Am J Digest Dis 2015;2(2):76-89
www.ajdd.us /ISSN:2329-6992/AJDD0015964

Original Article

Immunotherapy with immunomodulatory antibody for hepatocellular carcinoma

Liang Huang

Department of Surgery, University of Iowa, Iowa City, IA, USA

Received September 10, 2015; Accepted November 23, 2015; Epub December 25, 2015; Published December 30, 2015

Abstract: Investigators have been interested in immunotherapy for hepatocellular carcinoma for decades. Immunotherapy represents a new effective treatment for hepatocellular carcinoma. Its recent breakthrough is largely attributable to the advances in understanding of anti-tumor immune responses, tolerance, regulation of anti-tumor immune and the advent of newer therapies, including checkpoint antibodies and agonist antibodies to the co-stimulatory molecules targeting T cells. With the success of immunotherapy such as ipilimumab and programmed death-1 pathway-targeted agents, more and more preclinical data have offered the basis of immunomodulatory antibody therapy as an important treatment option for hepatocellular carcinoma. In this article, I attempted to review and summarize the mechanisms, implication and future of immune checkpoint therapy and costimulatory molecules for T cells. I also discussed some ongoing clinical trials with a focus on immune checkpoint antibody.

Keywords: Hepatocellular cancer, immunotherapy, review, PD-1, PD-L1

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, with a high mortality [1]. Most of the HCC are a consequence of long-term hepatitis B virus and/or hepatitis C virus infection [2]. The majority of HCC are not resectable at the time of diagnosis. In patients received operation, few are cured. Only a modest improvement in survival was found with the addition of chemotherapy and radiation therapy. Surgery and local ablative therapies such as radiofrequency ablation (RFA) and ethanol injection are the available curative treatment options while transarterial chemoembolization (TACE) and selective intra-arterial radiofrequency ablation and systemic chemotherapy are considered as palliative treatment in patients with more advanced disease [3]. For the patients who undergo resection alone, the overall 5-year survival rate remains poor. Thus, it is imperative to integrate alternative strategies to improve disease control and patient survivals. With the advances in understanding of the mechanism of immune tolerance, and regulation of anti-tumor immunity and the advent of targeted therapies, immunotherapy represents a new effective treatment for HCC patients.

Immunotherapy of HCC includes vaccines adoptive T cell therapy, cytokines and immunomodulatory antibodies. The HBV vaccine led to a decreased incidence of HBV-related HCC [4]. The vaccine and passive transfer of anti-cancer monoclonal antibodies and donor T cells are only modest effective treatments for HCC. Other available systemic treatment options for patients with HCC are sorafenib, the programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) inhibitors. Sorafenib has established as a promising treatment option for advanced HCC [5].

Several studies have shown specific $CD8^+$ T-cell responses against tumor-associated antigens (TAAs) in HCC patients and a clinical benefit of T-cell infiltration in the tumor tissue. However, the impact of TAA-specific $CD8^+$ T-cell responses on tumor control seems rather weak [6].

Given the success of immune checkpoint blockade with anti-cytotoxic T lymphocyte associated antigen 4 (CTLA-4) antibodies in melanoma and

other solid tumors [7, 8], it has become possible to incorporate various immunotherapeutic strategies into the plan of treatment. These immune therapies enable reprogramming of the immune system to allow effective recognition and killing of HCC cells. Activating the immune system for therapeutic benefit in HCC has become reality. Immunotherapy targeting HCC has proved its efficacy in halting progression of HCC and the survival of patients and it has emerged as a promising treatment strategy [9]. Continued advances in antibody development and T cell engineering should further help improve clinical outcomes.

Immunotherapy enhancing tumor-specific immune responses is currently under active investigation in HCC research. We reviewed and summarized the recent advances of the immune responses in HCC and the relationship of immune checkpoints. We will discuss currently ongoing and recent clinical trials and preclinical experiments aiming to enhance anti-tumor immunity by blocking immune inhibitory mechanisms or by providing co-stimulatory signals to immune system.

Tumor associated antigen (TAA) and immune checkpoint antibody in HCC

Many HCC-specific $CD8^+$ T cell responses against tumor associated antigens (TAA) have been identified in HCC patients [10, 11] and it is crucial to the development of immunotherapy. These TAAs include alpha-fetoprotein (AFP) [11, 12], NY-ESO-1 [13-15], synovial sarcoma X breakpoint 2 (SSX-2) [13, 14], telomerase reverse transcriptase (TERT) [10, 16], melanoma antigen gene-A (MAGE-A) [13], glypican-3 (GPC3) [17, 18], and cyclophilin B40 [10].

AFP is an oncofetal antigen primarily expressed in the fetal liver, yolk sac, and gastrointestinal tract, which is also elevated in hepatitis and HCC. GPC-3 is another fetal oncoprotein expressed in over 70% of HCCs. NY-ESO-1, SSX-2, and MAGE-A are Cancer/testis antigens. NY-ESO-1 is one of the most immunogenic TAAs expressed in melanoma, HCC, breast cancer and ovarian cancer [6]. TERT is involved in telomere elongation and human TERT-specific $CD8^+$ T-cell responses have been identified in HCC patients [16]. All these TAAs have been shown to elicit specific $CD8^+$ T-cell responses in HCC patients [11-16, 18-20].

However, these TAA specific $CD8^+$ T cell responses are very weak [6]. Potentiation of TAA-specific immune responses seems to be able to be achieved by application of multiple immune checkpoints blockade therapy [21]. It seems attractive and promising and may improve HCC control and suppress tumor regression. Moreover, there are many specific neo-antigens for individual tumors [22]. The checkpoint blockade cancer immunotherapy targeting these tumor-specific mutant antigens may also play an important role in future immunotherapy [21, 23]. These tumor-specific mutant antigens can also be incorporated into synthetic peptide vaccines and become personalized vaccines. Neoantigen-specific T cell reactivity could become successful cancer immunotherapies according to the data obtained over the past few years. The boosting of neoantigen-specific T cell immunity with personalized immunotherapies opens a new area and will further help control the HCC [24].

Immune suppression mechanisms in liver

A few mechanisms of immune suppression have been proposed, including immunoediting; defect of tumor antigen processing and presentation; inhibitory receptors expression on tumor-specific T cells; priming defects. A better understanding of the mechanisms of immune dysfunction in HCC will allow the development of new immunotherapeutic strategies for improved clinical benefit. Overcoming these mechanisms could induce or boost TAA-specific $CD8^+$ and $CD4^+$ T cell responses.

Natural immunosuppression and chronic hepatic inflammation induced immunosuppression

Most pathogens and antigens from the intestine and blood can be eliminated by immune system in liver and gut. However, it is agreed that pathogens (including HBV and HCV) can escape immune attack and persist in liver, and liver has developed immune tolerance toward some antigens to avoid autoimmune damage and maintain organ integrity [25, 26]. One mechanism accounting for the immune tolerance is related to various antigen presenting cells (APC). This is endogenous immune suppression in liver. Hepatocytes, liver sinusoidal endothelial cells (LSECs), and hepatic stellate cells (HSCs) all respond to the exogenous pathogenic input from gut and blood. They sig-

nal to the immunosuppression system consisting of Kupffer cells (KCs), Myeloid-derived Suppressor Cells (MDSCs), and liver dendritic cells (DCs). These cells then mediate both activation of regulatory T cells (Tregs) and suppression of effector T cells, NK cells.

This immune tolerance is maintained through interleukin 10 (IL-10) released by KC, or through transforming growth factor (TGF)-β released by KCs or endothelial cells [27-29]. These cells also mediate immunosuppression by expression of PD-L1 [30].

Liver infection could induce immune suppression by producing relatively incomplete $CD4^+$ T-cell activation. This incomplete $CD4^+$ T-cell activation consequently results in poor activation of $CD8^+$ T cells and its clonal exhaustion and premature death [25]. On the other hand, due to the immune tolerance, the magnitude of intrahepatic immune responses to viral infection (including Hepatitides A and B) are limited to avoid fatal hepatic necrosis [25]. IL-10 or TGF-β and/or Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (see **Figure 1**) as well as programmed death-1 (PD-1) (see **Figure 2**) contribute to the immunosuppression in the conditions of autoimmune disease [31], allograft rejection [32] and viral infection [26]. These molecules help convert regulatory T cells (Tregs) from infiltrating naïve $CD4^+$ T cells and/or effector $CD4^+$ T cells.

Another mechanism of immune tolerance is related to low levels of co-stimulatory molecules and the upregulation of immune checkpoints molecules (PD-L1 or PD-L2) on hepatocytes (HC), hepatic stellate cells (HSC), KC, LSEC and intrahepatic leucocytes (LC) [30]. PD-1 plays a role in facilitation of T cell apoptosis or T cell dysfunction [33] in liver, and leading to immune tolerance [30, 34, 35]. Blockade of B7-H1/PD-1 ligation in liver sinusoidal endothelial cells (LSECs) [33] and Kupffer cells [36] significantly reduced HSC's immunomodulatory activity. PD-1 expression in circulating $CD8^+$ T cells has been correlated with progression from cirrhosis to HCC [37]. The expression of PD-L1 as well as PD-L2 and PD-1 can be increased in chronic inflammation [36], and plays a role in the immunosuppression induced by chronic inflammation [38-40]. CTLA-4 [40] and T cell immunoglobulin domain and mucin domain-3 (Tim-3) [38, 41] have been linked to suppression of T cell effector function in chronic viral hepatitis.

Chronic infection leads to the expansion of Tregs and the enhancement of the suppressor function of Tregs, which suppress the anti-tumor immune response and inhibit tumor immuno-surveillance against HCC [42-44].

MDSCs with high expression of PD-1 and increased IL-10 secretion exert their immunosuppressive function in chronic infection and liver cancer conditions [45].

Combined PD-1 and CTLA-4 inhibition can help restore antigen-specific $CD8^+$ T cell functions in chronically inflamed livers [40].

Immunosuppression associated with the tumor bed microenvironment

Immune system plays a dual role by both eliminating cancer cells and suppressing tumor growth. On the other hand, it promotes tumor growth either by selecting for tumor cells that survived after immunity and or by providing a microenvironment that facilitate tumor progression [46].

HCC have evolved a variety of immune escape mechanisms and immunosuppression mechanisms, such as the generation of cells with immune suppressor functions, including Tregs [47], invariant natural killer T cells (iNKT) [48], myeloid-derived suppressor cells [49] and tumor-associated macrophages (TAMs) such as alternatively activated macrophages (M2) [50]. In addition, down-regulation of HLA-I by APCs leads to the failure of HCC-associated antigen presentation [51], ineffective tumor antigen processing [52] and the inability of the immune system to recognize liver cancer. Moreover, $CD4^+$ T helper cells diminished in tumor bed in late stage HCC compared with early stage HCC [53]. All these changes contribute to the tumor tolerance and tumor progression in HCC.

Up-regulation of Tregs was associated with poor survival [54, 55] and the use of treatments targeting Treg could serve as a therapeutic strategy for restoration of anti-tumor immune responses [56]. Targeting suppressor cells alone or in combination with other immunotherapy deserves further evaluated in HCC patients.

Many immunomodulatory molecules are also involved in tumor bed immune suppression. CTLA-4 is a member of the CD28:B7 immuno-

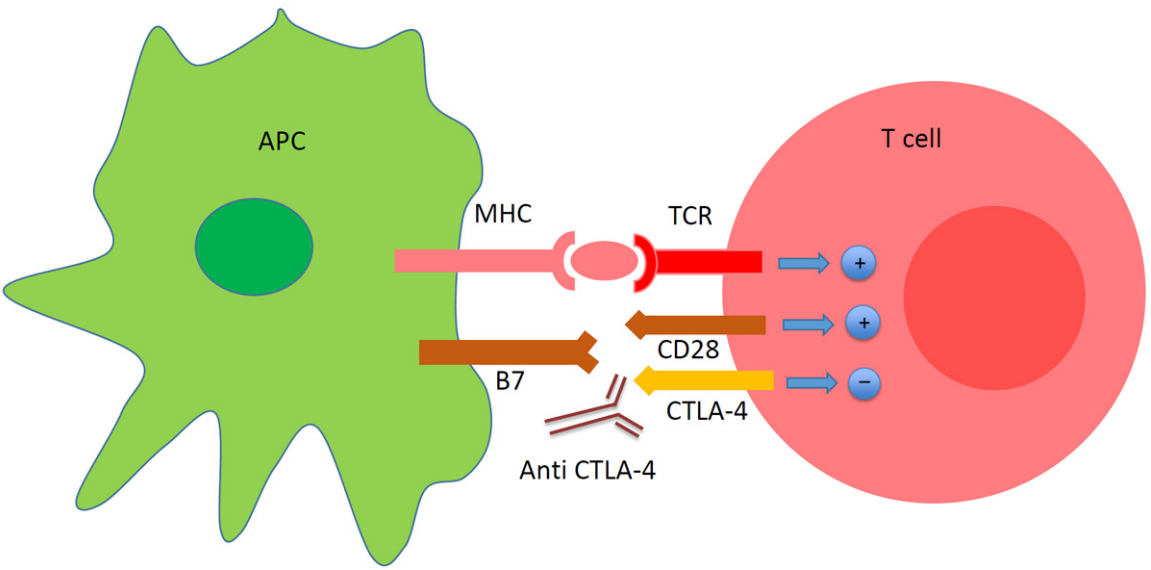

Figure 1. Mechanism of anti CTLA-4. The interaction between B7 and CTLA4 leads to inhibition of T cell activation by antigen presenting cell. By blocking CTLA-4, antibody augment T cell activation and expansion and promote the antitumor immunity. CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; MHC, major histocompatibility complex; TCR, T cell receptor; APC, antigen presenting cell.

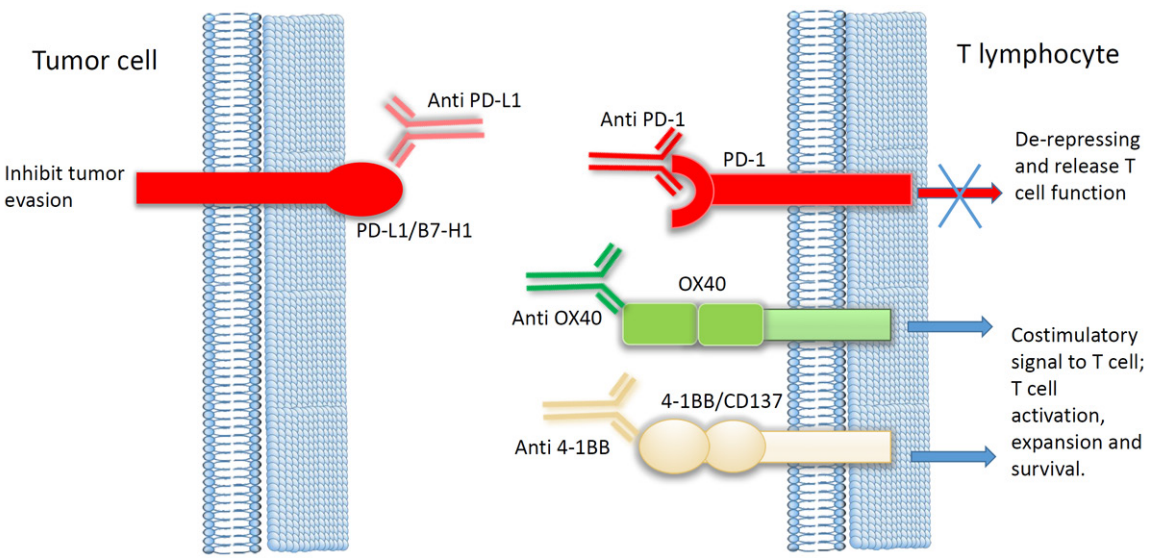

Figure 2. Mechanisms of inhibitory anti PD-1, anti PD-L1; agonistic anti OX40/CD134, anti 4-1BB/CD137 monoclonal antibodies. Antibodies against PD-1 and PD-L1 are activating CD4 and CD8 T cells while anti 4-1BB/CD137 and anti-OX40/CD134 offer co-stimulatory signals.

globulin superfamily and is the first immune checkpoint protein. Activation of T cells require 2 essential signals including both MHC binding with TCR antigen and the costimulatory signal the B7-1 (CD80) and B7-2 (CD86) molecules binding with CD28 [57]. CTLA-4 competes with CD-28 on T cells to bind B7 and down regulated T cell function [58], thus induces immunosuppression. Ligation of B7-1 and B7-2 with CTLA-4 transmit co-inhibitory signals down regulating T cell activation (see **Figure 1**).

The blockade of CTLA4 promotes T-cell immune responses against tumors [59]. The antitumor effect of CTLA-4 blockade involves both effector T cells and Treg [60].

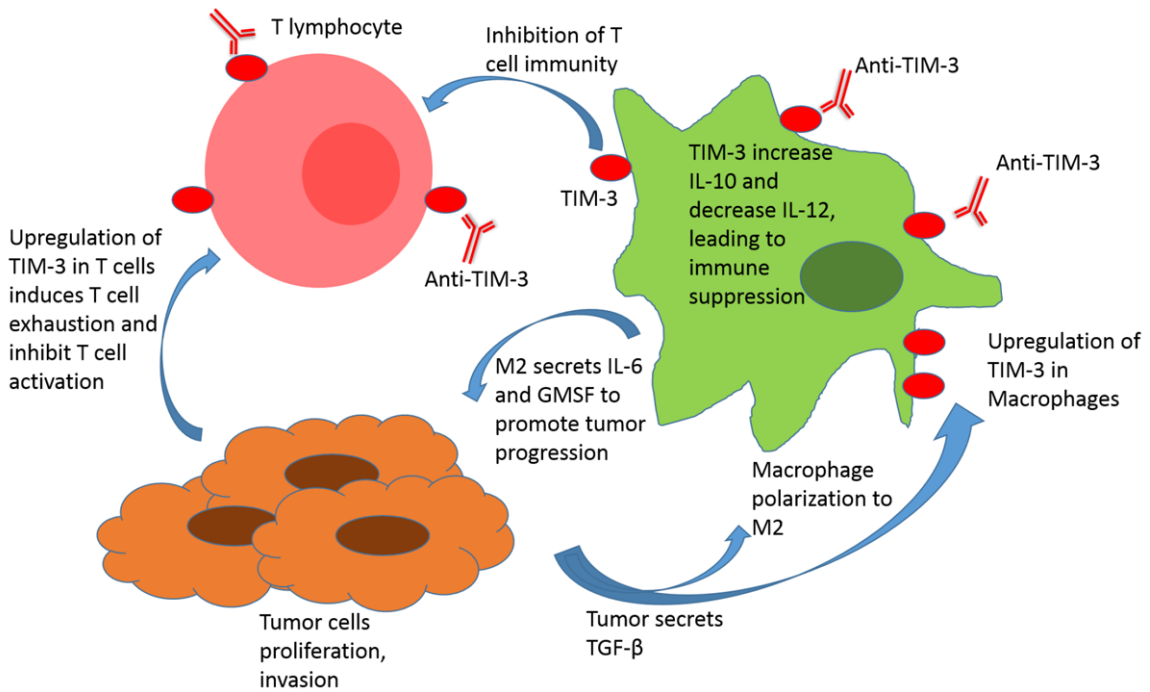

Figure 3. Mechanism of TIM-3, a new immune checkpoint inhibitor. Antibody against Tim-3 is activating CD4 and CD8 T cells while inhibit M2 (tumor associated macrophages), potentiating T cell antitumor immunity. TIM-3, T cell immunoglobulin domain andmucin domain-containing molecule-3; TGF-β, transforming growth factor-β. IL-6, interleukin-6; IL-12, interleukin-12 GMSF, Granulocyte macrophage colony-stimulating factor; M2, macrophage with alternatively activated phenotype.

These immune checkpoint blockades repress the activation of effector T cells and may contribute to HCC antigen immune escape in cancer-bearing host.

Another immunomodulatory molecule, Galectin-9, is expressed in liver and is involved in T cell development and homeostasis [61]. Galectin-9 binds to T cell immunoglobulin and mucin domain-containing molecule (Tim-3) on T cells. Tim-3/galectin-9 interactions promote death of Th1 T cells and induce tolerance [62] (see **Figure 3**). More recently, Tim-3 has been recognized to also have the potential to contribute to the functional inactivation of CD8 T cells in persistent HBC infections [63]. The upregulation of the Tim-3/Galectin-9 Pathway in chronic hepatitis B infection promotes T cell exhaustion and immune tolerance [38].

HCC immune tolerance can also be produced by decreased functions of co-stimulatory molecules. Expressions of B7-1 and B7-2 (immune costimulatory ligands) have been found reduced on HCC cells [64, 65], leading to a decrease of B7/CD28 mediated activation of effector T cells. More importantly, costimulatory molecules such as OX40, CD137 (4-1BB) are involved in modulation of immunity of chronic viral hepatitis and HCC (see **Figure 2**).

NK cells are well known to play an important role in antiviral immunity [66]. NK cells regulate T-cell responses by lysing antigen-presenting cells or by eliminating activated CD4 T cells. NK cells can regulate $CD4^+$ T-cell-mediated support for the antiviral $CD8^+$ T cells; thus modulating the control of viral pathogenesis and persistence. Both myeloid DCs and plasmacytoid DCs (pDCs) were identified in the modulation of NK activation [67, 68]. The interactions of pDCs and NK through the GITR/GITRL [69] and OX40/OX40L [70] pathways are believed to be able to regulate NK cell activity. NK cell activity can also be regulated through CD137 (4-1BB)/4-1BBL pathway [71]. It was suggested that in chronic HBV infected patients, an OX40L/IFN-α-dependent pathway was involved in the functional defect of plasmacytoid DCs in inducing NK cytolytic function [72]. Combination therapy of anti PD-1 and 4-1BB antibodies has demonstrated the ability to boost T cell immunity

Table 1. Studies applying immune checkpoints blockade for the treatment of HCC

Study	Phase	Target	Subject	Therapy	Results	Conclusions
Duffy, 2013	II	CTLA4	Patients	HCC patients received tremelimumab in combination with either TACE or RFA.	N/A	N/A
Sangro, 2013	II	CTLA4	20 HCC patients with chronic hepatitis C. Most patients were in the advanced stage and 43% had an altered liver function (Child-Pugh class B).	Tremelimumab at a dose of 15 mg/kg IV every 90 days. Twenty patients were assessable for toxicity and viral response and 17 were assessable for tumor response.	A good safety profile. Partial response rate was 17.6% and disease control rate was 76.4%. Time to progression was 6.48 months (95% CI 3.95-9.14). A significant drop in viral load was observed.	Tremelimumab is safe and presents antitumor and antiviral effect in patients with advanced HCC developed on HCV-induced liver cirrhosis.
Sangro, 2013	I	PD-1	Advanced HCC patients with or without chronic viral hepatitis	Dose escalation study of anti-PD-1 (nivolumab).	Evaluate safety and preliminary activity of nivolumab but not finished.	N/A
NCT00966251, 2009	I/II	PD-1	Advanced HCC patients not eligible for surgery, TACE or other systemic therapy.	Anti-PD-1 (CT-011, Pidilizumab).	Evaluate safety and tolerability.	Terminated because of slow accrual.
Moales-Kastresasa, 2013	Preclinical	PD-L1, OX40 (CD134) 4-1BB (CD137)	Mouse	Triple combination of antibodies (100 mg of each anti-CD137, anti- 0X40 and anti-B7-H1) or control rat IgG was administered intraperitoneally.	Antibodies extended survival mice bearing HCC. Antibody therapy synergize with adoptive T cell therapy too.	Anti PD-L1, anti OX40 and anti 4-1BB can improve survival of HCC mice in a CD8-dependent fashion.
Yan, 2015	Preclinical	Tim-3	Mouse	Tim-3 knockdown on macrophages. Time-3 blockade by neutralizing antibody, or small interfering RNA, or short hairpin RNA-expressing lentivirus.	Tim-3 interference in macrophages significantly inhibited the HCC growth both in vitro and in vivo.	Blockade of Tim-3 might be great potential in HCC therapy.

CTLA-4, cytotoxic T lymphocyte antigen-4; PD-1, programmed death-1; PD-L1, programmed death ligand-1; Tim-3, T cell immunoglobulin and mucin domain (TIM)-3; \N/A not available.

Table 2. Studies applying immune costimulatory therapy for the treatment of HCC

Study	Phase	Target	Therapy	Results	Conclusions
Pan, 2002	Preclinical	0X40 4-1BB	Combination therapy of a nti-4-1BB, anti-0X40 and Il-12.	Higher survival rate in mice with hepatic colon metastases. Immune activation results in higher survival rated in mice with large tumor burdens.	Il-12, anti-4-1BB and anti-0X40 antibodies may provide better treatment for patients with advanced cancers.
Qiu, 2006	Preclinical	4-1BBL PD-1	Plasmid encoding 4-1BB ligand and PD-1 were injected in to mice with HCC.	Both 4-1BBL and PD-1 alone can inhibit tumor growth. But the combination therapy inhibit completely in 42% of the mice. And the survival rate was significantly improved.	Combination therapy with 4-1BBL and PD-1 can exert a synergistic anti-tumor effect on HCC model.
Li, 2011	Preclinical	4-1BBL, B7-1, B7-2	Transduction of tumor cell line H22 with B7-1, B7-2 and 4-1BBL.	Strong cytotoxic T lymphocyte responses were observed. The tumor vaccines improved T-cell-mediated antitumor response.	Vaccine with gene transfer of B7-1, B7-2 and 4-1BBL demonstrated a therapeutic potential for HCC.
Gauttier, 2014	Preclinical	4-1BB	Anti-CD137 antibody was tested on 2 in situ models of HCC.	Tumor regression was observed in 40-60% of mice. The antitumor effect is T cell dependent. Antibody inhibited MDSC and Treg infiltration in tumor. MDSC depletion with anti4-1BB therapy resulted in recovery of 80% of the mice with HCC.	Agonistic anti-CD137 monoantibody is a promising strategy for HCC immunotherapy.

MDSC, myeloid derived suppressor cells; Treg, regulatory T lymphocytes.

against HBV infection [73]. However, CD137 overstimulation may be a contributing factor for development of HCC from chronic HBV infection [74].

Immune checkpoint therapy in HCC (Table 1)

Multiple immune checkpoint blocking agents were tested in various preclinical models. To date, most clinical therapies are using antibodies against PD-1, PD-L1 and CTLA-4 molecules. Most of the trials use a single antibody. These therapeutic strategies are in early phases of clinical trial development.

CTLA-4

Monoclonal antibodies that can block CTLA-4 (Ipilimumab) and PD1 have shown efficacy in inducing clinical responses and offered a clinical benefit for HCC patients [8]. Upregulation of CTLA-4 expression by T regulatory cells has been associated with immune tolerance in liver transplantation [32]. A clinical trial was done to evaluate the effect of anti CTLA-4 (tremelimumab) in patients with hepatocellular carcinoma (HCC) and chronic hepatitis C virus (HCV) infection [9]. Seventeen patients had a 76.4% disease control rate and 17.6% patients had partial response. Anti CTLA-4 treatment presented a good safety profile when used 15 mg/kg IV every 90 days in 21 patients. Tremelimumab extended the median overall survival time to 8.2 months with half of patients presenting transient transaminase elevation after the first dose. Enhancement of anti-HCV immune responses was associated with a drop in viral load. This antitumor and antiviral activity in patients with HCC encourages further research.

A new phase I clinical trial of tremelimumab combined with local therapy of HCC such as radiofrequency ablation or transarterial chemoembolization is being conducted (NCT0185-3618) because local therapy is presumed to release more TAA from necrotizing tumor tissue into blood which presumably lead to better priming of specific T cells to obtain a synergistic effects [75].

PD-1 and PD-L1

Evidence suggested that PD-L1 status may act as a predictor of recurrence of HCC and the therapy targeting the PD-L1/PD-1 pathway against HCC is attractive [76]. Also, PD-1 expression was higher and CD28 and CD127 expression levels were lower in tumor infiltrating lymphocytes (TILs) in chronic hepatitis B patients with HCC [77]. PD-1 blockage reverses immune dysfunction and hepatitis B viral persistence in a mouse animal model [78].

Blocking the PD-1 receptor on activated T cells with monoclonal antibodies (mAbs) has been shown to overcome immune resistance in tumors including melanoma, hepatocellular carcinoma. Nivolumab (BMS-936558), is a fully human IgG4 monoclonal antibody against PD-1 receptor and is under investigation in a phase I clinical trial (NCT01658878) [79]. This clinical trial will evaluate the safety and efficacy of nivolumab in patients with HCC with or without HBV or HCV-related hepatitis. Pidilizumab (CT-011) (another anti PD-1) and anti-PD-L1 antibodies were also evaluated in a phase I clinical trial (NCT00966251). However due to the slow accrual this trial was terminated early with no available results. PD-1 Antibodies have demonstrated antitumor activity and a better toxicity profile than ipilimumab [80].

LAG3 and TIM-3

Other immune checkpoint antibodies such as lymphocyte activation gene 3 (LAG3), T cell immunoglobulin domain and mucin domain-containing molecule-3 (TIM-3) and NK-inhibitory receptors [81, 82] have exhibited antitumor activity in animal models. These molecules inhibit T cell responses, particularly under conditions involving chronic inflammation. LAG-3 plays a role in the suppression of HBV-specific cell-mediated immunity in HCC patients, and provides a target to boost HCC treatment [81]. In a mouse model of HCC, the knockdown of TIM-3 in macrophages significantly inhibited the HCC tumor growth [83].

Agonistic antibodies against the costimulatory molecules in HCC (Table 2)

T cells require two signals for activation: signal 1 is provided by the interaction of the T-cell receptor (TCR) with major histocompatibility complex (MHC) peptide, while signal 2 is provided by costimulatory pathways like B7-CD28, OX40L-OX40, and CD137L-CD137 (4-1BB). Beside blocking negative regulatory molecules on

the surface of effector cells (such as PD-1), agonizing monoclonal antibodies to the stimulatory targets provide another promising way to enhance CD8 T cell responses by providing co-stimulation through ligation of tumor necrosis factor receptor (TNFR) family members, including OX40 (CD134) and 4-1BB (CD137). The results of clinical trials with other diseases investigating 4-1BB (CD137); OX40 (CD134), glucocorticoid-induced TNFR-related gene (GITR) are encouraging [84]. Costimulatory molecules such as CD244 (2B4) [85], CD28 [77], CD40 [86], CD137 (4-1BB) and CD134 (OX-40) [87] have been identified as factors involved in cancer immunity [85].

CD40

CD40 is a member of the TNFR family and it is important for the survival and function of B cells. Also CD40 expression in HCCs is related to the tumor cell resistance against TNFR-mediated apoptosis [86, 88]. CD40 is up-regulated in intrahepatic endothelial cells (IHEC) and epithelial cells during inflammatory liver disease and HCC [86]. Anti-CD40 modulates immune response by enhancing T cell migration to $CD40^+$ tumor by targeting blood vessels, promoting APC activation and stimulating a specific CTL response to cross-presented tumor antigens [89]. The combination of CD40 stimulation and interleukin-2 (IL-2) leads to synergistic antitumor responses in several models of advanced metastatic disease [90].

4-1BB (CD137)

4-1BB is an inducible cell surface molecule and is expressed on activated effector $CD8^+$ and $CD4^+$ T cells, NK cells, NK/T cells, Tregs, DCs, macrophages, neutrophils, and eosinophils. It belongs to the TNFR superfamily. Engagement of its ligand (CD137L) or an agonist antibody provides sufficient costimulation signal in a CD28-independent pathway for T cells [91]. CD137 signaling enhances T cell proliferation and Th1 cytokine production and rescues $CD8^+$ T cells from activation-induced cell death [92]. Anti 4-1BB protects tumor-infiltrating lymphocytes, cytotoxic T lymphocyte (CTL) and enhances cytolytic activity [92, 93].

Anti-CD137 and anti-OX40 provide T-cell costimulation in the mice with HCC. A triple combination of anti-CD137, anti-OX40 and PD-L1 antibodies improved the survival of mice bearing HCC in a CD8-dependent fashion and synergized with adoptive T-cell therapy [87]. In addition, targeting 4-1BB with an agonist antibody can promote tumor control in hepatic metastatic colon cancer [94]. Moreover, therapy targeting both immune modulatory molecules such as 4-1BB and OX-40 demonstrated encouraging results in preclinical experiment [87]. Anti 4-1BB was studied using two in-situ models of HCC in immunocompetent mice [95]. In one orthotropic HCC model, the treatment led to a long lasting protection and resulted in complete tumor regression in 40-60% of animals. And a combination treatment with a depletion of MDSC led to the recovery of 80% of the mice. These results proved that agonistic anti-4-1BB is a promising therapeutic strategy for HCC.

In another study with a murine HCC model, the density of $CD8^+$ T cells in the tissue around HCC was significantly increased when using combinatorial intratumor treatment of plasmids containing 4-1BB ligand and PD-1. The results demonstrated the treatment with 4-1BBL and PD1 could synergize antitumor effect in HCC [96]. Recently, PD-1, PD-L1 and 4-1BB ligand are introduced into HCC cell line to provide stimulatory signals for T-cells. A reduced tomorigenecity was found in these tumor cells expressing 4-1BBL [97].

OX40 (CD134)

OX40 is another tumor necrosis factor receptor expressed primarily on activated $CD4^+$ and $CD8^+$ T cells, neutrophils, dendritic cells, and Tregs. Ligation of OX40 and its ligand transmits a potent costimulatory signal. OX40 promotes T-cell activation, survival, proliferation, and cytokine production [98]. Engagement of OX40 with an agonist antibody can also deactivate the suppressive function of Tregs [99].

Combinations of OX40 agonist antibody with radiotherapy and other immunotherapy such as IL-12, IL-2, anti 4-1BB and anti CTLA-4 have improved immune control of tumors in mice bearing hepatic colon cancer metastases [100]. In HCC patient, Tregs are moderately or highly infiltrating in cirrhotic liver and tumor fragments, expressing OX40 and a T-helper (Th)1-suppressing phenotype; however, in non-cirrhotic liver only few Tregs express low levels of OX40 and become "Th1-like" cells. OX40

ligand, mostly expressed by M2-like monocytes and macrophages, could lead to OX40 + Treg proliferation and counteract the differentiation of Th1-like Tregs in HCV-infected patients with cirrhosis and HCC [101].

Combinatorial therapy of immunostimulatory monoclonal antibodies has been shown to synergize at eliciting relevant immune responses and treat HCC in mouse [102, 103]. Thus they are indicated to serve as targets for therapies aimed at enhancing antitumor immunity. By using agonist antibodies, these molecules can be utilized to augment anti-tumor immunity. Further clinical trials targeting these molecules are expected to emerge in the near future. In addition, combination of immune targeting agents along with inhibitory checkpoint blockage is the most promising strategy for clinical development because HCC-induced immune tolerance and chronic inflammation is associated with multiple immunosuppressive mechanisms.

Conclusions and perspectives

HCC-bearing hosts develop and maintain tumor immune tolerance with expression of several immune inhibitory checkpoints (PD-1, PD-L1, CTLA-4, LAG-3, and TIM-3). The up-regulation of immune checkpoints contributes to HCC progression and resistance to immunotherapy. Blockade of the T cell inhibitory receptors CTLA-4, PD-1 TIM-3, and LAG-3 simultaneously and synergistically augment T cell responses. Moreover, these receptors can also modulate the activity of B cells, natural killer cells, monocytes, macrophages, and dendritic cells. They could be promising targets for future HCC immunotherapy. Recently, other negative checkpoint regulators have emerged as targets of this exciting strategy of antibody blockade in cancer immunotherapy, such as V-domain immunoglobulin (Ig)-containing suppressor of T-cell activation (VISTA) [104], CD160 and B- and T-cell lymphocyte attenuator (BTLA) [105].

Multiple immune checkpoint combinations with new immune targets can be used to enhance anti-tumor immune response. For example, simultaneous treatments with immune checkpoint targeting therapies (anti CTLA-4, anti PD-1, and anti PD-L1 antibodies), or combinations of checkpoint blockage with antibodies targeting costimulatory (4-1BB, OX40) molecules would potentially result in enhanced immunity, leading to successful treatment development.

The use of inhibitory checkpoints blockade combined with conventional therapy such as embolization and radiofrequency ablation will also provide chances to overcome immune tolerance by giving additional boost to immune system activation [106, 107]. The mechanism could be by necrotizing tumor tissue and releasing tumor associated antigens into the blood stream and enhancing antigen specific T-cell priming. Thus, using the immunotherapy coupled with procedures can lead to regression of extrahepatic metastasis.

Emerging as a new category in HCC treatment, immunostimulatory antibodies have resulted in changes in the management of HCC. Further efforts are needed to explore more successful and exciting treatments for HCC in the future.

Address correspondence to: Dr. Liang Huang, Department of Surgery, University of Iowa, 200 Hawkins Drive, Iowa City, IA 52242, USA. E-mail: huangliangbj@hotmail.com

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

[2] Shlomai A, de Jong YP and Rice CM. Virus associated malignancies: the role of viral hepatitis in hepatocellular carcinoma. Semin Cancer Biol 2014; 26: 78-88.

[3] Ulahannan SV, Duffy AG, McNeel TS, Kish JK, Dickie LA, Rahma OE, McGlynn KA, Greten TF and Altekruse SF. Earlier presentation and application of curative treatments in hepatocellular carcinoma. Hepatology 2014; 60: 1637-1644.

[4] Park NH, Chung YH and Lee HS. Impacts of vaccination on hepatitis B viral infections in Korea over a 25-year period. Intervirology 2010; 53: 20-28.

[5] Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Haussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D and Bruix J. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008; 359: 378-390.

[6] Breous E and Thimme R. Potential of immunotherapy for hepatocellular carcinoma. J Hepatol 2011; 54: 830-834.
[7] Mellman I, Coukos G and Dranoff G. Cancer immunotherapy comes of age. Nature 2011; 480: 480-489.
[8] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A and Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363: 711-723.
[9] Sangro B, Gomez-Martin C, de la Mata M, Inarrairaegui M, Garralda E, Barrera P, Riezu-Boj JI, Larrea E, Alfaro C, Sarobe P, Lasarte JJ, Perez-Gracia JL, Melero I and Prieto J. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. J Hepatol 2013; 59: 81-88.
[10] Mizukoshi E, Nakamoto Y, Arai K, Yamashita T, Sakai A, Sakai Y, Kagaya T, Yamashita T, Honda M and Kaneko S. Comparative analysis of various tumor-associated antigen-specific t-cell responses in patients with hepatocellular carcinoma. Hepatology 2011; 53: 1206-1216.
[11] Thimme R, Neagu M, Boettler T, Neumann-Haefelin C, Kersting N, Geissler M, Makowiec F, Obermaier R, Hopt UT, Blum HE and Spangenberg HC. Comprehensive analysis of the alpha-fetoprotein-specific CD8+ T cell responses in patients with hepatocellular carcinoma. Hepatology 2008; 48: 1821-1833.
[12] Butterfield LH, Ribas A, Meng WS, Dissette VB, Amarnani S, Vu HT, Seja E, Todd K, Glaspy JA, McBride WH and Economou JS. T-cell responses to HLA-A*0201 immunodominant peptides derived from alpha-fetoprotein in patients with hepatocellular cancer. Clin Cancer Res 2003; 9: 5902-5908.
[13] Bricard G, Bouzourene H, Martinet O, Rimoldi D, Halkic N, Gillet M, Chaubert P, Macdonald HR, Romero P, Cerottini JC and Speiser DE. Naturally acquired MAGE-A10- and SSX-2-specific CD8+ T cell responses in patients with hepatocellular carcinoma. J Immunol 2005; 174: 1709-1716.
[14] Gehring AJ, Ho ZZ, Tan AT, Aung MO, Lee KH, Tan KC, Lim SG and Bertoletti A. Profile of tumor antigen-specific CD8 T cells in patients with hepatitis B virus-related hepatocellular carcinoma. Gastroenterology 2009; 137: 682-690.
[15] Korangy F, Ormandy LA, Bleck JS, Klempnauer J, Wilkens L, Manns MP and Greten TF. Spontaneous tumor-specific humoral and cellular immune responses to NY-ESO-1 in hepatocellular carcinoma. Clin Cancer Res 2004; 10: 4332-4341.
[16] Mizukoshi E, Nakamoto Y, Marukawa Y, Arai K, Yamashita T, Tsuji H, Kuzushima K, Takiguchi M and Kaneko S. Cytotoxic T cell responses to human telomerase reverse transcriptase in patients with hepatocellular carcinoma. Hepatology 2006; 43: 1284-1294.
[17] Hiroishi K, Eguchi J, Baba T, Shimazaki T, Ishii S, Hiraide A, Sakaki M, Doi H, Uozumi S, Omori R, Matsumura T, Yanagawa T, Ito T and Imawari M. Strong CD8(+) T-cell responses against tumor-associated antigens prolong the recurrence-free interval after tumor treatment in patients with hepatocellular carcinoma. J Gastroenterol 2010; 45: 451-458.
[18] Komori H, Nakatsura T, Senju S, Yoshitake Y, Motomura Y, Ikuta Y, Fukuma D, Yokomine K, Harao M, Beppu T, Matsui M, Torigoe T, Sato N, Baba H and Nishimura Y. Identification of HLA-A2- or HLA-A24-restricted CTL epitopes possibly useful for glypican-3-specific immunotherapy of hepatocellular carcinoma. Clin Cancer Res 2006; 12: 2689-2697.
[19] Liu Y, Daley S, Evdokimova VN, Zdobinski DD, Potter DM and Butterfield LH. Hierarchy of alpha fetoprotein (AFP)-specific T cell responses in subjects with AFP-positive hepatocellular cancer. J Immunol 2006; 177: 712-721.
[20] Shang XY, Chen HS, Zhang HG, Pang XW, Qiao H, Peng JR, Qin LL, Fei R, Mei MH, Leng XS, Gnjatic S, Ritter G, Simpson AJ, Old LJ and Chen WF. The spontaneous CD8+ T-cell response to HLA-A2-restricted NY-ESO-1b peptide in hepatocellular carcinoma patients. Clin Cancer Res 2004; 10: 6946-6955.
[21] Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, Mulder GE, Toebes M, Vesely MD, Lam SS, Korman AJ, Allison JP, Freeman GJ, Sharpe AH, Pearce EL, Schumacher TN, Aebersold R, Rammensee HG, Melief CJ, Mardis ER, Gillanders WE, Artyomov MN and Schreiber RD. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014; 515: 577-581.
[22] van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, van Dijk LJ, Behjati S, Hilkmann H, El Atmioui D, Nieuwland M, Stratton MR, Kerkhoven RM, Kesmir C, Haanen JB, Kvistborg P and Schumacher TN. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 2013; 31: e439-442.
[23] Robbins PF, Lu YC, El-Gamil M, Li YF, Gross C, Gartner J, Lin JC, Teer JK, Cliften P, Tycksen E, Samuels Y and Rosenberg SA. Mining exomic sequencing data to identify mutated antigens

recognized by adoptively transferred tumor-reactive T cells. Nat Med 2013; 19: 747-752.
[24] Schumacher TN and Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015; 348: 69-74.
[25] Crispe IN. Immune tolerance in liver disease. Hepatology 2014; 60: 2109-2117.
[26] Protzer U, Maini MK and Knolle PA. Living in the liver: hepatic infections. Nat Rev Immunol 2012; 12: 201-213.
[27] Knolle P, Schlaak J, Uhrig A, Kempf P, Meyer zum Buschenfelde KH and Gerken G. Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J Hepatol 1995; 22: 226-229.
[28] Knolle PA, Germann T, Treichel U, Uhrig A, Schmitt E, Hegenbarth S, Lohse AW and Gerken G. Endotoxin down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells. J Immunol 1999; 162: 1401-1407.
[29] Bissell DM, Wang SS, Jarnagin WR and Roll FJ. Cell-specific expression of transforming growth factor-beta in rat liver. Evidence for autocrine regulation of hepatocyte proliferation. J Clin Invest 1995; 96: 447-455.
[30] Muhlbauer M, Fleck M, Schutz C, Weiss T, Froh M, Blank C, Scholmerich J and Hellerbrand C. PD-L1 is induced in hepatocytes by viral infection and by interferon-alpha and -gamma and mediates T cell apoptosis. J Hepatol 2006; 45: 520-528.
[31] Tiegs G and Lohse AW. Immune tolerance: what is unique about the liver. J Autoimmun 2010; 34: 1-6.
[32] Li W, Kuhr CS, Zheng XX, Carper K, Thomson AW, Reyes JD and Perkins JD. New insights into mechanisms of spontaneous liver transplant tolerance: the role of Foxp3-expressing CD25+ CD4+ regulatory T cells. Am J Transplant 2008; 8: 1639-1651.
[33] Diehl L, Schurich A, Grochtmann R, Hegenbarth S, Chen L and Knolle PA. Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8+ T cell tolerance. Hepatology 2008; 47: 296-305.
[34] Yu MC, Chen CH, Liang X, Wang L, Gandhi CR, Fung JJ, Lu L and Qian S. Inhibition of T-cell responses by hepatic stellate cells via B7-H1-mediated T-cell apoptosis in mice. Hepatology 2004; 40: 1312-1321.
[35] Gu X, Wang Y, Xiang J, Chen Z, Wang L, Lu L and Qian S. Interferon- gamma triggers hepatic stellate cell-mediated immune regulation through MEK/ERK signaling pathway. Clin Dev Immunol 2013; 2013: 389807.
[36] Kassel R, Cruise MW, Iezzoni JC, Taylor NA, Pruett TL and Hahn YS. Chronically inflamed livers up-regulate expression of inhibitory B7 family members. Hepatology 2009; 50: 1625-1637.
[37] Shi F, Shi M, Zeng Z, Qi RZ, Liu ZW, Zhang JY, Yang YP, Tien P and Wang FS. PD-1 and PD-L1 upregulation promotes CD8(+) T-cell apoptosis and postoperative recurrence in hepatocellular carcinoma patients. Int J Cancer 2011; 128: 887-896.
[38] Nebbia G, Peppa D, Schurich A, Khanna P, Singh HD, Cheng Y, Rosenberg W, Dusheiko G, Gilson R, ChinAleong J, Kennedy P and Maini MK. Upregulation of the Tim-3/galectin-9 pathway of T cell exhaustion in chronic hepatitis B virus infection. PLoS One 2012; 7: e47648.
[39] Peng G, Li S, Wu W, Tan X, Chen Y and Chen Z. PD-1 upregulation is associated with HBV-specific T cell dysfunction in chronic hepatitis B patients. Mol Immunol 2008; 45: 963-970.
[40] Nakamoto N, Cho H, Shaked A, Olthoff K, Valiga ME, Kaminski M, Gostick E, Price DA, Freeman GJ, Wherry EJ and Chang KM. Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. PLoS Pathog 2009; 5: e1000313.
[41] Moorman JP, Wang JM, Zhang Y, Ji XJ, Ma CJ, Wu XY, Jia ZS, Wang KS and Yao ZQ. Tim-3 pathway controls regulatory and effector T cell balance during hepatitis C virus infection. J Immunol 2012; 189: 755-766.
[42] Zhang HH, Mei MH, Fei R, Liu F, Wang JH, Liao WJ, Qin LL, Wei L and Chen HS. Regulatory T cells in chronic hepatitis B patients affect the immunopathogenesis of hepatocellular carcinoma by suppressing the anti-tumour immune responses. J Viral Hepat 2010; 17 Suppl 1: 34-43.
[43] Cabrera R, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C and Nelson DR. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. Hepatology 2004; 40: 1062-1071.
[44] Aalaei-Andabili SH and Alavian SM. Regulatory T cells are the most important determinant factor of hepatitis B infection prognosis: a systematic review and meta-analysis. Vaccine 2012; 30: 5595-5602.
[45] Huang A, Zhang B, Yan W, Wang B, Wei H, Zhang F, Wu L, Fan K and Guo Y. Myeloid-derived suppressor cells regulate immune response in patients with chronic hepatitis B virus infection through PD-1-induced IL-10. J Immunol 2014; 193: 5461-5469.
[46] Schreiber RD, Old LJ and Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011; 331: 1565-1570.
[47] Fu J, Xu D, Liu Z, Shi M, Zhao P, Fu B, Zhang Z, Yang H, Zhang H, Zhou C, Yao J, Jin L, Wang H, Yang Y, Fu YX and Wang FS. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology 2007; 132: 2328-2339.

[48] Cariani E, Pilli M, Zerbini A, Rota C, Olivani A, Pelosi G, Schianchi C, Soliani P, Campanini N, Silini EM, Trenti T, Ferrari C and Missale G. Immunological and molecular correlates of disease recurrence after liver resection for hepatocellular carcinoma. PLoS One 2012; 7: e32493.

[49] Hoechst B, Ormandy LA, Ballmaier M, Lehner F, Kruger C, Manns MP, Greten TF and Korangy F. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. Gastroenterology 2008; 135: 234-243.

[50] Yeung OW, Lo CM, Ling CC, Qi X, Geng W, Li CX, Ng KT, Forbes SJ, Guan XY, Poon RT, Fan ST and Man K. Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma. J Hepatol 2015; 62: 607-616.

[51] Matsui M, Machida S, Itani-Yohda T and Akatsuka T. Downregulation of the proteasome subunits, transporter, and antigen presentation in hepatocellular carcinoma, and their restoration by interferon-gamma. J Gastroenterol Hepatol 2002; 17: 897-907.

[52] Kurokohchi K, Carrington M, Mann DL, Simonis TB, Alexander-Miller MA, Feinstone SM, Akatsuka T and Berzofsky JA. Expression of HLA class I molecules and the transporter associated with antigen processing in hepatocellular carcinoma. Hepatology 1996; 23: 1181-1188.

[53] Alisa A, Ives A, Pathan AA, Navarrete CV, Williams R, Bertoletti A and Behboudi S. Analysis of CD4+ T-Cell responses to a novel alpha-fetoprotein-derived epitope in hepatocellular carcinoma patients. Clin Cancer Res 2005; 11: 6686-6694.

[54] Fu J, Zhang Z, Zhou L, Qi Z, Xing S, Lv J, Shi J, Fu B, Liu Z, Zhang JY, Jin L, Zhao Y, Lau GK, Zhao J and Wang FS. Impairment of CD4+ cytotoxic T cells predicts poor survival and high recurrence rates in patients with hepatocellular carcinoma. Hepatology 2013; 58: 139-149.

[55] Zhou J, Ding T, Pan W, Zhu LY, Li L and Zheng L. Increased intratumoral regulatory T cells are related to intratumoral macrophages and poor prognosis in hepatocellular carcinoma patients. Int J Cancer 2009; 125: 1640-1648.

[56] Greten TF, Ormandy LA, Fikuart A, Hochst B, Henschen S, Horning M, Manns MP and Korangy F. Low-dose cyclophosphamide treatment impairs regulatory T cells and unmasks AFP-specific CD4+ T-cell responses in patients with advanced HCC. J Immunother 2010; 33: 211-218.

[57] Baxter AG and Hodgkin PD. Activation rules: the two-signal theories of immune activation. Nat Rev Immunol 2002; 2: 439-446.

[58] Linsley PS, Bradshaw J, Greene J, Peach R, Bennett KL and Mittler RS. Intracellular trafficking of CTLA-4 and focal localization towards sites of TCR engagement. Immunity 1996; 4: 535-543.

[59] Callahan MK and Wolchok JD. At the bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. J Leukoc Biol 2013; 94: 41-53.

[60] Peggs KS, Quezada SA, Chambers CA, Korman AJ and Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med 2009; 206: 1717-1725.

[61] Rabinovich GA and Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nat Rev Immunol 2009; 9: 338-352.

[62] Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB and Kuchroo VK. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol 2005; 6: 1245-1252.

[63] Golden-Mason L, Palmer BE, Kassam N, Townshend-Bulson L, Livingston S, McMahon BJ, Castelblanco N, Kuchroo V, Gretch DR and Rosen HR. Negative immune regulator Tim-3 is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional CD4+ and CD8+ T cells. J Virol 2009; 83: 9122-9130.

[64] Fujiwara K, Higashi T, Nouso K, Nakatsukasa H, Kobayashi Y, Uemura M, Nakamura S, Sato S, Hanafusa T, Yumoto Y, Naito I and Shiratori Y. Decreased expression of B7 costimulatory molecules and major histocompatibility complex class-I in human hepatocellular carcinoma. J Gastroenterol Hepatol 2004; 19: 1121-1127.

[65] Tatsumi T, Takehara T, Katayama K, Mochizuki K, Yamamoto M, Kanto T, Sasaki Y, Kasahara A and Hayashi N. Expression of costimulatory molecules B7-1 (CD80) and B7-2 (CD86) on human hepatocellular carcinoma. Hepatology 1997; 25: 1108-1114.

[66] Waggoner SN, Cornberg M, Selin LK and Welsh RM. Natural killer cells act as rheostats modulating antiviral T cells. Nature 2012; 481: 394-398.

[67] Gerosa F, Gobbi A, Zorzi P, Burg S, Briere F, Carra G and Trinchieri G. The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. J Immunol 2005; 174: 727-734.

[68] Swiecki M, Gilfillan S, Vermi W, Wang Y and Colonna M. Plasmacytoid dendritic cell ablation impacts early interferon responses and antiviral NK and CD8(+) T cell accrual. Immunity 2010; 33: 955-966.

[69] Hanabuchi S, Watanabe N, Wang YH, Wang YH, Ito T, Shaw J, Cao W, Qin FX and Liu YJ. Human plasmacytoid predendritic cells activate NK cells through glucocorticoid-induced tumor necrosis factor receptor-ligand (GITRL). Blood 2006; 107: 3617-3623.

[70] Liu C, Lou Y, Lizee G, Qin H, Liu S, Rabinovich B, Kim GJ, Wang YH, Ye Y, Sikora AG, Overwijk WW, Liu YJ, Wang G and Hwu P. Plasmacytoid dendritic cells induce NK cell-dependent, tumor antigen-specific T cell cross-priming and tumor regression in mice. J Clin Invest 2008; 118: 1165-1175.

[71] Baessler T, Charton JE, Schmiedel BJ, Grunebach F, Krusch M, Wacker A, Rammensee HG and Salih HR. CD137 ligand mediates opposite effects in human and mouse NK cells and impairs NK-cell reactivity against human acute myeloid leukemia cells. Blood 2010; 115: 3058-3069.

[72] Martinet J, Dufeu-Duchesne T, Bruder Costa J, Larrat S, Marlu A, Leroy V, Plumas J and Aspord C. Altered functions of plasmacytoid dendritic cells and reduced cytolytic activity of natural killer cells in patients with chronic HBV infection. Gastroenterology 2012; 143: 1586-1596. e1588.

[73] Fisicaro P, Valdatta C, Massari M, Loggi E, Ravanetti L, Urbani S, Giuberti T, Cavalli A, Vandelli C, Andreone P, Missale G and Ferrari C. Combined blockade of programmed death-1 and activation of CD137 increase responses of human liver T cells against HBV, but not HCV. Gastroenterology 2012; 143: 1576-1585. e1574.

[74] Wang J, Zhao W, Cheng L, Guo M, Li D, Li X, Tan Y, Ma S, Li S, Yang Y, Chen L and Wang S. CD137-mediated pathogenesis from chronic hepatitis to hepatocellular carcinoma in hepatitis B virus-transgenic mice. J Immunol 2010; 185: 7654-7662.

[75] Duffy AG, Ulalannan SV, Fioravanti S. A pilot study of tremelimumab, a monoclonal antibody against CTLA-4, in combination with either transcatheter arterial chemoembolization (TACE) or radiofrequency ablation (RFA) in patients with hepatocellular carcinoma (HCC). J Clin Oncol 2014; 32: e515133.

[76] Gao Q, Wang XY, Qiu SJ, Yamato I, Sho M, Nakajima Y, Zhou J, Li BZ, Shi YH, Xiao YS, Xu Y and Fan J. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. Clin Cancer Res 2009; 15: 971-979.

[77] Hsu PN, Yang TC, Kao JT, Cheng KS, Lee YJ, Wang YM, Hsieh CT, Lin CW and Wu YY. Increased PD-1 and decreased CD28 expression in chronic hepatitis B patients with advanced hepatocellular carcinoma. Liver Int 2010; 30: 1379-1386.

[78] Tzeng HT, Tsai HF, Liao HJ, Lin YJ, Chen L, Chen PJ and Hsu PN. PD-1 blockage reverses immune dysfunction and hepatitis B viral persistence in a mouse animal model. PLoS One 2012; 7: e39179.

[79] Sangro B, Crocenzi TS, Welling TH, et al. Phase I dose escalation study of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in patients (pts) with advanced hepatocellular carcinoma (HCC) with or without chronic viral hepatitis. J Clin Oncol 2013; 31: TPS3111.

[80] Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL, Gilson MM, Wang C, Selby M, Taube JM, Anders R, Chen L, Korman AJ, Pardoll DM, Lowy I and Topalian SL. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 2010; 28: 3167-3175.

[81] Li FJ, Zhang Y, Jin GX, Yao L and Wu DQ. Expression of LAG-3 is coincident with the impaired effector function of HBV-specific CD8(+) T cell in HCC patients. Immunol Lett 2013; 150: 116-122.

[82] Li H, Wu K, Tao K, Chen L, Zheng Q, Lu X, Liu J, Shi L, Liu C, Wang G and Zou W. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. Hepatology 2012; 56: 1342-1351.

[83] Yan W, Liu X, Ma H, Zhang H, Song X, Gao L, Liang X and Ma C. Tim-3 fosters HCC development by enhancing TGF-beta-mediated alternative activation of macrophages. Gut 2015; 64: 1593-604.

[84] Schaer DA, Hirschhorn-Cymerman D and Wolchok JD. Targeting tumor-necrosis factor receptor pathways for tumor immunotherapy. J Immunother Cancer 2014; 2: 7.

[85] Wu Y, Kuang DM, Pan WD, Wan YL, Lao XM, Wang D, Li XF and Zheng L. Monocyte/macrophage-elicited natural killer cell dysfunction in hepatocellular carcinoma is mediated by CD48/2B4 interactions. Hepatology 2013; 57: 1107-1116.

[86] Sugimoto K, Shiraki K, Ito T, Fujikawa K, Takase K, Tameda Y, Moriyama M and Nakano T. Expression of functional CD40 in human hepatocellular carcinoma. Hepatology 1999; 30: 920-926.

[87] Morales-Kastresana A, Sanmamed MF, Rodriguez I, Palazon A, Martinez-Forero I, Labiano S, Hervas-Stubbs S, Sangro B, Ochoa C, Rouzaut A, Azpilikueta A, Bolanos E, Jure-Kunkel M,

Gutgemann I and Melero I. Combined immunostimulatory monoclonal antibodies extend survival in an aggressive transgenic hepatocellular carcinoma mouse model. Clin Cancer Res 2013; 19: 6151-6162.

[88] Ahmed-Choudhury J, Russell CL, Randhawa S, Young LS, Adams DH and Afford SC. Differential induction of nuclear factor-kappaB and activator protein-1 activity after CD40 ligation is associated with primary human hepatocyte apoptosis or intrahepatic endothelial cell proliferation. Mol Biol Cell 2003; 14: 1334-1345.

[89] Toes RE, Schoenberger SP, van der Voort EI, Offringa R and Melief CJ. CD40-CD40Ligand interactions and their role in cytotoxic T lymphocyte priming and anti-tumor immunity. Semin Immunol 1998; 10: 443-448.

[90] Berner V, Liu H, Zhou Q, Alderson KL, Sun K, Weiss JM, Back TC, Longo DL, Blazar BR, Wiltrout RH, Welniak LA, Redelman D and Murphy WJ. IFN-gamma mediates CD4+ T-cell loss and impairs secondary antitumor responses after successful initial immunotherapy. Nat Med 2007; 13: 354-360.

[91] Watts TH. TNF/TNFR family members in costimulation of T cell responses. Annu Rev Immunol 2005; 23: 23-68.

[92] Lee HW, Park SJ, Choi BK, Kim HH, Nam KO and Kwon BS. 4-1BB promotes the survival of CD8+ T lymphocytes by increasing expression of Bcl-xL and Bfl-1. J Immunol 2002; 169: 4882-4888.

[93] Hernandez-Chacon JA, Li Y, Wu RC, Bernatchez C, Wang Y, Weber JS, Hwu P and Radvanyi LG. Costimulation through the CD137/4-1BB pathway protects human melanoma tumor-infiltrating lymphocytes from activation-induced cell death and enhances antitumor effector function. J Immunother 2011; 34: 236-250.

[94] Lee H, Park HJ, Sohn HJ, Kim JM and Kim SJ. Combinatorial therapy for liver metastatic colon cancer: dendritic cell vaccine and low-dose agonistic anti-4-1BB antibody co-stimulatory signal. J Surg Res 2011; 169: e43-50.

[95] Gauttier V, Judor JP, Le Guen V, Cany J, Ferry N and Conchon S. Agonistic anti-CD137 antibody treatment leads to antitumor response in mice with liver cancer. Int J Cancer 2014; 135: 2857-2867.

[96] Qiu H, Zhang H, Feng ZH, Geng H and Zhang GM. [Therapeutic effects of simultaneous expression of 4-1BBL and sPD-1 on experimental murine hepatoma]. Zhonghua Gan Zang Bing Za Zhi 2006; 14: 505-509.

[97] Li G, Wu X, Zhang F, Li X, Sun B, Yu Y, Yin A, Deng L, Yin J and Wang X. Triple expression of B7-1, B7-2 and 4-1BBL enhanced antitumor immune response against mouse H22 hepatocellular carcinoma. J Cancer Res Clin Oncol 2011; 137: 695-703.

[98] Pardee AD, McCurry D, Alber S, Hu P, Epstein AL and Storkus WJ. A therapeutic OX40 agonist dynamically alters dendritic, endothelial, and T cell subsets within the established tumor microenvironment. Cancer Res 2010; 70: 9041-9052.

[99] Croft M. Control of immunity by the TNFR-related molecule OX40 (CD134). Annu Rev Immunol 2010; 28: 57-78.

[100] Pan PY, Zang Y, Weber K, Meseck ML and Chen SH. OX40 ligation enhances primary and memory cytotoxic T lymphocyte responses in an immunotherapy for hepatic colon metastases. Mol Ther 2002; 6: 528-536.

[101] Piconese S, Timperi E, Pacella I, Schinzari V, Tripodo C, Rossi M, Guglielmo N, Mennini G, Grazi GL, Di Filippo S, Brozzetti S, Fazzi K, Antonelli G, Lozzi MA, Sanchez M and Barnaba V. Human OX40 tunes the function of regulatory T cells in tumor and nontumor areas of hepatitis C virus-infected liver tissue. Hepatology 2014; 60: 1494-1507.

[102] Morales-Kastresana A, Labiano S, Gutgemann I and Melero I. Combinations of immunostimulatory antibodies with synergistic effects against spontaneous cancer. Oncoimmunology 2014; 3: e27812.

[103] Han Y, Chen Z, Yang Y, Jiang Z, Gu Y, Liu Y, Lin C, Pan Z, Yu Y, Jiang M, Zhou W and Cao X. Human CD14+ CTLA-4+ regulatory dendritic cells suppress T-cell response by cytotoxic T-lymphocyte antigen-4-dependent IL-10 and indoleamine-2,3-dioxygenase production in hepatocellular carcinoma. Hepatology 2014; 59: 567-579.

[104] Lines JL, Sempere LF, Broughton T, Wang L and Noelle R. VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy. Cancer Immunol Res 2014; 2: 510-517.

[105] Baksh K and Weber J. Immune checkpoint protein inhibition for cancer: preclinical justification for CTLA-4 and PD-1 blockade and new combinations. Semin Oncol 2015; 42: 363-377.

[106] Ayaru L, Pereira SP, Alisa A, Pathan AA, Williams R, Davidson B, Burroughs AK, Meyer T and Behboudi S. Unmasking of alpha-fetoprotein-specific CD4(+) T cell responses in hepatocellular carcinoma patients undergoing embolization. J Immunol 2007; 178: 1914-1922.

[107] Mizukoshi E, Yamashita T, Arai K, Sunagozaka H, Ueda T, Arihara F, Kagaya T, Yamashita T, Fushimi K and Kaneko S. Enhancement of tumor-associated antigen-specific T cell responses by radiofrequency ablation of hepatocellular carcinoma. Hepatology 2013; 57: 1448-1457.