plenty of animal and clinical studies.

In addition to promoting angiogenesis and increasing vessel permeability, the VEGF signaling pathway has also been found to play a crucial role in cancer immunity.[20,23–25] In general, several mechanisms have been postulated for the immunosuppressive activity of the activation of the VEGF signaling pathway. Pathological angiogenesis generates abnormal vessels that may restrict

the infiltration of immune cells into tumors.[23] The upregulated level of VEGF could also result in the immunosuppressive tumor microenvironment (TME) by inhibiting the trafficking, proliferation, and effector function of cytotoxic T lymphocytes (CTLs) as well as the maturation and antigen presentation of dendritic cells (DCs). This, in turn, promotes immunosuppressive cell recruitment and proliferation, leading to a hypoxic and low-pH TME.[23] Thus, inhibition of the VEGF–VEGFR interaction could not only normalize vasculature but also enhance antitumor immunity.[20] The addition of ICIs could further prevent the immune evasion of tumors and has a potential synergic effect with VEGF/VEGFR inhibitors.[20] Recently, Shigeta *et al.*[26] tested dual anti-PD-1/VEGFR-2 therapy in orthotopic murine models of HCC. They found that an anti-PD-1 antibody and an anti-VEGFR-2 antibody synergized in vessel normalization and immune microenvironment reprogramming, overcoming resistance to any of the individual treatments and thereby enhancing tumor elimination.[26]

Recently released data from the IMbrave150 study demonstrated that atezolizumab plus bevacizumab resulted in higher ORR and prolonged OS and PFS when compared with sorafenib monotherapy.[19] There are a number of clinical trials focusing on various combination regimens of VEGFR inhibitors and ICIs currently underway (Table 1). Here we will review these clinical studies and mainly focus on those with preliminary results.

Bevacizumab

After bevacizumab treatment, CD8+ T-cell proliferation is significantly increased while the presence of PD-L1+ tumor cells, PD-1+ cells, regulatory T (Treg) cells, and tumor-associated macrophages (TAMs) is significantly decreased in glioblastoma tissues.[27] Bevacizumab could also increase CD8+ T-cell populations in tumor, reduce Treg cell populations in the blood, and sustain the circulation of the effector T cells.[28–30] *In vitro* and *in vivo* administration of bevacizumab was shown to inhibit VEGF-mediated increases in Treg cells.[31] Bevacizumab treatment resulted in a significant reduction of myeloid-derived suppressor cells (MDSCs), thereby restoring effective antitumor immunity.[32–34] Previous studies also found that bevacizumab could abrogate the inhibition of monocytes differentiation into DCs.[35–37] Bevacizumab was shown to enhance the maturation and number of DCs in the peripheral blood of patients with lung, breast, and colorectal cancers.[38] Moreover, atezolizumab in combination with bevacizumab enhanced antigen-specific T-cell migration in metastatic renal cell carcinoma (RCC).[39] Similarly, in patients with melanoma, a significant increases in circulating CD4+ and CD8+ T cells occurred with ipilimumab plus bevacizumab *versus* ipilimumab alone.[40] To summarize, bevacizumab has significant effects on a variety of immune cells and contributes to the restoration of an immunosupportive TME.

These findings provide important rationales for the combination of bevacizumab and ICIs in cancer treatment. As previously mentioned, bevacizumab plus atezolizumab has recently become a new first-line treatment for advanced HCC (NCT03434379).[19] Other clinical trials are also testing its efficacy (NCT02715531, NCT04180072, NCT04563338, NCT04487067, and NCT04732286).[41] Other combinations of bevacizumab and ICIs, for instance, bevacizumab plus nivolumab (NCT04393220), bevacizumab plus durvalumab (NCT02519348), bevacizumab plus toripalimab (NCT04605796, NCT04723004), and bevacizumab plus HX-008 (NCT04741165) are also under investigation in clinical trials.

Sorafenib

Sorafenib can decrease suppressive immune cell populations, including Treg cells and MDSCs, and enhance functions of tumor-specific effector T cells.[42–44] Sorafenib can also restore classical macrophage polarization in the TME of HCC, which correlates with a reduction in tumor burden.[45,46] Sorafenib may also inhibit macrophage-induced hepatocarcinoma growth by acting on various targets.[47,48] Similar to bevacizumab, sorafenib could abrogate the differentiation of human monocytes into DCs.[35] In mouse models of various cancer types, sorafenib induced increased infiltration of CD4+ and CD8+ T cells into tumors.[44,49–51] By contrast, in HCC mouse models or studies using peripheral blood mononuclear cells (PBMCs), the number and function of CD4+ and CD8+ T cells may be depressed directly by sorafenib or indirectly by the elevation of MDSCs and Treg cells following sorafenib treatment.[52,53] For natural killer (NK) cells, low-dose sorafenib may enhance NK cell effector functions, such as interferon gamma (IFN-γ)/tumor necrosis factor alpha (TNF-α) production,

degranulation, and lytic functions,[46,54] whereas high-dose sorafenib generally inhibits NK cell proliferation and function.[55–58] On experimental models of HCC, sorafenib in combination with nivolumab showed a stronger tumor growth inhibition as opposed to sorafenib or nivolumab monotherapy.[59]

Therefore, it is reasonable to test the combination of sorafenib and ICIs in clinical studies. Clinical trials of combined efficacy of sorafenib plus pembrolizumab (NCT03211416), nivolumab (NCT03439891), PDR001 (NCT02988440), toripalimab (NCT04069949), and atezolizumab (NCT04770896) are now ongoing.

Lenvatinib

In murine models of diverse cancers, lenvatinib monotherapy was found to increase the population of CD8+ T cells in TME and this effect was further enhanced when combined with an anti-PD-1 antibody.[60–62] Lenvatinib enhanced the antitumor activity of PD-1 blockade by upregulating memory T-cell population and enhanced T helper type 1 (Th1) immune response.[63,64] It was also found that the antitumor activity of lenvatinib was associated with enhanced tumor immune infiltration and activation of NK cells in HCC models.[65] More importantly, lenvatinib may decrease TAMs in RCC, colon cancer, and thyroid cancer tumor models, and exhibited synergistic antitumor effects in combination with anti-PD1 therapy.[64,66–68]

In a phase Ib clinical trial (NCT03006926), lenvatinib plus pembrolizumab was assessed as a first-line treatment in advanced HCC.[69] The combination therapy showed promising antitumor activity with an ORR of 36.0% [95% confidence interval (CI): 26.6–46.2%] and a tolerable safety profile.[69] Another phase Ib clinical trial of lenvatinib plus nivolumab, in patients with unresectable HCC, reported an ORR as high as 76.7% (95% CI: 57.7–90.1%) and manageable adverse events (NCT03418922).[70] A phase II trial of lenvatinib plus nivolumab in HCC is currently underway (NCT03841201).[71] A phase III trial aiming to compare the efficacy of the combination of lenvatinib and pembrolizumab with lenvatinib monotherapy, as first-line treatment for HCC, is also now underway (NCT03713593).[72] Other clinical trials on the combination of lenvatinib and ICIs are listed in Table 1.

Regorafenib

Regorafenib was previously reported to decrease macrophage accumulation in several murine models of colorectal cancer.[73,74] When administered in mice, regorafenib suppressed melanoma progression in a CD8+ T-cell-dependent manner.[75] Recently, regorafenib was found to promote tumor immunity by targeting the RET–Src axis to further inhibit JAK1/2–STAT1 and MAPK signaling as well as attenuate IFN-γ-induced PD-L1 and indoleamine 2,3-dioxygenase 1 (IDO1) expression.[76]

For HCC, early stage clinical trials of regorafenib plus avelumab (NCT03475953), pembrolizumab (NCT03347292, NCT04696055), nivolumab (NCT04170556, NCT04310709), sintilimab (NCT04718909), and tislelizumab (NCT04183088) are ongoing at present.

Cabozantinib

Cabozantinib rendered tumor cells more sensitive to immune-mediated killing and contributed to a more permissive immune environment in experimental study.[77] Cabozantinib treatment also resulted in a significant reduction of MDSCs in mouse models.[34] In clinical trials, cancer patients treated with cabozantinib were found to have increased CD8+ T cells and decreased Treg cells and MDSCs.[78,79] Furthermore, cabozantinib was reported to trigger a neutrophil-mediated anticancer innate immune response that resulted in tumor clearance.[80] Cabozantinib could downregulate M1 macrophages to prevent bone metastasis of prostate carcinoma[81] and reverse c-MET-induced immunosuppressive PD-L1 upregulation.[82]

In one cohort from the CheckMate 040 study, the triple combination of nivolumab, ipilimumab, and cabozantinib led to an ORR of 26% with tolerable toxicity.[83] There are also ongoing clinical trials testing cabozantinib plus pembrolizumab (NCT04442581), nivolumab (NCT04514484), atezolizumab (NCT03170960, NCT03755791), and durvalumab (NCT03539822) for HCC patients.

Ramucirumab

Ramucirumab-containing therapies for gastric cancer (GC) patients displayed significantly increased CD8+ T-cell infiltration, decreased

Treg cell infiltration, and reduced PD-1 expression by CD8+ T cells.[84]

A phase Ib study (NCT02572687) showed that ramucirumab plus durvalumab yielded increased antitumor activity without unexpected toxicity in HCC patients, especially those with high PD-L1 expression.[85]

Others

SHR-1210 (anti-PD-1 antibody) and apatinib combination therapy demonstrated an ORR of 50.0% (95% CI: 24.7–75.4%) in advanced HCC patients (NCT02942329).[86] A phase Ib trial (NCT03289533) evaluating avelumab plus axitinib in treatment-naive patients with HCC showed an ORR of 31.8% (95% CI: 13.9–54.9%).[87]

As shown in Table 1, many other combinations of anti-VEGF/VEGFR antibodies and ICIs are now under investigation in clinical trials.

TGF-β inhibitors

The TGF-β pathway has miscellaneous functions in cancers, including regulating cell growth, differentiation, apoptosis, motility and invasion, extracellular matrix production, angiogenesis, and immune response.[88,89] In HCC, TGF-β signaling has been demonstrated to be significant in a subset of patients based on gene expression profiling.[90] It is found that TGF-β1, an isoform of TGF-β, can stimulate α3-integrin expression in noninvasive HCC cells at the transcriptional level, transforming them into a motile and invasive phenotype.[91] In an experimental model of hepatocyte transmigration using hepatic sinusoidal endothelial cells and malignant hepatocytes to mimic vascular invasion, TGF-β was shown to be crucially involved in blood vessel invasion of HCC cells. This suggests its key role in the dissemination and metastasis of HCC.[92] In addition, TGF-β was found to contribute to HCC invasion and metastasis by inducing FGFR4 expression through the extracellular-signal-regulated kinase (ERK) pathway.[93] TGF-β signaling also functions as a master regulator for immune cell proliferation, differentiation, development, and survival.[94,95] In the liver, TGF-β signaling plays a crucial role in regulating immune cells to maintain a balance between immune tolerance and activation[96–98] (Figure 2). TGF-β activity has been identified as essential for the pathogenesis of HCC, including the activation of cancer-associated fibroblasts (CAFs).[96–98]

The potent immunosuppressive function of Treg cells is a major hurdle in generating an effective antitumor response in HCC.[99] Tumor cells and all stromal cells, including MDSCs, TAMs, DCs, CAFs, hepatic stellate cells (HSCs), and liver sinusoidal endothelial cells (LSECs) modulate Treg cell activity in HCC *via* the TGF-β pathway. MDSCs play a critical role in tumor-induced liver immunosuppression by inhibiting NK cell functions through membrane-bound TGF-β and promoting Treg cell expansion.[100,101] TGF-β, in the TME, promotes the expression of T-cell immunoglobulin mucin receptor 3 (TIM3) on macrophages and facilitates the alternative activation of macrophages *via* TIM3, thereby promoting tumor growth *via* the nuclear factor-κB (NF-κB)-interleukin 6 (IL-6) axis.[102] TGF-β promotes the differentiation of M2 macrophages, which repress CD8+ T-cell, NK cell, and DC functions and increase Treg cell functions.[103] A subset of DCs selectively promote the proliferation of Treg cells in a TGF-β dependent manner.[104] TGF-β expressed and activated by cancer cells, or other cells in the TME, promote cancer progression through its effects on CAFs.[105] CAFs are important components of the HCC TME, generating cyclooxygenase-2 (COX2), IL-8, and other cytokines that, together, stimulate TAMs to release TNF-α and platelet-derived growth factor (PDGF).[96–98] HSCs are activated by TGF-β and induce immune tolerance *via* enhancing the expansion of MDSCs, attenuation of effector T-cell functions, and augmentation of Treg cells.[106,107] LSECs are the most efficient liver cell type in TGF-β-dependent Treg cell induction and produce the chemokine C-X-C motif chemokine ligand 16 (CXCL16) to control the accumulation of C-X-C motif chemokine receptor 6 (CXCR6) + NKT cells.[108,109]

TGF-β has been demonstrated to induce forkhead box P3 (Foxp3), a transcriptional factor expressed predominantly on Treg cells, through a Smad2/3-dependent mechanism.[110] Foxp3+ Treg cells are highly enriched in tumors of HCC patients and correspond to poorer clinical outcomes.[111] Treg cells also secrete TGF-β, as well as adenosine and IL-10, to suppress effector T cells, such as CD8+ CTLs,[112] which play a critical role in the antitumor immune response by the production of various effector molecules including IFN-γ, IL-2, and TNF-α.[113] CD28 is a

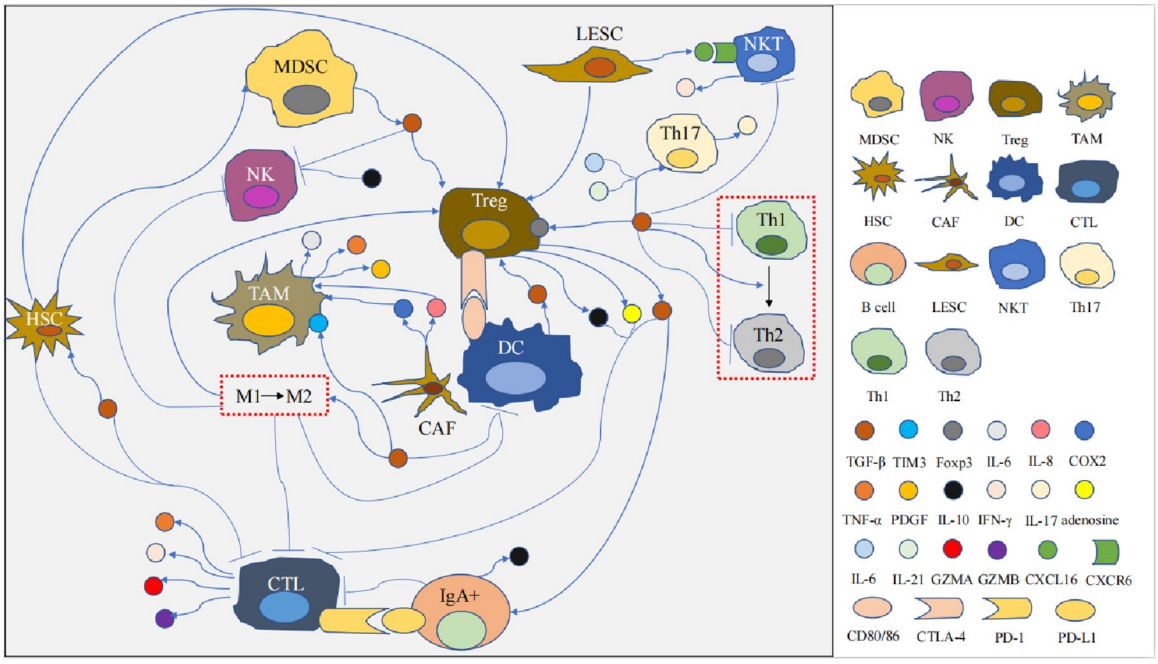

Figure 2. Interactions between TGF-β and immune cells in the tumor microenvironment of hepatocellular carcinoma.

co-stimulatory molecule that interacts with the CD80/CD86 complex on antigen-presenting cells (APCs). The immune checkpoint molecule expressed on Treg cells, CTLA4, can compete with CD28 to downregulate the CD80/CD86 complex through CTLA-4-mediated trans-endocytosis, thus inhibiting the antitumor immunity.[114,115] TGF-β has also been implicated in inducing Treg cell polarization.[116] Accordingly, inhibition of the TGF-β signaling decreased Treg cell infiltration in tumor tissues and induced HCC tumor regression.[116] Therefore, targeting Treg cells, such as by blocking the TGF-β signaling or the membrane-bound receptor CTLA-4, may be a particularly effective immune-based approach for improving the immune response against HCC.

IFN-γ is a cytokine that supports the proliferation and differentiation of myeloid cells and CD8+ T cells. In numerous immune cell types, TGF-β suppresses IFN-γ expression *via* the Smad pathway, thereby inhibiting the antitumor activity of CD8+ T cells.[117,118] Th1 and Th2 lineages produce cytokines that support the growth and functions of CD8+ cytotoxic T cells, B cells, and macrophages. TGF-β restricts the specification of Th1 and Th2 cells by repressing T-bet expression and inducing MSC and Sox4 expression, respectively.[118] Additionally, TGF-β was demonstrated to promote a shift of Th1 toward Th2 cell differentiation, resulting in a less efficient antitumor immune response.[119] Th17 cells are a proinflammatory Th cell subtype that expresses IL-17 and contribute to non-alcoholic steatohepatitis (NASH) and HCC.[120,121] Through the activation of Smad2/3, TGF-β cooperates with IL-6/IL-21 to positively regulate the generation of Th17 cells from naive CD4+ T cells.[120,121] Intratumoral TGF-β suppresses NKT cells, which are responsible for recruiting effector immune cells to the tumor by producing large amounts of IFN-γ.[122] NKG2D is one of the NK cell activating receptors. Active NKG2D can induce the activation, cytokine production, degranulation, and cytotoxic potential in NK cells.[123] Downregulation of NKG2D or upregulation of the inhibitory receptor NKG2A is associated with increased generation of immunosuppressive cytokines, such as TGF-β and IL-10, contributing to NK cell dysfunction in HCC.[124–129] Although TGF-β promotes chemotaxis of eosinophils and mast cells, it inhibits effector functions of NK cells and antigen presentation by DCs.[95,130] During B-cell maturation, TGF-β stimulates class switch recombination, which converts IgM-expressing B cells into IgA-expressing (IgA+) cells with regulatory activity.[131] IgA+ cells are involved in nonalcoholic fatty liver disease (NAFLD)-associated HCC by co-expressing PD-L1 and IL-10 and by restraining cytotoxic CD8+ T lymphocytes.[132]

TGF-β enhances antigen-induced PD-1 expression on tumor-infiltrating lymphocytes (TILs) through Smad3-dependent transcriptional activation.[133] And TGF-β induced epithelial–mesenchymal transition (EMT) increases PD-L1 expression in tumor cells.[134] The engagement of the co-inhibitory receptor PD-1 or its ligand PD-L1 blocks T-cell antigen receptor (TCR) signaling and inhibits T-cell proliferation and secretion of cytotoxic mediators, including granzyme A (GZMA), granzyme B (GZMB), TNF-α, and IFN-γ, which all collectively lead to T-cell exhaustion.[135,136] Thus, increased TGF-β signaling may allow tumors to evade host immune responses by upregulating PD-1 expression, and inhibition of TGF-β signaling may directly enhance antitumor immunity.[133] Tumors, particularly immune-excluded tumors that are intrinsically resistant to anti-PD-1/PD-L1 therapy, display elevated TGF-β signaling.[137–139] Indeed, anti-PD-1 treatment can induce a competing TGF-β-driven immunosuppressive program in a mouse model.[140] Together, these findings provide an important rationale for blocking the TGF-β signaling in order to circumvent the aggressiveness and resistance to therapies of HCC, especially in the era of immune-oncology.

Pharmacological agents targeting the TGF-β pathway, including receptor kinase inhibitors and neutralizing antibodies that inhibit the interactions of TGF-β ligands with their receptors, have already demonstrated promising antitumor efficacy in early clinical trials with an acceptable safety profile in a variety of cancers, including HCC.[141] Dual blockade of TGF-β signaling and PD-1/PD-L1 checkpoint successfully reduced TGF-β signaling in stromal cells, promoted T-cell infiltration into tumor centers, elicited vigorous antitumor immunity, and contributed to tumor regression in mouse models.[137,140,142–144] In addition, M7824, a bifunctional fusion protein consisting of an α-PD-L1 antibody moiety, based on avelumab linked to the extracellular domain of TGF-β receptor II, was developed.[145,146] This novel agent blocked signaling from the immune checkpoint PD-L1 surface protein and reduced TGF-β signaling within the TME by binding to all three TGF-β isoforms.[145,146] Its dual anti-immunosuppressive function led to the activation of the innate and adaptive immune systems, upregulation of PD-L1 levels in tumor cells, and induction of tumor regression in mouse models.[145,146] Another bifunctional fusion protein that blocks both TGF-β and CTLA-4 has also been developed, which was more effective in reducing tumor-infiltrating Tregs and inhibiting tumor progression compared with CTLA-4 antibody.[147] Early clinical trials of M7824 in patients with metastatic or locally advanced solid tumors, including HCC, have shown encouraging efficacy and a manageable safety profile (NCT02517398, NCT02699515) (Table 1).[148,149] A clinical trial of galunisertib (a systemic TGF-β receptor 1 inhibitor) in combination with nivolumab for HCC is ongoing (NCT02423343) (Table 1). Other clinical trials testing BCA101 (EGFR/TGF-β fusion monoclonal antibody) plus pembrolizumab (NCT04429542) or NIS793 (TGF-β inhibitor) plus PDR001 (spartalizumab, PD-1 inhibitor) (NCT02947165) are also ongoing (Table 1).

MET inhibitors

c-MET (MET) is a tyrosine kinase receptor with a single known ligand, hepatocyte growth factor (HGF). In HCC, overexpression of c-MET and HGF was noted in 20% and 33% of human samples, respectively.[150] The MET/HGF axis is involved in HCC progression by promoting cellular proliferation, survival, and invasion.[151,152] The MET/HGF signaling pathway is also associated with tyrosine kinase inhibitor resistance,[153,154] as patients with plasma HGF concentrations above 3279.1 pg/ml derived no obvious benefit from sorafenib compared with placebo in the pivotal phase III SHARP trial.[155] Therefore, the MET/HGF pathway may be used as a potential therapeutic target for the treatment of HCC. Moreover, the MET/HGF pathway has complex immunomodulatory effects (Figure 3). It can act as an immunosuppressive stimulus by negatively affecting DCs and T lymphocytes, or as an immune-positive stimulus by promoting the recruitment of DCs, B cells, and T lymphocytes.[156–161]

To date, the potential interplay between the HGF/MET pathway and DCs has been extensively investigated. It is well known that DCs are involved in the presentation of tumor-associated antigens (TAAs) and the activation of CD4+ Treg cells that control CD8+ cytotoxic T-cell activity. Several studies have found that HGF is able to enhance this function and plays a positive role in the immune response.[156,162–164] However, it has also been shown that HGF can act as a potent negative regulator of DC function and can induce an increase of Treg cells, a decrease of IL-17-producing T cells,[156] and an increase in

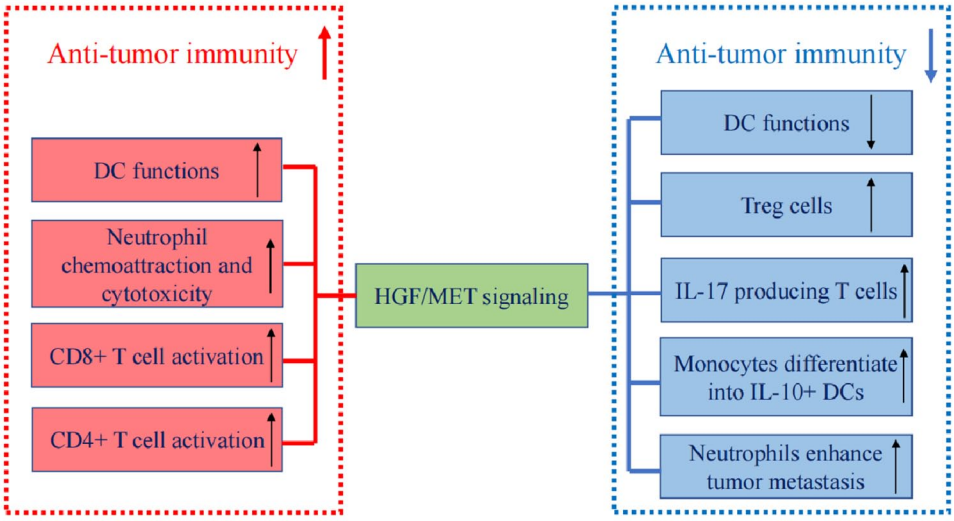

Figure 3. Immunomodulatory effects of HGF/MET signaling.

IL-10 and TGF-β, which are known for their immunosuppressive effects.[157] The inhibition of DC functions, together with the decrease of CD8+ T cells and the increase of Treg cells, leads to a decline of anticancer immunity.[156] Moreover, HGF favors the differentiation of monocytes into IL-10+ regulatory DCs. This inhibitory effect has also been demonstrated in monocytes, particularly with an induction toward differentiation into IL-10+ regulatory DCs.[165]

Besides APCs, the impact of HGF/MET signaling on granulocytes has also been highlighted in previous studies. MET is required for neutrophil chemoattraction and cytotoxicity, while its deletion in mouse neutrophils enhances tumor growth and metastasis.[166] This phenotype is associated with reduced neutrophil infiltration to both primary and metastatic tumors in clinical samples.[166] Mechanistically, tumor-derived TNF-α or other inflammatory stimuli can induce MET in neutrophils, which results in neutrophil transmigration across an activated endothelium and inducible nitric oxide synthase production, thereby promoting cancer cell killing and abating tumor progression.[166] This mechanism may partially explain the resistance to MET inhibitors in some patients, which is an important issue for clinicians in developing treatment strategies.[166] This example reveals the complex role of MET in tumors regarding its immunomodulatory effects, which is of great importance when testing its combination with immunotherapy. In HCC, tumor neutrophils actively enhanced the metastasis of malignant cells *in vitro* and *in vivo via* HGF/c-MET interaction.[167]

c-MET inhibition promoted perforin expression in CD8+ T cells and contributes to the activation of the immune system.[168] c-MET inhibition impaired the reactive mobilization and recruitment of neutrophils into tumors and draining lymph nodes, potentiating T-cell antitumor immunity.[169] Furthermore, in immunocompetent mice, the addition of MET inhibitors to immunotherapy increased the number of active T cells and changed their phenotype by reducing the proportion of exhausted T cells.[169] These results were independent of MET expression in the tumor models used, suggesting that c-MET inhibitor co-treatment may improve the response to cancer immunotherapy in settings beyond c-MET-dependent tumors.[169]

MET was identified as a broadly expressed TAA that can be recognized by CD8+ cytotoxic T cells, which then triggered activation of the immune system against cancer cells overexpressing MET.[170] Similarly, Kumai *et al.*[171] showed that MET expression itself behaves as a TAA, which was able to activate CD4+ T cells and to induce tumor cell killing in NK/T-cell lymphoma (NKTCL) cell lines. Particularly, in this model, MET elicited a specific antitumor immune response with three newly identified MET-induced T-cell epitopes. The activation of T cells was stronger in the presence of MET inhibitors since it caused a reduction of the synthesis of

TGF-β from tumor cells. Additionally, the presentation of MET-derived peptides by major histocompatibility complex class II (MHCII) to CD4+ T cells was influenced by chaperon processing and autophagy, thus proposing an innovative potential role of autophagy inducers as immune activators. Finally, since HGF/MET stimulation increases the proliferation of NKTCL cells *in vitro*, MET inhibition again displayed a dual role: direct tumor killing for MET-dependent cell survival and antitumor immune activation.[171]

PDL-1/2 expression was found to correlate significantly with c-MET expression in GC patients.[172] c-MET was shown to upregulate PD-L1 expression, contributing to immune escape of tumor cells in various cancers including GC, non small cell lung cancer (NSCLC), RCC, and HCC.[82,173–179] However, there are also some preclinical studies showing the opposite effect of c-MET in terms of the regulation of PD-L1 expression. MET-mediated phosphorylation and activated glycogen synthase kinase 3β (GSK3B) can lead to decreased expression of PD-L1.[180] Exposure of liver cancer cell lines to MET inhibitors increased their expression of PD-L1 and inactivated co-cultured T cells.[180] In combination with a MET inhibitor, anti-PD-1 and anti-PD-L1 produced additive effects to slow the growth of HCCs in mice.[180] Similarly, exposure of NCSLC cell lines to the c-MET inhibitor, tivantinib, increased their PD-L1 expression, which in turn caused cells to become more resistant to T-cell killing. This provides a rationale for the use of the combination therapy of c-MET inhibitors and ICIs in NSCLC.[181] Moreover, a novel bispecific antibody targeting both MET and PD-1 was developed and studied in multiple cancer cell-type models.[182,183] The antibody exhibited strong anti-proliferative and anti-metastatic effects *in vitro* and *in vivo* and reduced the production of inflammatory chemokines such as IL-6 and TNF-α, thus suggesting an important therapeutic potential, although it is still in the preclinical model stage.[182,183] Besides, clinical trial of APL-101 (c-MET inhibitor) plus nivolumab or APL-501 (PD-1 inhibitor) (NCT03655613) and capmatanib (MET inhibitor) plus PDR001 (NCT02795429) are recruiting patients (Table 1).

FGFR4 inhibitors

FGFR4 is the predominant FGFR expressed in the liver and has been identified as a promising target in HCC.[184] Fibroblast growth factor 19 (FGF19) can bind to and activate FGFR4 to induce hepatocyte proliferation.[185] The activation of the FGF19/FGFR4 pathway suppresses E-cadherin expression and hence promotes EMT, contributing to the aggressiveness of HCC.[186] FGF19 was significantly overexpressed in HCC specimens and was independently associated with tumor progression and poor prognosis in HCC patients.[187,188] Additionally, FGF19 was found to be strongly associated with the immune checkpoint signature (CD274, PDCD1, BTLA, CTLA4, HAVCR2, IDO1, and LAG3) in lymphoepithelioma-like HCC (LEL-HCC).[189] As previously described, FGFR4 expression was upregulated by TGF-β through the ERK pathway, in HCC cell lines, which contributed to the metastatic dissemination of HCC *in vivo*.[93] More importantly, FGF19/FGFR4 signaling plays an important role in the resistance of HCC to sorafenib,[190,191] making it of particular interest given the current widespread use of MKIs in HCC patients. A clinical trial testing FGF401 (FGFR4 inhibitor) plus PDR001 (NCT02325739), and another clinical trial testing fisogatinib (FGFR4 inhibitor) plus CS1001 (PD-1 inhibitor) (NCT04194801), in HCC, are both currently ongoing (Table 1).

Epigenetic drugs

Epigenetic events play a crucial role in tumor development, progression, and metastasis, providing another potential target for cancer treatment.[192–194] Owing to their effect on immune response and cancer immunity,[195] there are strong rationales for the potential combination of epigenetic drugs and ICIs in cancers. Epigenetic modifiers function importantly in priming and enhancing the therapeutic effect of the host immune system against cancer.[196,197] PBMCs and T-cell DNA methylation in HCC suggested that a broad molecular signature was involved in tumor progression, which was highly enriched in immune function-related genes such as PD-1.[198] Another study also found that highly upregulated DNA methyltransferases (DNMTs) were positively correlated with PD-L1 overexpression in sorafenib-resistant HCC cells.[199] Knockdown of PD-L1 induced DNMT1-dependent DNA hypomethylation and restored the expression of methylation-silenced Cadherin 1, a metastasis suppressor in HCC.[199]

Epigenetic drugs, such as DNMT inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi), were found to upregulate the expression of inhibitory immune checkpoints in either

immune or cancer cells, which enhance the response to immune checkpoint therapy.[196,197,200–207] DNMTi enhanced the therapeutic efficacy of PD-L1 blockade and increased tumor-infiltrating CD8+ T cells and Th1-type chemokine expression in ovarian cancer in C57/BL6 mice.[106] In a mouse model of fibrosis-associated HCC, the combination of i-BET762 (BET inhibitor) and anti-PD-L1 therapy was found to be able to repress monocytic MDSCs, enhance TILs, and lead to tumor eradication and prolonged survival.[208] The therapeutic effect of a HDACi belinostat in combination with immune checkpoint blockades (ICBs) was highlighted in a murine model of HCC, which was associated with enhanced IFN-γ production by antitumor T cells and a decrease in Treg cells.[209] Hong *et al.*[210] demonstrated that epigenetic therapy targeting EZH2 and DNMT1 could be a potential strategy to augment immunotherapy for HCC by stimulating T-cell trafficking into the TME. Guadecitabine, a second-generation DNMTi, showed synergic antitumor effects with immune checkpoint therapy and provided a rationale for such combination treatment.[211] As a result, a clinical trial of durvalumab plus guadecitabine (NCT03257761) for HCC patients is currently ongoing (Table 1).

Others

A phase I study (NCT02476123) of the anti-CCR4 antibody mogamulizumab in combination with nivolumab in patients with advanced or metastatic solid tumors, including HCC, demonstrated acceptable antitumor activity and safety profile.[212]

There are now also a lot of combination therapies of other molecular targeted therapies and immune checkpoint therapies for HCC under investigation, such as pembrolizumab plus bavituximab (NCT03519997), nivolumab plus SF1126 [phosphatidylinositol 3-kinase (PI3K) inhibitor] (NCT03059147), nivolumab plus copanlisib (PI3K inhibitor) (NCT03735628), nivolumab plus abemaciclib [cyclin-dependent kinase 4 (CDK4) inhibitor] (NCT03781960), nivolumab plus cabiralizumab [colony-stimulating factor 1 receptor (CSF1R) antagonist] (NCT04050462), nivolumab plus BMS-986253 (IL-8 inhibitor) (NCT04050462, NCT04123379), pembrolizumab/nivolumab/atezolizumab/avelumab plus ALT-803 [IL-15 receptor (IL-15R) agonist] (NCT03228667), nivolumab plus BMS-813160 (CCR2 antagonist) (NCT04123379), nivolumab plus CC-122 (NCT02859324), etc.

Conclusion

Plenty of novel agents are under development and investigation in the post-sorafenib era of advanced HCC treatment. Among them, molecular targeted drugs and ICIs are by far the most promising. Various combination strategies of molecular targeted therapies and immune checkpoint therapies are being tested and have generated some encouraging preliminary data. We are continuing to experiment with various therapeutic approaches to improve the clinical outcome of advanced HCC. One of the biggest challenges in order to optimize treatment outcomes is the development of predictive biomarkers for both monotherapies and combination therapies to accurately identify patients most likely to respond to particular treatments. In HCC, MKIs have been used for more than a decade and ICIs have been approved for several years, yet there are still no satisfactory biomarkers for these two therapies. For combination therapies, a better understanding of the mechanisms of synergistic therapeutic effects would aid in the design of more effective treatment regimens, such as dosing and sequencing strategies. Finally, the prevention and management of toxicities of combination therapies should be taken seriously in clinical practice.

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Author contributions

Conceptualization, ZLL, and JC; Methodology, ZLL, and JHL; Software, ZLL; Formal Analysis, ZLL and JHL; Investigation, ZLL; Writing – Original Draft Preparation, ZLL and JC; Writing – Review and Editing, JC and DS; Supervision, JC. All authors have read and agreed to the published version of the manuscript.

Conflict of interest statement

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