Ther Adv Gastroenterol
2017, Vol. 10(10) 805–814
DOI: 10.1177/1756283X17722061

Current immunotherapeutic strategies in hepatocellular carcinoma: recent advances and future directions

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Abstract: Hepatocellular carcinoma (HCC) is a common and serious health problem with high mortality. Treatment for HCC remains largely unsatisfactory owing to its high recurrence rates and frequent accompanying cirrhosis. In addition, the unique immune environment of the liver promotes tolerance, which, in conjunction with immune evasion by the disease, makes HCC a less promising target for conventional immunotherapy. However, recent advances in the immunotherapy have led to novel approaches to overcome these obstacles by manipulating and enhancing tumor-specific immune responses against HCC by using various modalities, such as cancer vaccines and immune checkpoint blockade. These treatments have shown both safety and promising outcomes in patients with HCC of various etiologies and tumor stages. Furthermore, combined strategies have been assessed to achieve optimal outcomes, by using immunotherapies with or without conventional treatments. This review briefly covers the background, recent advances, current issues, and future perspectives on immunotherapy in the field of HCC treatment.

Keywords: hepatocellular carcinoma, immunotherapy, clinical trial

Received: 25 March 2017; revised manuscript accepted: 20 June 2017.

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Hepatocellular carcinoma (HCC) is one of the most common cancers and is the third leading cause of cancer death worldwide.[1] However, despite remarkable advances in both the diagnosis and management of HCC, only 30–40% of HCC patients are eligible for potentially curative therapies, which include surgical resection, transplantation or percutaneous ablation. Moreover, patients almost inevitably experience recurrences even after potentially curative treatments.[2] Several adjuvant therapies have been attempted to improve the outcome of patients with early-stage HCC, showing less satisfactory results until recently.[3,4] For patients with intermediate-stage HCC, transarterial chemoembolization (TACE) offers survival advantages, although it is largely considered to be a palliative therapy and its use is often limited by the development of vascular invasion or extrahepatic spread.[5] Systemic therapies are indicated for a substantial portion of patients with advanced HCC. Sorafenib, which is an orally administered small molecule tyrosine kinase inhibitor, has been proven effective in patients with advanced HCC and has thus been approved as a first-line systemic therapy.[6] Further, regorafenib has recently demonstrated its efficacy as a second-line agent.[7] However, therapies for patients with intermediate- or advanced-stage HCC are largely less satisfactory. Recently, several immunotherapeutic agents including immune checkpoint blockade have shown promising results in patients with certain malignancies such as melanoma and non-small cell lung cancer; these agents have also been tested in patients with HCC.[8]

Immunologic characteristics of the liver and the basics of immunotherapy for hepatocellular carcinoma

Liver immunity and tolerance

The liver is known to be an immunologically privileged organ. The unique dual vasculature of the liver allows entrance of nutrients and pathogen-derived molecules from the portal vein. In the

face of constant and tremendous exposure to gut-borne pathogens, the immune effectors of the liver play a pivotal role in host defense and also constitute a unique immunologic milieu of self-tolerance.[9] This self-tolerance is organized *via* complex interactions among nonparenchymal cells such as liver sinusoidal endothelial cells (LSECs), Kupffer cells, dendritic cells, and lymphocytes. For example, LSECs, which also function as antigen-presenting cells (APCs), express high levels of programmed death-ligand 1 (PD-L1) and low levels of the costimulatory molecules CD80 and CD86, thereby resulting in limited activation of CD4+ and CD8+ T lymphocytes.[10] In addition, LSECs exhibit downregulated major histocompatibility complex (MHC) expression and mediate reduced activation of T cells by dendritic cells (DCs).[11] These tolerogenic mechanisms of LSECs result in decreased immune surveillance by the liver. One other component contributing to the development of immune tolerance is the action of Kupffer cells, stationary resident macrophages of the liver. Kupffer cells, which express low levels of MHC, promoted expansion of inhibitory forkhead box P3 (FoxP3)- and CD25-expressing regulatory T cells (Tregs).[12] The combined interactions of all of these cells induce tolerance, which in turn appears to act as a barrier to effective immunity against the development of liver cancer.

Immunosuppression in chronic liver inflammation

The liver is a unique organ in that it promotes tolerance rather than active, inflammatory immunity in order to avoid the chronic inflammation that would otherwise result from its constant exposure to antigens from portal venous blood. Immune tolerance in the liver, which is a physiologic phenomenon, might hinder an adequate immune response against tumor cells. With respect to cell-mediated immunity, the immune microenvironment facilitates T-cell exhaustion in the chronically inflamed liver, as is the case in viral or autoimmune hepatitis and steatohepatitis.[13] Optimal T-cell activation to eliminate pathogens or tumor cells requires well-organized ligand–receptor interactions between T cells and APCs *via* multiple stimulatory and inhibitory signals. These include interactions between cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and CD80 or CD86, programmed death 1 (PD-1) and PDL1 or PDL2, and T-cell immunoglobulin domain and mucin domain-3 (TIM-3) and galectin-9.[14] In recent studies, correlation was observed between PD-1–PDL1 interaction and the extent of hepatic inflammation, where treatment with anti-PD-1 or anti-PD-L1 antibody restored T-cell-mediated antiviral response in animal models of hepatitis B infection.[15] Other inhibitory molecules such as CTLA-4 are also overexpressed on CD8+ T cells in patients with chronic hepatitis B.[16] In chronic hepatitis C, both CTLA-4 and PD-1 contributed to T-cell exhaustion, which was reversed by combined PD-1/CTLA-4 blockade.[17] In short, these data suggest that an immunosuppressive environment with T-cell exhaustion results from chronic inflammation related to well-known HCC risk factors.

Immune evasion in hepatocellular carcinoma

Tolerance to tumor antigens develops from decreased recognition of tumor cells and functional suppression of the immune system, resulting in tumor progression.[18] Although immune evasion in HCC has not been fully elucidated, several mechanisms have been suggested. These include dysfunctions in antigen presentation and the resulting inadequate activation of immune effector cells, and altered immune checkpoint molecules and cytokine profiles. Failure of HCC-associated antigen presentation results from decreased expression of MHC class I molecules and ineffective tumor antigen processing.[19,20] Changes in the immune cell population in HCC include the emergence of CD14/CTLA-4+ regulatory DCs that suppress T-cell response; increases in Tregs, invariant natural killer T cells, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages; and diminished CD4+ T helper cells.[21–24] Of these, MDSCs disrupt immune surveillance by suppressing effector T cells, impairing natural-killer-cell function, and expanding Tregs, thereby contributing immune suppression in HCC.[22,25] In addition to suppression of T-cell response in hepatitis C, MDSCs are also involved in sustaining immune tolerance in hepatitis B.[26] A recent study demonstrated that neutrophil count was a predictor of MDSC levels as well as overall survival in HCC patients, suggesting higher neutrophil count as a potential indication for MDSC-targeted therapy.[27]

Dysregulated immune checkpoint molecules also perform an important role in immune evasion by

HCC. These molecules include CTLA-4, PD-1/PD-L1, lymphocyte-activation gene 3 (LAG-3), TIM-3, and galectin-9.[28–31] In particular, increased expression of PD-1 by CD8+ T cells was observed in patients with HCC, which was in turn associated with disease progression after curative hepatic resection.[32] Increased levels of immunosuppressive cytokines (IL-4, IL-5, IL-8, and IL-10) are associated with suppression of the immune activating cytokines IL-1, tumor necrosis factor (TNF), and interferon gamma (IFN-γ), thereby contributing to poor prognosis and aggressive tumor characteristics.[33] Increased levels of IL-10 in patients with advanced HCC was associated with immune dysfunction as well as shorter survival.[34]

Spontaneous immune responses in hepatocellular carcinoma

Although HCC is not considered an immunogenic tumor, spontaneous immune responses do occur against tumor-associated antigens (TAAs), including alpha-fetoprotein (AFP), glypican-3 (GPC3), melanoma antigen gene A (MAGE-A), and New York-esophageal squamous cell carcinoma-1 (NY-ESO-1).[35] TAA-specific T-cell responses correlated with improved outcomes in HCC patients, with longer survival and lower risk of recurrence after surgery in patients with tumors expressing multiple TAAs and TAA-specific CD8+ T-lymphocyte infiltrates.[36] In addition, decreased recurrence and improved survival have been reported after curative treatments including surgery, liver transplantation or radiofrequency ablation (RFA) in the presence of CD4+/CD8+ tumor-infiltrating lymphocytes and cytotoxic or activating killer-cell immunoglobulin-like receptor-positive natural killer (NK) cells.[37,38] Therefore, TAA-directed immune responses may be a promising therapeutic target.

Recent advances in immunotherapy in hepatocellular carcinoma

The aforementioned immunological features of HCC support immune tolerance induced by both the tumor and the organ itself. This immune tolerance in turn promotes disease progression instead of anticancer immunity and tumor control. Tumor-specific immune responses are mostly inadequate for the control of HCC because of the presence of Tregs, dysfunctional tumor-specific CD8+ T lymphocytes, and immune evasion. However, immunotherapeutic strategies have developed targeted immune responses that counterbalance the equilibrium between progression and control of the tumor. Here, we summarize recent reports on the representative types of immunotherapy for HCC.

Cancer vaccine for hepatocellular carcinoma

The fundamental goal of cancer vaccination is to induce tumor-specific immune responses by effector T lymphocytes in order to reduce tumor burden and prevent tumor relapse. DCs play pivotal roles in presenting TAAs to T cells instead of exerting direct effect on tumor cells.[39] However, DC-induced immune responses can be interrupted by tumor-produced cytokines such as IL-6, IL-10, or vascular endothelial growth factor, or by Tregs induced by immature DCs.[40] Additional measures for DC vaccines have been adopted to overcome the immunosuppressive effects of tumors, including use of mature DCs by *ex vivo* activation and adding strong stimuli for activation.[41] Until now, DC vaccines have been tested in patients with HCC in several studies with different designs, such as *ex vivo*-matured DCs pulsed with tumor lysate in patients with advanced HCC and adjuvant DC vaccines following microwave ablation or TACE.[42–44] Most recently, a phase I/IIA study using a multiple TAA (AFP, GPC-3, and MAGE-1)-pulsed adjuvant DC vaccine after primary treatment (surgery, ablation, or TACE) showed safety and extension of time to progression (TTP) in the vaccination group.[45] Collectively, studies using DC vaccines have shown safety with few adverse events and some promising results, yet warrant more examination.

Adoptive cell therapy

Adoptive cell therapies using tumor-infiltrating lymphocytes and T-cell receptor (TCR) grafting have drawn attention in the field of anticancer immunotherapy.[46] An important limitation of TCR-based immunotherapy is the fact that T cells that recognize TAA epitopes with high avidity are negatively selected during T-cell development because TAAs are usually self-proteins of fetal tissues. However, recent researches have focused on allo-restricted T-cell stimulation to overcome this tolerance phenomenon. For example, GPC3, which is expressed in approximately 75% of HCCs, is known to be associated with poor prognosis.[47] Using *in vitro* and murine xenograft models, Dargel and colleagues elegantly

identified GPC3-specific TCR, and demonstrated killing of GPC3-positive hepatoma cells by primary CD8+ T cells expressing the TCR.[48] They used DCs from HLA-A2-negative donors which were cotransfected with GPC3 and HLA-A2 RNA in order to stimulate and expand antigen-specific T cells to circumvent GPC3 tolerance. To date, however, human studies on the application of adoptive cell therapy using TCR are limited in the field of immunotherapy in HCC, and more experiences are required.

Cytokine-induced cells have shown promising results in the field of adoptive immunotherapy for HCC. Randomized trials with cytokine-induced killer (CIK) cells demonstrated survival benefit in patients with previous surgical resection or percutaneous ablation.[49–52] Collectively, these data suggest that CIK therapy seems to be more efficacious for patients with early-stage HCC than advanced HCC, and also, it requires validation in different patient populations, since most of the trials were conducted in Asia.[53]

Immune checkpoint blockade

Outstanding achievements with immune checkpoint inhibitors in several malignancies have been spotlighted recently, which has led to clinical trials of those therapeutics in patients with HCC, as summarized in Table 1.[8] (see Figure 1 which shows a case with complete response with immune checkpoint inhibitor).

Tremelimumab, a fully humanized IgG2 monoclonal antibody against CTLA-4, has been shown to be well tolerated, with promising outcomes in a phase II study.[54] The participants in this study comprised 20 patients with hepatitis C virus (HCV)-related HCC with 57.1% at an advanced stage, 42.9% with Child–Pugh class B, and 23.8% with prior sorafenib failure. The disease control rate was 76.4%, achieving partial response in 17.6% of the patients, and the median TTP was 6.48 months. Although transient grade 3 or 4 transaminitis frequently developed, no correlation was observed with circulating cytokines or HCV viral load. In fact, decreased viral load was shown, suggesting an antiviral effect of immune checkpoint blockade and possible usefulness in HCC patients with viral etiology.

More encouraging results have emerged from studies investigating anti-PD-1/PD-L1 blockade. Interim analysis of a phase I/II dose-escalation trial investigating nivolumab, a fully humanized IgG4 monoclonal antibody to PD-1, reported safety with a relatively lower rate of hepatotoxicity as well as promising efficacy. An overall objective response rate of 19%, including two complete responses, was reported for HCC patients with various etiologies (viral in half), 70% of whom had extrahepatic metastases and the majority of whom were pretreated with sorafenib.[56] Neither serious hepatic dysfunction nor autoimmune diseases were found. Notably, sustained response was observed in all patients, with disease control and an absence of developing progressive disease due to resistance. Objective responses were also observed in patients with nonviral etiology and the overall survival rate at 1 year was 62%. In 2017, results of a dose-escalation and expansion study of nivolumab in advanced HCC ($n = 262$) were presented (CheckMate 040), [ClinicalTrials.gov identifier: NCT01658878].[57] Briefly, treatment-related adverse events of grade 3–4 were observed in 25% of the study population. The objective response rate was 20% in 214 patients in the dose-expansion phase, with a median response duration of 9.9 months, and the disease control rate was 64%. The 9-month overall survival rate in the expansion phase was 74%. A global phase III randomized control trial of nivolumab *versus* sorafenib as first-line treatment in patients with advanced HCC (CheckMate 459), [ClinicalTrials.gov identifier: NCT02576509] is ongoing.[58]

There are two other ongoing studies investigating another anti-PD-1 humanized IgG4 antibody, pembrolizumab (KEYNOTE-224 and KEYNOTE-240), [ClinicalTrials.gov identifiers: NCT02702414 and NCT02702401].[59,60] The KEYNOTE-224 study is a single-arm, multisite, phase II study to evaluate the efficacy and safety of pembrolizumab in ~100 patients with previously treated, advanced HCC. The primary endpoint is objective response rate. The KEYNOTE-240 study is a randomized, double-blind, placebo-controlled phase III study in patients with previously-treated, advanced HCC, in which ~408 patients are randomly assigned 2:1 to receive pembrolizumab or placebo. Primary objectives are progression-free survival and increased overall survival.

Oncolytic viral therapy

In oncolytic viral therapy, viruses are designed to be preferentially replicated in cancer cells,

Table 1. Recent clinical trials of immune checkpoint inhibitors in hepatocellular carcinoma.

Drug	Trial/ClinicalTrials.gov identifier	Phase	Design	Outcome/status
Tremelimumab	NCT01008358[54]	I	Monotherapy; HCV ($n = 21$)	DCR 76.4%; TTP 6.48 months
	NCT01853618[55]	I	Combination with RFA, cryoablation or TACE; $n = 32$	PFS 57.1% at 6 months, 33.1% at 12 months; TTP 7.4 months; OS, 12.3 months
Nivolumab	CA209-040 [NCT01658878][56]	I/II	Monotherapy; HCV ($n = 12$), HBV ($n = 11$) or uninfected ($n = 24$); prior sorafenib in 68%	ORR 19%; DCR 67%
	CheckMate 040 [NCT01658878][57]	I/II	Cohort 1 (dose escalation), cohort 2 (dose expansion); $n = 262$	TRAEs (grade 3–4) 25%; ORR 20% (duration of response 9.9 months); 9-month OS 74%
			Cohort 3 (*versus* sorafenib)	Completed
			Cohort 4 (plus ipilimumab)	Completed
	CheckMate 459 [NCT02576509][58]	III	726 (nivolumab *versus* sorafenib)	Ongoing
Pembrolizumab	KEYNOTE-224 [NCT02702414][59]	II	~100 (monotherapy single arm)	Ongoing
	KEYNOTE-240 [NCT02702401][60]	III	408 (*versus* placebo)	Ongoing

Note: As of June 2017, available results of the abovementioned clinical trials were obtained from relevant conference proceedings or journal articles, and cited as references; otherwise, status is either completed or ongoing (according to ClinicalTrials.gov).
Abbreviations: DCR, disease control rate; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NCT, national clinical trial; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TRAE, treatment-related adverse event; TTP, time to progression.

thereby resulting in cancer cell lysis and triggering antitumor immunity. JX-594, an oncolytic and immunotherapeutic vaccinia virus, is engineered with inactivation of the thymidine kinase gene for cancer specificity, and insertion of human granulocyte-macrophage colony-stimulating factor and β-galactosidase transgenes for immune stimulation.[61,62] In a randomized, dose-finding phase II study in patients with advanced HCC, treatment with JX-594 demonstrated tolerable safety profile with no treatment-related death, and intrahepatic disease control was achieved in 50% at both low and high dose.[63] The overall survival was longer in the high-dose group than in the low-dose group (14.1 months *versus* 6.7 months, $p = 0.020$). Further studies on the synergistic or additive anticancer activity of JX-594 in combination with other agents are anticipated. A phase III randomized study comparing JX-594 followed by sorafenib *versus* sorafenib in patients with advanced HCC is under way [ClinicalTrials.gov identifier: NCT02562755]. Another fascinating strategy is the combination of oncolytic virus and immune checkpoint blockade, because immunosuppressive environment is restored and tumors relapse following the clearance of the oncolytic virus by the host immune response. Recently, a combination of oncolytic vaccinia virus and anti-CTLA-4 antibody showed enhanced tumor response in mouse models of renal and colorectal cancer.[64] Likewise, a phase I/IIa trial is ongoing to evaluate the safety and efficacy of the combination of JX-594 and nivolumab as the first-line treatment of in patients with advanced HCC [ClinicalTrials.gov identifier: NCT03071094].

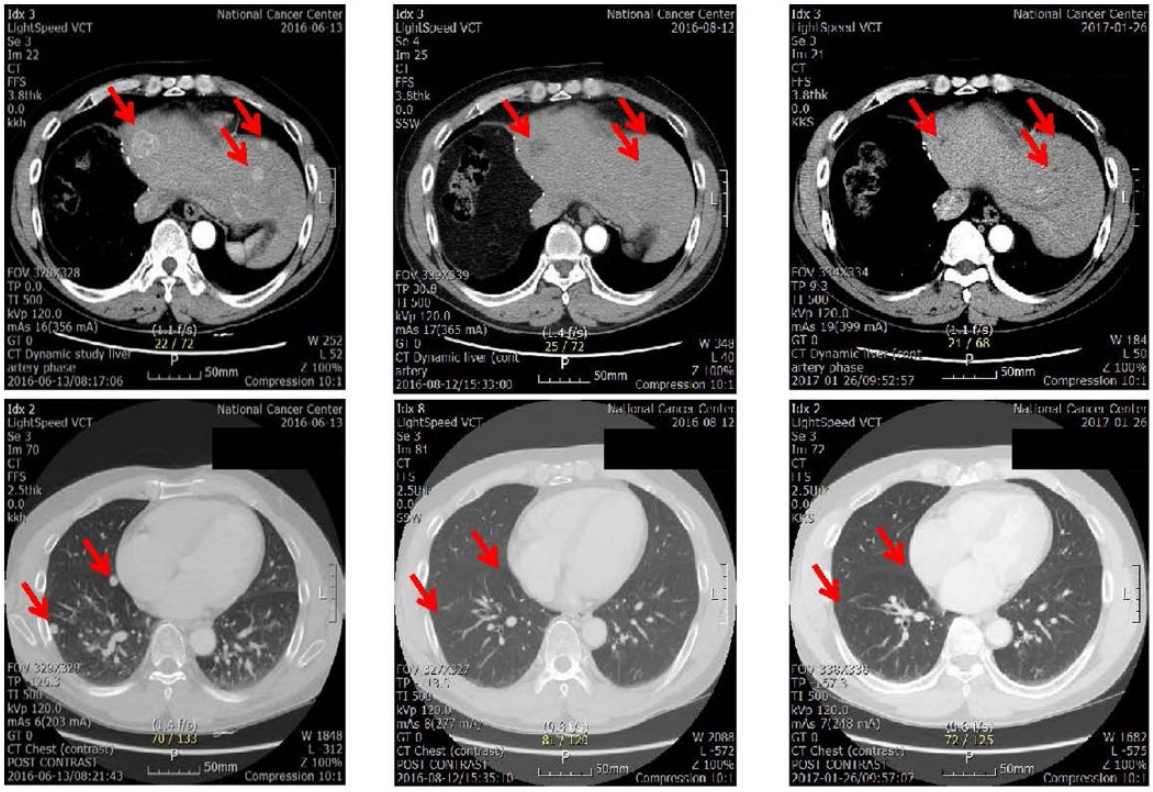

Figure 1. A case of immune checkpoint inhibitor treatment. A 58-year-old male patient had right lobectomy for a 17 cm-sized hepatocellular carcinoma and multiple recurrence in remained liver and metastasis in the lung developed after 4 months. He enrolled in a clinical trial with immune checkpoint inhibitor; 2 months later, computed tomography revealed complete remission in modified RECIST, a response that continued.

Can immunotherapy be useful in combination strategies to improve the outcomes of hepatocellular carcinoma patients?

Because recent studies of immunotherapy in HCC have shown some promising results with acceptable safety, it is reasonable to assess combined approaches such as immunotherapy plus conventional treatments or a combination of immunotherapeutic agents together.

Tumor stage-oriented treatment is a conventional strategy for patients with malignancies including HCC. Treatments with curative intent are provided to patients with early-stage HCC, including surgical resection, local ablation, or transplantation, and palliative treatments are administered to patients with intermediate or advanced-stage HCC, for example, TACE or sorafenib.[1] Tumor recurrence is the most troublesome issue in patients treated for early-to-intermediate-stage HCC with potentially curative treatments. The strategy of combined locoregional treatment with systemic treatment has been investigated in several clinical trials with various study designs. Despite some promising results in preliminary studies on the combination of locoregional treatment plus sorafenib, large-scale phase III trials failed to prove a definitive benefit of adjuvant sorafenib combined with surgical resection, RFA, or TACE.[65–67] With the advent of newer immune-based therapies, the theoretical advantages of combined locoregional therapy and immunotherapy such as immune checkpoint blockade have been postulated and studied. For example, RFA has been reported to upregulate tumor-cell expression and presentation of TAAs in animal models, suggesting the possibility of augmented efficacy when combined with immunotherapy to enhance tumor cell recognition by effector T cells.[68] A recent pilot study by Duffy *et al.* demonstrated intriguing results with combined ablation (RFA, cryoablation or TACE) and tremelimumab.[55] Progression-free survival rates were 57.1% at 6 months and 33.1% at 12 months in 32 patients (75% being Barcelona Clinic Liver Cancer stage

C), without observing any dose-limiting toxicities. The median TTP was 7.4 months and the median overall survival was 12.3 months (Table 1). Biopsies showed increases in CD8+ T cells in patients presenting clinical improvements. This study took a meaningful step forward in terms of exploring a combination approach using locoregional therapy and immune checkpoint blockade. However, whether locoregional therapy truly contributed to the outcome improvement remains unanswered due to the single-arm study design and the assessment of relative contribution of different treatment modalities in such combinations seems to be of critical relevance.

Sorafenib, an antiangiogenic, multitargeted tyrosine kinase inhibitor, is the current standard of care in patients with advanced HCC, yet provides limited survival advantages. Immunomodulatory effects of sorafenib have been observed in recent studies, for example, increased activation of effector T cells with blocking Treg function, and restoration of DC differentiation, amongst others.[69,70] Chen *et al.* reported that sorafenib-induced hypoxia promoted immunosuppression *via* upregulation of PD-L1 expression and Tregs in mouse models.[71] Stromal cell-derived 1 alpha (SDF1α) was also involved in the recruitment of immunosuppressive cells, with reversal of this phenomenon being achieved *via* inhibition of the SDF1α receptor (C-X-C receptor type 4), which in turn resulted in improved outcomes. More interestingly, greater antitumor activity was observed when a PD-L1 antibody was added to this combination to additionally induce a cytotoxic CD8+ T-cell response. Hence, further studies of combined treatment of antiangiogenic agents with immune checkpoint blockade are urgently warranted.

Will immunotherapy be a new game-changer in hepatocellular carcinoma?

HCC is a common malignancy worldwide and remains a serious global health problem as current standard treatments are largely unsatisfactory. Tumor recurrence and *de novo* carcinogenesis are major obstacles to improved outcomes. In addition, heterogeneity in the pathogenesis and biological behavior of HCC has led to failure of newer systemic agents to improve outcomes over sorafenib until very recently.[7] Immunotherapy has emerged into a spotlight in the treatment of various malignancies, showing efficacy through reduction of existing tumor burdens, as well as prevention of the development of new cancer. Moreover, conventional standard treatments also have immunomodulating potential, allowing logical anticipation of additive or synergistic effects in boosting anticancer immunity when combined with immunotherapies such as cancer vaccines or immune checkpoint blockade. The acceptable safety profiles of the immunotherapeutic agents examined in recent clinical studies support this expectation, albeit further investigation is necessary. Questions still remain regarding identification of the best patient population for immune-based treatments, considering the presence of HCC subclasses with distinct tumor biology.[72] In addition, disparity in responses to immunotherapy might result from various host or tumor factors such as etiology or concentration of checkpoint molecules or immune effectors in tumor microenvironment, which necessitate further investigation on potential biomarkers, as well. Hopefully, results of ongoing and forthcoming studies examining various combinations of immunotherapies with or without conventional therapies are anticipated with keen interest, toward the development of safe and more effective treatments for patients with this deadly disease.

Funding

This study was supported by a grant from the National Cancer Center, Korea (#1510520).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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