Review

DOI: 10.5582/ddt.2015.01054

Immunotherapy for hepatocellular carcinoma

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Summary **Hepatocellular carcinoma (HCC) is the most common type of hepatic malignancies, with poor prognosis. Treatment for HCC are limited, especially for patients with advanced disease who are not eligible for curative hepatectomy or hepatic transplantation. Mechanisms of immune response during tumor development have been investigated for decades. The efficacy and safety of immunotherapy have also been tested in clinical treatment of malignancies. Here we reviewed the immunotherapy strategies for HCC, as well as the particularity of liver immune system and the immune tolerance of HCC. Vaccines, adaptive therapy, immune checkpoint blockades and cytokines are included. We hope this review will give us an integral concept on HCC immunotherapy and help the readers to understand the mechanism of immune tolerance in liver cancer.**

Keywords: Hepatocellular carcinoma, immune tolerance, immunotherapy

1. Introduction

Primary liver cancer is the second leading cause of cancer related death worldwide, with an increasing incidence rate. Asia and Africa have the highest incidence rates of liver cancer all over the world, and China accounts for more than 50% of the whole burden (*1*). Hepatocellular carcinoma (HCC) is the most common type of hepatic malignancies, accounting for approximately 85% of primary liver cancer (*2*). Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is considered as the main risk factor for HCC, which induces a chronic inflammation microenvironment within infected liver (*3*). Other cause factors, including aflatoxin contact, alcohol consumption, obesity, tobacco abusing, *et al*., are also involved in the carcinogenesis and progression of HCC (*4*).

Although public health measures such as HBV vaccine immunization and health education have resulted in a decrease of HCC incidence (*3*), patients' median survival is approximately 6 to 20 months after establishing of diagnosis. Early detection of the disease makes better outcome for HCC patients. Partial hepatectomy is considered as an optimal treatment for patients with adequate liver function and no evidence of portal hypertension or vascular invasion. For other patients with earlier stage HCC but unfavorable liver function, liver transplantation is also a curative procedure (*5*). However, majority of HCC patients developed advanced-stage disease at first diagnosis. Transcatheter arterial chemoembolization (TACE) and chemotherapy are main options for patients with more advanced disease as palliative procedure, but the efficiency is undesirable (*6*). Additionally, sorafenib is the only multi-kinase inhibitor approved by Food and Drug Administration (FDA) for HCC treatment. In a phase III clinical trial, HCC patients receiving sorafenib had a better overall survival (OS) than patients receiving placebo (10.7 months *vs*. 7.9 months) (*7*).

Different from other organs, liver is considered as a lymphoid organ and chronic inflammation in HBV or HCV infected liver would also promote tumor development. Since new therapy strategy is urgent, immunotherapy has been paid more attention in recent years. We will discuss the complicated immune microenvironment within liver and focus on the current immunotherapy strategies for HCC. We hope this review would give a new horizon on HCC immunotherapy.

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2. The liver as a lymphoid organ

The liver has unique vasculature and distinctive dual blood supply, with large blood flow volume (1.5 L per minute). It receives blood from both the systemic circulation (25%, transmitting oxygen *via* hepatic artery) and the portal vein (75%, draining venous blood from the digestive tract, the pancreas and the spleen) (*8*). Thin wall capillaries formed by fenestrated, basement membrane absent liver sinusoidal endothelial cells (LSECs) separate the bloodstream from the hepatocytes and create a space so called hepatic sinusoid (*9*). Mixed blood from portal vein and hepatic artery imported into the hepatic sinusoid. Under physiological conditions, the liver undertakes multiple tasks, including metabolism, detoxification and immune reaction, within hepatic sinusoid. Myriad antigens and dietary component carried by the venous blood from gastrointestinal tract enter the sinusoid *via* blood vessels of the portal triad. The incomplete sinusoidal wall and low velocity of blood flow facilitate the material exchange and immune reaction.

Classic immune organs, such as spleen, lymph nodes and thymus, are well known since their anatomy and histology have been found to be related to immune function. However, organ like liver whose parenchymal cells may not carry the first physiological task as immunoreactivity still performs potential immunological functions. Hepatocytes are the parenchymal cells of liver cells accounting for 80% of total cells, and the remaining 20% are non-parenchymal cells, including LSECs, hepatic stellate cells (HSCs), Kupffer cells (KCs), dendritic cells (DCs) and lymphocytes. These cells have different functions and differential sources, along with hepatocytes together to regulate local and systemic immune function.

LSECs accounting for 50% of hepatic non-parenchymal cells and constitutively express scavenger receptor and mannose receptor that are responsible for recognition and elimination of pathogens, as well as major histocompatibility complex (MHC) I and MHC II and costimulatory molecule (*e.g.* CD80 and CD86). Pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) also express on LSECs (*10,11*). Furthermore, LSECs are considered as professional antigen presenting cells (APCs) and responsible for the defense against foreign antigens from dietary (*12*). However, it maintains a capability to induce immune tolerance. Antigen presentation through MHC molecules to T cells results in an upregulation of specific molecules, including the B7 family member programmed death ligand 1 (PD-L1) (*13*). Inhibitory signaling is transmitted by the formation of PD-L1 and its receptor programmed cell death protein 1 (PD-1), inducing T cell tolerance. Another tolerance-inducing mechanism is induction of rapid tolerization of antigen-specific CD8+ T cell. LSECs antigen cross-presentation to CD8+ T cell will induce rapid proliferation but no effector cytokine production (such as interferon-γ, IFN-γ, or interleukin-2, IL-2), which means a cellular cytotoxicity reduction (*14*). Suppressive cytokine interleukin-10 (IL-10) secreted by liver-resident macrophage KCs also can lead to an induction of LSECs antigen presentation capacity (*15*). Since liver is continuously exposed to massive molecule derived from food and commensal gut flora, hepatic immune tolerance is essential to maintain the immune homeostasis within the whole body.

HSCs are cells with an astral phenotype, located between hepatocytes and LESCs. These HSCs comprise 5-8% of total liver cells (*16*). Under normal conditions, HSCs serve as a storage place for vitamin A and lipid. HSCs act as immune cells by expressing antigen presenting associated molecules, similar to LSECs and KCs, including MHC I, MHC II, CD80 and CD86 (*17,18*). In another study, HSCs failed to perform as APCs since expression of key molecules required for antigen presentation were not observed (*19*). HSCs may participate in immune regulation by other manners. Under chronic inflammatory environment, HSCs differentiate to a more active phenotype, which is myofibroblasts and will promote formation of cirrhosis. Activated HSCs express the immunological modulator PD-L1 and can inhibit T cell responses by inducing T cell apoptosis (*20*).

KCs are special macrophages located within liver and the second majority of hepatic non-parenchymal cells (35%). KCs adhere to LSECs and directly capture pathogens from blood stream. To accomplish its mission as macrophage, KCs express immune receptors such as TLRs, scavenger receptors, complement receptors and so on, Activation of these receptors will activate KCs, which stimulates cytokines production, allowing KCs to function as immune sentinel (*21*). Studies have demonstrated that absence of KCs leaded to severe bacterial infection and even host death, indicating that KCs are essential for immunologic defense (*22*). KCs can eliminate pathogens by recruiting neutrophils, which indicates its capability of pathogen clearance and immune cell recruitment. Molecules associated with antigen presentation also express on KCs, such as MHC I, MHC II, as well as costimulatory molecules. Since the particularity of hepatic physiology by the myriad antigens it will encounter, KCs induce immune tolerance under physiological conditions (*23*). Continuous exposure to lipopolysaccharide can inhibit KCs to activate lymphocytes, which also stimulates KCs to release IL-10 (*23,24*). Prostaglandin E2 produced by KCs abrogates activation of antigen-specific CD4+ T cells (*25*). KCs interacting with regulatory T cells (Tregs), increase IL-10 production by Tregs, promote induction of systemic tolerance (*26*).

DCs locate in the portal triad in a high number, surrounding the central vein (*27*). According to their

different surface markers, they can be divided into five subpopulation, with the two main subpopulation myeloid and lymphoid DCs (*28,29*). Liver DCs internalize antigens and present them to regional lymph node to accomplish their tasks as APCs, but unlike DCs from other tissues, liver DCs appear to be poor activator of T cells response (*29,30*). Studies have demonstrated that cytokine milieu within liver (high IL-10 and low IL-12) contribute to the 'immature' status of DCs (*30*). Furthermore, interaction with LSECs and hepatocytes reduces the capacity of DCs to activate T cells, induced by high production of IL-10 by DCs (*31*). DC-derived IL-10 also promotes a shift from Th1-type responses to Th2-type responses, further suppressing cellular immunity and promoting the development of Tregs (*32*).

Stationary hepatic lymphocytes include significant numbers of natural killer (NK), T cells, B cells, and natural killer T (NKT) cells. They together play important roles in detection, elimination and response to potential pathogens. Among these cells, NK cells comprise the majority of total liver-resident lymphocytes (20-30%), while the percentage of NK cells is less than 5% seen peripheral blood. Enriched NK cells perform duties as a critical sentinel by surveillance for infection, killing of infected hepatocytes, or even for malignant transformation cells (*33*). Activated NK cells release cytotoxic granules containing perforin and granzyme in a cell-directed manner, which will kill target cells. NK cells produce a large amount of cytokines (such as IFN-γ) after being stimulated, which also enhance immune response (*34*). The conventional T cells express CD4 or CD8 molecule, along with a diverse type of T cell receptors (TCR) consisted by α and β chain. In the liver, the number of CD8+ T cells is one to two times the number of CD4+ T-cells, while the ratio is reversed in peripheral blood (*35*). The percentage of γδ T cells in the liver lymphocyesis approximately 20%, which is much higher than it in the blood (*36*). However, the role γδ T cells may play in maintaining liver immune homeostasis still remains unknown γδ TCR can bind to ligands in both an MHC-dependent and MHC-independent fashion (*37*). γδ T cells in the liver take part not only in bacterial infection, but also in tumor immunity. The protective role was performed by Vγ4 γδ T cells by IFN-γ and perforin production after activation, while Vγ1 γδ T cells, another principle subpopulation of γδ T cells, play a regulatory role in tumor immunity by IL-4 production (*38,39*). Some T cells do not express CD4 or CD8. These cells are known as "double negative" T cells and found in the liver, expressing αβ or γδ TCR, which may participate in liver autoimmunity (*40,41*). NKT cells are a particular group of T lymphocytes that express both NK and T cell surface markers. They are also enriched and important immunological component in liver. NKT cells express restricted TCR repertoire and recognize lipid presented by CD1 molecule (*42*). Cytokine production of NKT cells is fast and efficient, ensuring NKT cells to complete its task. NKT cells participate in immune procedure in liver injury, inflammation, fibrosis, and regeneration (*43*). IFN-γ and IL-4 are the main cytokine that produced by NKT cell, which involve in regulating innate and adaptive immunity (*42*). NKT cells also have the capacity to patrol the hepatic vasculature and search for pathogens (*44*).

Hepatic immunity is considered to be immunological tolerance rather than immunity. Since liver is continually exposed to abundant antigens and microbes contained in dietary, to maintain the immune homeostasis, complicated immunological tolerogenic activity is required in hepatic environment to not response to harmless molecules. This can not only reduce the rejection rate of allogeneic liver graft, but also weaken the immunosurveillance, which is detrimental in the case of HCC progression.

3. Immune escape mechanism of HCC

HCC has a unique self-protection mechanism to escape from the host's immunosurveillance. Secretion of immunosuppressive cytokines, abnormal expression of antigens and changes in the local immune microenvironment facilitate the HCC cells to avoid from immune attack (*45*). Evidence has also demonstrated that immunosuppressive factors expressed by tumor cells that inhibit APC or T cell function, which suppress the antigen presentation and immune response, facilitate the immune escaping of tumor cells.

Transforming growth factor-beta (TGF-β) is well known as a typical immunosuppressive factor. It has dual function: one is to inhibit tumor proliferation and initiate tumor cell differentiation and apoptosis in the early stage of tumorogenesis, the other is its immune suppressive potential in advanced stage disease. Moreover, TGF-β also has capability of angiogenesis promotion and epithelial-mesenchymal transition (EMT) induction (*46-48*), which facilitates tumor invasion and metastasis. TGF-β1 is a subtype of TGF-β, and a principal isoform in humans, which is considered as a biomarker for the occurrence and development of tumor. TGF-β1 is also a polypeptide cytokine abundant in the liver, with high biological activity. The expression of TGF-β1 are abnormally elevated in liver cancer (*49*), which mainly involves the inhibition of innate immune and stimulation of Tregs generation to destroy the anti-tumor immune response, resulting in progression of malignancies (*50*).

Another immunosuppressive cytokine is IL-10, which belongs to Th2-type cytokines, produced by monocyte-derived macrophages, Tregs and tumor cells. IL-10 plays a variety of ways in immunosuppression, promote tumor cell escape from immunosurveillance. It can activate the naive CD4+ T cells, and inhibit Th1 cells secretion, thereby affecting the maturation and function of Tregs. It

also reduces the expression of MHC II molecule, as well as CD80/86 or other costimulatory molecules on APCs, and decrease the ability of antigen-presenting. IL-10 also indirectly induces cytotoxic T cells (CTL) into anergy state (*51*).

Tumor antigens refers to new antigens occurred in tumor development or antigens abnormally expressed by tumor cells, which can induce anti-tumor immune response. If the difference between antigens expressed by tumor cells and normal proteins is small, or the antigens have low antigenicity, sufficient immune response will not be induced to remove the tumor cells. Alpha fetal protein (AFP) is an antigen associated with HCC, which synthesized by fetal liver and down-regulated for expression after birth. Malignant transformation will activate the expression of associated genes and the synthesis of the protein is restarted, so AFP is often overexpressed in HCC tumor cells. But due to the immune tolerance the system has established in fetal stage, only high level of AFP cannot induce sufficient immune response to kill tumor cells (*52*).

4. Immunotherapeutic strategies for HCC

As mentioned above, the instinct of hepatic immune system and the immune tolerance induced by HCC tumor cells result in disease progression rather than anti-tumor immunity. The targets involved in this procedure provide us an entry point for study of HCC immunotherapy.

Although AFP protein is considered as a tumor associated antigen (TAA) with low immunogenicity and well tolerated by the host immune system, it is the first target investigated in HCC vaccine therapy. Multiple strategies were used to overcome the limitation of AFP to generate sufficient immune response. In the first AFP vaccine clinical trial, 6 HLA-A*0201 HCC patients with elevated serum AFP were immunized with intradermal vaccinations of four AFP peptides (*53*). These peptides were derived from human AFP with HLA-A*0201-restriction and previously found to stimulate specific T cell responses in cultured peripheral blood lymphocytes (*54*). The result showed all of the patients (6/6) generated T cell responses to most or all of the peptides (*53*). In a subsequent phase I/II trial, AFP peptide-pulsed DCs was administrated and transient T cell response was detected in 6/10 HCC patients (*55*). Another TAA used in HCC vaccine study is glypican-3 (GPC3), which is overexpressed in more than 80% of HCC. HLA-A24-restricted $GPC3_{298-306}$ and HLA-A02-restricted $GPC3_{144-152}$ peptides were proven to induce specific CD8+ CTLs in HLA-A02 and HLA-A24 restricted HCC patients, respectively (*56*). Based on these encouraging results, a phase I clinical trial used these two peptides was performed. After GPC3 peptide vaccine administration, GPC3-specific CTL response was able to detected in 30 patients out of 33 patients. Overall survival was positively associated with GPC3-specific CTL response (*57*). Cell-free vaccines based on AFP and GPC3 DNA vaccines were both tested and showed anti-tumor effect and survival improvement in preclinical research (*58,59*). Elevated expression of telomerase was found in HCC, which makes telomerase a possible target for vaccine treatment. In a phase II study of GV1001, low-dose cyclophosphamide and GM-CSF were used, but did not lead to any responses. Additionally, decreasing in the number of CD4+CD25+Foxp3+ Tregs was observed in this trial (*60*). DC vaccines was found to induce antigen-specific CTLs (*61*), activate NK cells and inhibit Tregs in HCC patients (*62*). DCs fused with allogeneic hepatocellular carcinoma cell line HepG2 activated CD4+ and CD8+ T cells, and CTLs induced by the fusion cells were able to kill autologous HCC (*63*). An encouraging outcome was observed in a phase II clinical trial based on DC vaccine. DCs pulsed with autologous tumor lysates were administered. Among 31 treated patients, 4 patients (12.9%) exhibited partial response, 17 patients (54.8%) had stable disease. The overall 1-year survival rate of all 31 patients was 40.1% (*64*). In another phase II clinical trial, DCs pulsed with lysates of HepG2 cell line containing multiple antigens. 25 patients received at least 3 doses. The radiologically determined disease control rate was 28%. However, the survival was not favorable, with median survival of only 168 days (*65*). New vaccine treatment strategies were under investigation. Fusion antigen also performed better immunogenicity. A combination of full-length HBV core protein and melanoma antigen gene-A induced full development of antitumor response against the epitopes (*66*). Moreover, fusion antigen base on heat shock protein 65 containing different epitopes that involve initiating mechanisms in the immune response also acquired anti-tumor response in HCC bearing BALB/c mouse model (*67*). A highly immunogenic AFP created by computer-guided methodical epitope-optimization showed sufficient anti-tumor effects in mouse HCC model by activating CD8+ T cells (*68*). A phase II, open-label, randomized study on JX-594 for advanced HCC showed desirable result (*69*). JX-594 is an artificial genetic recombination vaccinia virus vaccine (*70-72*). JX-594 is designed to induce virus replication-dependent oncolysis and tumor-specific immunity (*73-75*). Low- or high-dose JX-594 was injected into liver tumors for two different groups of advanced HCC patients. JX-594 replication and granulocyte-macrophage colony-stimulating factor (GM-CSF) expression resulted in oncolysis and induction of anti-tumor immunity. Both doses showed tumor shrink in injected and distant non-injected tumors, with mild side effect such as influenza-like symptoms. Median survival was 14.1 months compared to 6.7 months on the high and low dose, respectively (*69*). In addition, a phase IIb trial on JX-594 is now recruiting advanced

HCC patients who failed sorafenib to detect therapy associated OS and recurrence-free survival (RFS) (NCT01387555).

Adoptive cell transfer (ACT) indicates autologous immune cells transfusion, which are extracted from patient's tumor or peripheral blood, then activated and expanded *in vitro*. This strategy is now promising and well developed in the treatment of solid tumors. ACT has showed considerable anti-tumor effects on HCC in several clinical trials.Cytokine-induced killer (CIK) cells are *in vitro* activated autologous and allogeneic T cells, which have acquired non-specific anti-tumor cytotoxicity and CD56 overexpression, and representing a cell population with double T and NK phenotype (*76*). Positive results were reported in studies on CIK adjuvant immunotherapy. A retrospectively study indicated that CIK cell treatment declined recurrence and metastasis in HCC patients after TACE and radiofrequency ablation (RFA) (*77*). In a randomized, controlled trial, postoperative CIK cell therapy was found to reduce the recurrence and metastasis of HCC. However, there was no improvement on OS (*78*). 150 patients who had undergone curative resection of HCC were enrolled in a randomized clinical trial. Among these patients, 76 patients accepted adoptive immunotherapy, and the remaining 74 patients underwent no adjuvant treatment. The median follow-up was 4.4 years. The trial showed that adoptive immunotherapy declined the frequency of tumor recurrence by 18%, with a better recurrence-free survival and disease-specific survival. No difference was observed in OS between treated and untreated groups (*79*). Several other studies demonstrated the same results (*80-82*). Combination with DC vaccine is another considerable strategy. After curative resection, HCC patients were treated with an autologous tumor lysate-pulsed DC vaccine combined activated T cell transfer combination. It was reported that HCC patients benefit from combination therapy. The median RFS and OS were 24.5 months and 97.7 months in the patients receiving combination therapy and 12.6 months and 41.0 months in the group receiving surgery alone (*83*). Other approaches such as NK cells or Chimeric antigen receptor-T cells (CAR-T) is also considered as a potential treatment for solid tumor. NK cells were found involved in the anti-tumor effect in HCC xenograft mouse models (*84,85*). Although CAR-T therapy has been evaluated in the treatment of hematological malignancies such as lymphoid leukemia (*86,87*) and acute myeloid leukemia (*88*), there is rare evidence for the application of CAR-T in HCC immunotherapy. The safety and efficiency of ACT should be considered and tested by further studies. The combination of immunotherapy also provides approach for in the development of new adaptive immune therapies.

With the deepening of the research, inhibitors targeted immune checkpoints promote the development of solid tumor immune therapy. Co-inhibitory signals transduced by PD-1 or CTLA-4 turn down the T-cell activation induced by antigen presentation. Blockage of such signals will gain an increasing in anti-tumor response. Among many investigated immune checkpoints, PD-1, PD-L1 and CTLA-4 molecules have been identified and antibodies against these targets were used in clinical. Ipilimumab (anti-CTLA-4), pembrolizumab and nivolumab (anti-PD-1) have been approved by the FDA for the treatment of melanoma. Tremelimumab is a monoclonal antibody that blocks CTLA-4. A phase II, non-controlled, multicenter clinical trial enrolled 21 patients with HCC and chronic HCV infection. Each patient received 15 mg/kg tremelimumab every 90 days until tumor progression or severe toxicity. Partial response rate was 17.6%, and disease control rate was 76.4%, with median OS of 8.2 months. A good safety profile was recorded. 45% of patients suffered above grade 3 transaminase toxicity after the first tremelimumab dose, which was not observed in the following doses. In most of the patients, tremelimumab induced a progressive decrease in viral load (*89*). Another phase I/II clinical trial is now under way to test the tremelimumab in combination with local therapies such as TACE or RFA (NCT01853618). Anti-PD-1 and anti-PD-L1 antibodies interfere with the signal transduction by the binding of PD-1 and PD-L1, which inhibits T cell activation and cytokine release (*90*). Among PD-1/PD-L1 targeted treatments, nivolumab is fully human IgG4 monoclonal antibody targeting PD-1 receptor. An active phase I dose escalation clinical trial is now recruiting. Safety and preliminary activity of nivolumab on patients with HCC with or without HBV or HCV infection will be detected in this trial (NCT01658878). A new PD-1 blockade pidilizumab (CT-011) was evaluated in a phase I clinical trial (NCT00966251), which unfortunately terminated because of slow accrual without reporting any results. In addition to PD-1 and CTLA-4, other potential checkpoints, like VISTA, OX40, TIM-3, LAG-3 and BTLA were under investigation (*91*). Preclinical studies have indicated anti-tumor activity of LAG3, TIM-3 and NK-inhibitory receptors, although efficacy and safety in HCC patients has not yet been reported (*92,93*). Studies on immune modulatory molecules such as CD244 (2B4), CD137 (4-1BB), and OX-40 are in progress (*94,95*). Immune checkpoint blockade therapy is considered to be a strategy with a bright future. Notably, CLTA-4 immune checkpoint involves in inhibition of antigen presenting procedure carried by DCs, which decreases CD4+ T cell activation to a specific antigen and increases the IL-10 production by DCs (*96*). Thus will strongly downregulate the antigen presenting capability (**97**). We suggest that combination of vaccine and immune checkpoint inhibitor will enhance TAA-specific immune activation.

Cytokine therapy showed mediate response for

treatment of HCC. Interferon (IFN) is used in the treatment of HCC infection and also shows anti-tumor activity. Several randomized clinical trials on IFN have been completed with mixed results. Although HCC patients may benefit from IFN, more attention should be paid on its side effect. Intratumoral application based on adenovirus-based approach may overcome these limitation (*98*). Chemokines are considered to regulate immune cell function by interacting with the receptors on the membrane. Tumor infiltrating immune cells, including T cells, NK cells and NKT cells, showed enhanced expression of certain receptors (***99***). Preclinical studies indicate that overexpression of certain chemokine genes, such as CXCL10 and CCL5 in HCC tissue predicted a better prognosis, which is correlated with CTL and NK cells (*100*). As we have discussed above, TGF-β is an immunosuppressor in HCC progression. There is a new cytokine targeting therapeutic approach, a novel small molecule inhibitor of TGF-b receptor I, LY2157299, is underinvestigation for HCC treatment. 109 HCC patients were enrolled in a phase II clinical trial. Median OS was 36 weeks. Median OS were 93.1 weeks and 29.6 weeks in AFP responders (> 20% decline from baseline) and non AFP responders, respectively. The trial is still active to further investigate the combination with sorafenib (NCT01246986).

5. Conclusion

Preclinical researches and clinical trials offer many opportunities for the development of HCC treatment. Immune therapeutic strategies such as vaccines, immune checkpoint blockade and ACT, have been proved safe and effective. Clinical application of immune checkpoint blockade provides a new version in malignancy immune therapy, which is also important in HCC. Combination of immune checkpoint blockade such as PD-1/CTLA-4 antibody and other immunotherapy approaches will be a trend and acquire excellent clinical benefits. More translational studies and randomized, controlled trials are needed to promote the development of HCC immunotherapy.

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(Received August 11, 2015; Revised October 23, 2015; Accepted October 26, 2015)