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**Immunotherapy for hepatocellular carcinoma: From basic research to clinical use**

Hong YP *et al*. Immunotherapy for HCC

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**Abstract**

Hepatocellular carcinoma (HCC) is a common cancer worldwide with poor prognosis. Few strategies have been proven efficient in HCC treatment, particularly for those patients not indicated for curative resection or transplantation. Immunotherapy has been developed for decades for cancer control, and is attaining more attention as a result of encouraging outcomes of new strategies such as chimeric antigen receptor T cells and immune checkpoint blockade. Right in the front of new era of immunotherapy, we reviewed the immunotherapy in HCC treatment, from basic research to clinical trials, covering anything from immunomodulators, tumor vaccines, and adoptive immunotherapy. The mechanisms, efficacy and safety as well as the approach particulars were unveiled in assist the readers to gain a concise but extensive understanding of immunotherapy of HCC

**Key words:** Interferon; Chemokine; Tumor vaccine; Adoptive immunotherapy; Checkpoint blockade; Chimeric antigen receptor

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**Core tip:** The paper supplied a comprehensive review on immunotherapy for hepatocellular carcinoma from basic experiments to clinical trials. The development of interferon, chemokine, tumor vaccine, adoptive immunotherapy including NK, NKT, CAR-T as well as Treg was summed up.

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**INTRODUCTION**

Hepatocellular carcinoma (HCC) accounts for 95% of primary liver cancer[1], which is the second most common cause of cancer-associated death worldwide and is estimated to be responsible for around 746000 deaths in 2012[2]. Only liver resection and liver transplantation are considered curative, with poor efficiency of other modalities such as radiofrequency ablation (RFA) and transarterial chemoembolization (TACE). However, very few patients are indicated for liver resection, and donors of liver are usually exceptional. Postoperative recurrence frequently occurs to result in dismal prognosis in most affected individuals[3].

Immunotherapy has been explored in HCC for decades[4], and is placed high expectations due to the recent progress in other malignancies such as melanoma. Different from other organs, liver shows its distinguished characteristics, such as an “immune organ”, and patients with HCC presented unique anti- or pro-tumor responses during the development and progression of HCC[5]. Immunotherapy can be categorized into several types according to their distinct strategies. For instance, immunomodulators and tumor vaccines are used to enhance the immune response to HCC as an indirect way; adoptive immunotherapy introduces great amount of effective immune cells to directly remove tumor cells. In this review, we summarized the critical immune characteristics of liver, and covered the immunotherapy of HCC in animal models as well as clinical trials (Figure 1).

**ROLES OF IMMUNE SYSTEM IN CARCINOGENESIS AND PROGRESSION OF HCC**

The inherent immune tolerance of liver hinders immune surveillance and therefore, makes it possible the carcinogenesis of HCC. Liver confronts abundant xenogenous antigens within blood from gut *via* portal vein. Specific mechanisms with regards to immune tolerance are activated to inhibit unneeded immune responses. Unfortunately, these mechanisms such as recruitment of regulatory T cells (Tregs) and myeloid-derived suppressor cells, as well as overexpression of inhibitory ligands such as programmed death ligand 1 (PD-L1) contribute to weaken anti-tumor immune responses[6].

As an “immune privileged” organ, multiple pathways exist within liver to maintain its function. Not only can hepatocytes cause T cell anergy in certain conditions, but many other nonparenchymal cells including stellate cells, hepatic dendritic cells, and liver sinusoidal endothelial cells also induce tolerance or apoptosis of effective or naive T cells. In particular, HCC lacks major histocompatibility complex (MHC) class II, which is the activator of CD4+ T helper (Th) cells[6].

Clinically, various cytokines dysregulate and contribute to HCC progression[7]. Increased immunosuppress cells in patients parallel with poorer prognosis. Th17 and its secretory product interleukin 17 (IL-17) promote angiogenesis of HCC, and recruit neutrophil to enhance angiogenesis[8,9]. Effector function of CD8+ T cells is prone to be impaired by increased Tregs, which predicts poor prognosis of HCC patients[10]. In addition, the function impairment of other cells like natural killer (NK) cell also contributes to tumor progression[11].

**IMMUNOMODULATORS**

*Cytokines*

Various cytokines involve in immune responses. Certain cytokines can directly inhibit tumor cell growth, or enhance the capacity of relevant immune cells to delay tumor development.

Interferon is well known for immunomodulation, anti-proliferation, and anti-angiogenesis. They were found decreased in serum of patients with HCC. All three types of interferon (IFN-α, IFN-β, IFN-γ) have been proved effective to inhibit HCC by inducing tumor cell apoptosis or autophagy[12-14]. However, the efficiencies among different types of interferon are under debate. Although some investigators addressed that IFN-β showed better anti-HCC effects[15], the use of IFN-α in HCC treatment were more frequently reported. However, IFN-α alone did not show satisfactory survival benefit in patients with unresectable HCC, confirmed by randomized controlled trials (RCTs) [16,17]. IFN-α-2b also failed to decrease the risk of postoperative recurrence[18]. In contrast, interferon showed some benefits when combined with other modalities such as chemotherapy, curative resection, and TACE[19-22]. Two meta-analyses revealed that adjuvant interferon therapy after curative therapy for HCC could improve both overall survival and recurrence-free survival[23,24]. Combination of IFN-α with sorafenib was also reported to be efficient in a mouse model[25], but this has not been tested in human. In addition, interferon treatment may gain further benefits for HCC patients with hepatitis B or C virus infection from removing the viruses[26,27]. Currently, two registered clinical trials regarding IFN-α are still recruiting participants. One multicenter RCT is planning to test IFN-α as an adjuvant therapy in HCC with low miR-26 expression (NCT011681446), and the other phase II trial is trying to combine IFN-α with fluorouracil to treat HCC patients underwent liver resection (NCT01834963). Generally, IFN-α showed demonstrated equivocal effects and should only be applied to selected patients as supportive or adjuvant therapy within the assumption of current evidence.

***Interleukins***

Interleukins have been applied to enhance anti-tumor responses of immune system. However, few studies concerning these interleukins were performed in human. Small-scaled clinical studies evaluated the efficacies of IL-2 or IL-12 alone or combined together in HCC treatment, but the results were inclusive[28,29]. Other sorts of interleukins were also studied. For instance, IL-37 was found to selectively recruit NK cells to conduct anti-tumor activity in HCC patients[30]. IL-24 was reported to show *in vivo* anti-tumor activity in the presence of apoptin[31]. However, clinical trials are lacking to determine the therapeutic effects of interleukins in human.

***Chemokines***

Chemokines regulate activities and behavior of cells including hepatocytes, immune cells, and the tumor microenvironment. By mediating pro- and anti-inflammatory responses, chemokines can regulate leukocyte recruitment, angiogenesis, and tumor progression[32]. Several chemokine associating signaling including CXCR4/CXCL12, CCR6/CCL20 axes were evident to promote HCC[33,34]. Blockade of these signalings by relevant receptors seems logical to control HCC.

Chemokines can regulate the function of immune cells by interacting the receptors on the membrane of these cells. Tumor infiltrating anti-tumor cells including T cells, NK cells, and natural killer T (NKT) cells showed enhanced expression of certain chemokine receptors[35]. Consistently, overexpression of certain chemokine genes such as CXCL10, CCL5, and CCL2 in HCC tissue correlated with Th1, CTL, and NK cells, and predicted better prognosis[36]. Regulated release of chemokines or genetic modification of chemokine receptors in immune cells may enhance anti-tumor immune response. Unfortunately, only preclinical data can be found in this field.

**IMMUNE CHECKPOINT BLOCKADE THERAPY**

Due to the great achievements acquired in melanoma, immune checkpoint blockade therapy sheds light on other solid tumor including HCC. Co-inhibitory signals diminish the intensity of anti-tumor response even though HCC specific antigen has been presented with MHC receptors. To overcome these, immune checkpoints should be a promising approach to restore anti-tumor function of immune cells. Many immune checkpoints have been identified in the lab. In addition to PD-1 and CTLA-4, which have been intensively studied nowadays, other checkpoints like TIM-3, OX40, VISTA, LAG-3 and BTLA are also potential[37].

Anti-CTLA-4 antibody blocks the binding of CTLA-4 and CD80/86, which defunct antigen-presenting cell (APC) and results in suppressed anti-tumor immune responses mediated by T cells[38,39]. Basically, the efficacy of CTLA-4 blockade correlates with the immunogenicity of the tumor. A phase I trial of tremelimumab (anti-CTLA-4 monoclonal antibody) in HCC patients has been reported in 2013[40]. The study enrolled 21 patients with advanced HCC not amenable to percutaneous ablation or TACE, and showed that tremelimumab was well tolerated. Partial response was found in 17.6% of the patients, and 45% of the cases had stable disease for more than 6 months. Another phase I clinical trial of tremlimumab combined with RFA or TACE is now ongoing (NCT01853618; Table 1).

Anti-PD-1 and anti-PD-L1 antibodies interfere the binding of PD-1 and PD-L1/2, which inhibits T cell proliferation and cytokine release[41]. Although CTLA-4 and PD-1 were found predominantly expressed in T cells with anti-tumor function[42, 43], and showed similar effects when used alone, different mechanisms and indicated patients concerning the two pathways were suggested by clinical observations[44]. Unfortunately, the phase I/II trial (NCT00966251) of a new PD-1 blockade CT-011 was terminated because of slow accrual. Another phase I trial of nivolumab (anti-PD-1 monoclonal antibody) is ongoing. This trial plans to recruit three cohorts of patients stratified by viral infection-HCV, HBV and no viral infection.

With the satisfactory effects in animal models, more and more trials are being conducted to investigate the role of immune checkpoint blockade therapy in HCC treatment (Table 1). Immune checkpoint blockade therapy is considered as a strategy with bright future, and is casted into the limelight by oncologists worldwide.

**TUMOR VACCINE**

Although prophylactic vaccine for HCC such as HBV vaccine showed great contribution to the decrease of HCC patients[45], the therapeutic vaccine for HCC remains arriving. Numerous approaches happen to be investigated, seeking to trigger the host immune system to remove cancer cells. The most important constraint for progression of tumor vaccine is lacking of tumor specific antigens or tumor associated antigens (TAA). With all the evolving understanding of tumor heterogeneity, it appears to be unprecedentedly challenging to exterminate cancer cells by tumor vaccine alone. On the other hand, it could possibly be an effective apporach to exhibit positive aspects in certain patients and plays important roles in regimens.

***Peptide based vaccine***

Alpha-fetoprotein (AFP) and glypican-3 (GPC3) are two frequently used TAAs in HCC vaccines. GPC3 can be overexpressed in more than 80% of HCC, and AFP can be positive in 60%-80% of HCC. Other HCC biomarkers that might be candidates for vaccine involved antigen include squamous cell carcinoma antigen, heat shock protein 70, NY-ESO-1b, *etc*[46,47].

AFP is rare in healthy adults, but can be highly expressed in HCC, making it an ideal target for anti-HCC immunotherapy. Actually, AFP is currently the most well studied target antigen for HCC immunotherapy. The earliest relevant clinical trial can be found more than a decade ago, testing AFP-specific T cell response to an AFP-derived peptide in six patients[48]. GPC3(144-152) (FVGEFFTDV) and GPC3(298-306) (EYILSLEEL) peptides were proved to induce specific CD8+ CTLs in HCC patients with HLA-A2 and HLA-A24 restriction, respectively[49]. Encouraged by this result, a phase II trial of GPC3 based vaccine as adjuvant therapy for patients after operation or RFA was registered and is now ongoing (UMIN-CTR: 000002614).

Proper design of epitopes with cross-recognition of wild-type antigens can enhance immune responses. To overcome the limitation of weak immune responses induced by native TAAs, He Y and colleagues created a highly immunogenic AFP *via* computer-guided methodical epitope-optimization. This genetic modified AFP vaccine showed amazing anti-tumor effects in xenograft and diethylnitrosamine-induced mouse model of HCC by means of activating CD8+ T cells[50]. Polypeptide or fusion peptide was another method to amplify anti-tumor immune responses. A combination of full-length HBV core protein, HBV-X protein (HBx)52-60, HBx140-148, AFP158-166, and melanoma antigen gene-A271-279 is an example for HCC vaccine[51]. Innovative creation of fusion peptide containing different epitopes that involving multiple steps of immune response was also proved to inhibit HCC in animals[52].

For peptide based tumor vaccine, the choice of peptide is critical for clinical response. Not all proteins that contribute to tumor progression are suitable for vaccine development. For instance, although expression and activity of telomerase was found up-regulated in most HCCs[53,54], telomerase peptide did not lead to any complete or partial responses in a phase II study on advanced HCC[55]. Additionally, the origin of peptide affects induction efficiency of CLTs and consequent anti-tumor effects. Peptides originated from endogenously presented antigen are thought to be sparse on tumor cells, and is inefficient to induce CTLs[56]. Intratumoral peptide injection was thus developed to enhance tumor cell antigenicity[56], however, this need further investigation.

***DNA based vaccine***

DNA based vaccine assumes that DNA directly injected into body undergoes transcription and translation in host cells, and the expressed peptide induces immune responses. Theoretically, all peptide vaccines can be transformed to DNA vaccines. AFP and GPC3 DNA vaccines were both developed and tested in the lab, showing tumor growth inhibition and survival improvement in mouse models[57-59]. In a recent small-scale clinical observation, two HCC (stage II) patients after locoregional therapy underwent AFP DNA vaccine and adenovirus boost immunization. This approach was confirmed to be safe and well tolerated; however, both patients experienced HCC recurrence after merely nine and eighteen months, correspondingly[60]. To our best information, no clinical trial with regard to DNA vaccine on HCC was reported or ongoing currently.

***Tumor vaccine using antigen presenting cells***

APC plays a key role in anti-tumor function of immune responses. Dendritic cells (DCs) are the most potent APC, and are closely related to HCC. Numerous studies have proved that DCs from peripheral blood and lymph nodes of HCC patients were decreased, with an immature phenotype and an impaired function[61-63]. A study revealed that the more DCs were detected in HCC nodules, the better the prognosis would be. Infiltration of DCs in HCC nodules was strongly associated with the prognosis of HCC patients after surgical resection[64]. The composition of DCs in the hepatic lymph nodes of HCC patients was aberrant which may be one of the causes of the inadequate T cell response against HCC in these patients[65]. In addition, some tumor-derived factors, such as vascular endothelial growth factor, granulocyte macrophage colony-stimulating factor, IL-6 and IL-10 would influence the differentiation, number, and phenotype of DCs[66].

Given the importance in cancer development, DCs are increasingly applied for vaccination in various cancers including HCC. DC based vaccine was reported to not only induce tumor antigen-specific CTLs[67], but also activate NK cells, and inhibit Tregs in HCC patients[51,68]. Logically, DCs pulsed with tumor tissue of individual patient should be used. However, the tumor tissue is not always available. Therefore, peptides or cell line lysate was commonly used to substitute tumor tissue by many investigators. At least in HCC, this replacement strategy was proved feasible and safe[69]. DCs infused with cancer cells, or transfected with total RNA of cancer cells, or transfected with designed plasmids were all able to mature and prime Th1 cells and CTLs[69-71].

Based upon these characteristics and success in preclinical studies, many clinical trials were being carried out to evaluate the efficacy of DCs based immunotherapy to treat HCC patients. Two phase I studies showed immunization by tumor lysate pulsed DCs was feasible for end stage HCC patients[72,73]. Another clinical trial of DC vaccine pulsed with autologous tumor lysate addressed that 12.9% of advanced HCC patients had PR and 54.8% had SD[74]. Notably, monthly boost vaccination resulted in a significantly better 1-year survival[74]. In another RCT on advanced HCC, DCs pulsed with HepG2 cell lysate resulted in 13.3% patients with PR and 60% with SD after 6 mo of treatment[75]. However, the proportion of clinical response regarding this therapy is relatively low. As an illustration, one phase II study using DCs pulsed with tumor lysate in HCC revealed only one out of 39 patients exhibited PR[76]. Furthermore, a phase I/II study using a multiple TAA-pulsed DC vaccine showed clinical response in only one out of five patients with advanced HCC[77]. There are also some studies evaluated the efficacy of DCs immunotherapy combined with local radiation[78] or TACE[79,80], but the results showed that DC infusion could not prevent HCC recurrence. Therefore further studies are needed to increase the efficacy of this therapeutic approach. A new phase I trial on DC vaccine for HCC was registered last year and is now recruiting participants (NCT 01974661; Table 2).

**ADOPTIVE IMMUNOTHERAPY**

Adoptive immunotherapy now becomes promising in the scenario of potential approaches for the treatment of solid tumors, which are refractory to the conventional therapies. An increasing number of literatures discussed the efficacy of adoptive immunotherapy to control tumors. Meanwhile, many clinical trials have demonstrated that adoptive immunotherapy showed potentially promising anti-tumor effects on various cancers including HCC.

***Lymphokine-activated killer cells***

First reported in the early 1980s, lymphokine-activated killer (LAK) cells are cytotoxic effector lymphocytes whose cytolytic activities are not restricted by MHC and are capable of killing tumor cells as well as NK-resistant tumor cell lines. There are actually constrained studies concerning the effectiveness of LAK cells for HCC treatment. A report stated that dealing with LAK cells cultivated by IL-2 reduced recurrence rate in postoperative HCC patients[81]. Another study found that LAK cells based immunotherapy was not an ideal adjuvant strategy after hepatic resection[82].Results from a clinical trial indicated that tumor-specific CTL therapy is more effective than LAK cell therapy in advanced HCC patients[83]. The enthusiasm of study on LAK for HCC treatment significantly declined since last decade.

***Cytokine-induced killer cells***

Cytokine-induced killer (CIK) cells exhibit potent, non-MHC restricted cytolytic activities against susceptible tumor cells of both autologous and allogeneic origins[84]. CIK cells are characterized by expression of both T cell biomarker CD3 and NK cell biomarker CD56[85], and can be generated from the human peripheral blood mononuclear cells (PBMC) induced by IFN-γ, anti-CD3 antibody and IL-2[84,86,87].

Compared with other immune cells, CIK cells possess some advantages. Firstly, CIK cells have higher proliferation rate and can be obtained from cancer patients by *in vitro* culture[88]. Secondly, CIK cells have strong cytolytic activities and cover a broad spectrum of targeted tumors, including those are insusceptible to LAK cells or NK cells[89]. Finally, CIK cells show minimal toxicity and do not cause graft-versus-host disease[84,90]. These merits make CIK cells a preferential adoptive immunotherapy for selected cancer patients[91].

Recently, escalating proofs from clinical trials demonstrated that CIK cells adoptive transfer demonstrated a substantial anti-tumor effect in patients with solid tumors and hematological malignancies[92-94]. Some reports showed that CIK adjuvant immunotherapy significantly improved the outcomes of HCC patients[95-101]. In these studies, CIK cells transfusion reduced the relapse rate of HCC in patients after TACE and RFA therapy, and prolonged the disease-free survival and OS for HCC patients after radically resection or TACE. A meta-analysis including 13 RCTs evaluated the efficacy of CIK immunotherapy in the treatment of HCC, and revealed a significant superiority in prolonging the OS and progressive-free survival of patients[102]. Recently, another meta-analysis assessed the efficacy of CIK therapy after TACE or TACE plus RFA, showing that CIK therapy combined with TACE plus RFA treatment was associated with higher 1-year RFS rate and 1-, 2-year OS rates[103]. However, due to limited number of patients in this field, the efficacy of CIK immunotherapy for HCC is still not convincible.

CIK cells represent an encouraging tool within the cancer adoptive immunotherapy, but more basic researches and clinical trials with high quality are urgently desired. To date, we have witnessed at least four registered clinical trials regarding CIK therapy for HCC ongoing (Table 3).

***Natural killer cell***

Natural killer (NK) cells, which belong to innate immune system, predominantly reside in the liver and play a critical role in the host defense against tumorigenesis[104,105]. The carcinogenesis is under close surveillance of NK cells and other members of immune system. In addition to the capability of killing tumor cells directly, NK cells are able to release immunomodulatory cytokines which can activate leukocytes of both innate and adaptive immune system[105]. Unfortunately, cytotoxicity of NK cells was significantly inhibited in patients with advanced HCC[104]. In line with this, NK cells derived from the HCC patients displayed a reduced cytotoxicity against HCC cell lines after stimulation with IL-2 *in vivo*[106]. The authors suggested that functional defects of NK cells might be responsible for the failure of anti-tumor immune responses. The NK cells from HCC patients were also impaired in their IFN-γ production and cytotoxicity, and this functional impairment was found to be associated with increased Tregs[107]. Meanwhile, myeloid-derived suppressor cells inhibited NK cell cytotoxicity and cytokine secretion[108]. These evidences suggested that HCC patients could be benefited from reactivation of NK cells. In a mouse model, administration of IL-12 and IL-18 increased NK cells in the liver, and resulted in reduction of intrahepatic tumor nodules[109]. Similar outcome was obtained in an additional research, which established that activation of NK cells increased survival in a xenograft mouse model[110]. Thus the approach of enhancing function of NK cells could possibly be accomplished at human HCC treatment. Although many solid evidences turned out the role of NK cells in anti-tumor reaction, there are, however, insufficient clinical studies to corroborate the efficacy of NK cell immunotherapy in HCC. Recently, a study has demonstrated that RFA could activate the peripheral blood circulating NK cells in HCC patients[111]. Two ongoing clinical trials are trying to assess NK cells therapy combined with liver resection (NCT02008929) or liver transplantation for HCC (NCT01147380; Table 3). In the future, it would be of great interest to investigate the efficacy of NK cells combined with other strategies to improve the immunotherapy in HCC.

***Natural killer T cells***

Natural killer T (NKT) cells are a heterogeneous group of T cells, having a range of characteristics different from conventional T cells. Human NKT cells are found in small numbers in healthy liver (0.5%) and blood (0.02%)[112,113], however they are critical players in the regulation of anti-tumor immunity[114-116]. NKT cells are best known for their immunosuppressive functions, however they can interact with many other immune cells such as DC, macrophage as well as NK cells, and the outcome of NKT cell stimulation depends on these interactions and the cytokine milieu[115]. NKT cells can manifest anti-tumor effects mediated by their reactivation with exogenous cytokines or ligands. But recently, the natural role of NKT cells in anti-tumor immunity was reported[112,115]. A phase I trial using autologous NKT cells to treat advanced HCC is now ongoing (NCT01801852; Table 3). Further investigation is needed to elucidate the role of NKT cells in human HCC.

***Chimeric antigen receptor-T cells***

One of the most important aims of T cell engineering is to generate tumor-targeted T cells through the genetic transfer of antigen specific receptors. T cell engineering was consisted of either physiological, MHC-restricted T cell receptors (TCRs) or non-MHC-restricted chimeric antigen receptors. The conception of the chimeric antigen receptor (CAR) originally generated from the growing understanding of the barriers to the effective immune therapy of various types of cancers. T cells armed with CARs (CAR-T cells) are able to recognize the cell surface antigens directly and are not blunted by tumor variations possessing lower surface expression of major MHC antigens which considered as a commonly mechanism of tumor immune escape[117, 118]. The three basic elements of CAR are an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain.

Up to now, three generations of CARs have been developed. The first-generation CAR only contains a T cell signaling domain that transmits the activation signal. It owns a feature of a single signaling domain most commonly derived from the CD3ζ component of the TCR/CD3 complex. The full activation of T cells need multiple signals, and it is obviously that the signaling from these first-generation CARs only provided the so-called “signal one” that could drive T cell effector functions; however, since absence of further signals, the T cells are unable to fully engage their effector machinery[119]. Considering this modular nature of the CAR, later designs aimed to add additional signaling domains which would increase the potency of the CARs and the consequent effector function of T cells. The second-generation CARs incorporate a single co-stimulatory molecule endodomain, such as the endodomain of CD28 or 4-1BB[120]. The third-generation CARs incorporate at least two co-stimulatory molecule endodomains, such as the endodomains of CD28 and 4-1BB[121]. Ligation of CD28 on CAR-T cells through the expression of B7 co-stimulatory ligands on target cells or co-expression of the CD28 molecule together with the scFv (specific monoclonal antibody) and CD3ζ domain of the CAR was shown to promote the proliferation of CAR-modified T cells and anti-tumor activity[122-124].

Plenty of studies have evaluated the efficacy of CAR-T cell treatment in hematological malignancies such as lymphoid leukemia[125,126], and acute myeloid leukemia[127, 128]. Nonetheless, the clinical efficacy of CAR-T cells continues to be marginal in solid tumors as compared to leukemia. So far, as we now have acknowledged, there are not any clinical trials reported evaluating the efficacy of CAR-T in HCC. Only one research stated that genetically modified T cells could be used to reconstitute virus-specific T cell immunity in chronic HBV patients and target tumors in HBV-related HCC. They found TCR re-directed HBV-specific T cells prouduced from PBMC of hepatitis B related HCC patients were capable of recognizing HCC tumor cells[129].

Besides the failed clinical trials of CAR-T cells in renal cell cancer and ovarian cancer[130-132], the safety and tolerance of CAR-T cells likewise need further assessment since trial cease and demise of patients were being often documented[124,133,134]. In a clinical trial of renal cell cancer, the patients administrated with a maximum of 10 infusions of a total 0.2 to 2.1 × 109 CAR-T cells manifested liver enzyme disturbances and finally stopped the treatment[133]. A patient administrated with CAR-T cells based on a Her2/neu-specific CARs died soon after the treatment[124]. These data from renal carcinoma seemed to indicate a dim future of CAR-T applications in solid tumors. To achieve a better clinical response, CAR-T cells need to overcome two major barriers, which are insufficient T-cell migration into the lesions and highly immunosuppressive microenvironments within the tumors[118,135,136].

Considered collectively, the original studies have highlighted two important lessons to all of us. To begin with, clinical anti-tumor responses of T cells seem to be proportional to T cell persistence. Consequently, we ought to enhance the T cell persistence and then try to efficiently traffic adequate quantities of CAR-T cells from the peripheral blood towards tumor tissues. Once arrived, these CAR-T cells must functionally respond against tumor cells within such immunosuppressive context. Secondly, understanding and defining the specific target is crucial for the safety of CAR-T treatment. Increasing evidences demonstrated that a careful choice of target antigen, including an understanding of accessibility and expression level must be under consideration for future CAR-T clinical trials.

***Treg based immunotherapy***

Treg mediated immunosuppression is the essential mechanism accountable for tumor immune evasion, and could be the primary hurdle of tumor immunotherapy[137]. Tregs characterized by the CD4+CD25+FoxP3+, playing a critical role in immune homeostasis and suppress function of immune cells such as CD8+T cells, CD4+ T cells and NKT cells. Many reports have demonstrated that the amount of Tregs in solid tumor decreased and inversely related to the prognosis of HCC patients[10,138-141].

Targeting Tregs has been of great interest to change the immune suppression milieu and enhance tumor specific immune responses. Depletion of Tregs using anti-CD25 monoclonal antibody has shown substantial anti-tumor effects in murine tumor models[142]. Nonspecific approach of CD25+ T cell depletion by injection of PC61 antibody was also tested on an orthotropic HCC model, leading to a significant protection against tumor development[143]. No such strategy has been performed on human till now.

**CONCLUSION**

An array of translational research and pilot clinical trials have revealed that immunotherapy is safe and tolerant for patients with cancers. The efficacies are also offered in some type of immunotherapy in selected patients. In HCC, more studies, including basic and clinical researches, are urgently required to improve the outcomes of immunotherapy with best-cost performance. Currently, immune checkpoint blockade and CAR-T strategies are specifically expected. In addition, it is become obvious that incorporating standard anti-tumor therapies with immunotherapy is a most likely effective alternative. Ultimately, more substantial randomized, controlled trials are required to authenticate the efficacy of immunotherapy for HCC patients.

**REFERENCES**

1 **Lau WY**. Primary liver tumors. *Semin Surg Oncol* 2000; **19**: 135-144 [PMID: 11126378]

2 **Cancer IAfRo.** GLOBOCAN 2012: estimated cancer incidence, motality and prevalence worldwide in 2012. Available from: http: //globocan.iarc.fr/Pages/fact\_sheets\_cancer.aspx. Accessed in 26 August, 2014

3 **Bruix J**, Sherman M; American Association for the Study of Liver D. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

4 **Greten TF**, Manns MP, Korangy F. Immunotherapy of HCC. *Rev Recent Clin Trials* 2008; **3**: 31-39 [PMID: 18474013 DOI: 10.2174/157488708783330549]

5 **Flecken T**, Spangenberg HC, Thimme R. Immunobiology of hepatocellular carcinoma. *Langenbecks Arch Surg* 2012; **397**: 673-680 [PMID: 21479622 DOI: 10.1007/s00423-011-0783-x]

6 **Miamen AG**, Dong H, Roberts LR. Immunotherapeutic approaches to hepatocellular carcinoma treatment. *Liver Cancer* 2012; **1**: 226-237 [PMID: 24159587 DOI: 10.1159/000343837]

7 **Budhu A**, Wang XW. The role of cytokines in hepatocellular carcinoma. *J Leukoc Biol* 2006; **80**: 1197-1213 [PMID: 16946019 DOI: 10.1189/jlb.0506297]

8 **Kuang DM**, Zhao Q, Wu Y, Peng C, Wang J, Xu Z, Yin XY, Zheng L. Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma. *J Hepatol* 2011; **54**: 948-955 [PMID: 21145847 DOI: 10.1016/j.jhep.2010.08.041]

9 **Zhang JP**, Yan J, Xu J, Pang XH, Chen MS, Li L, Wu C, Li SP, Zheng L. Increased intratumoral IL-17-producing cells correlate with poor survival in hepatocellular carcinoma patients. *J Hepatol* 2009; **50**: 980-989 [PMID: 19329213 DOI: 10.1016/j.jhep.2008.12.033]

10 **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, Wang FS. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology* 2007; **132**: 2328-2339 [PMID: 17570208 DOI: 10.1053/j.gastro.2007.03.102]

11 **Li T**, Yang Y, Hua X, Wang G, Liu W, Jia C, Tai Y, Zhang Q, Chen G. Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO. *Cancer Lett* 2012; **318**: 154-161 [PMID: 22182446 DOI: 10.1016/j.canlet.2011.12.020]

12 **Herzer K**, Hofmann TG, Teufel A, Schimanski CC, Moehler M, Kanzler S, Schulze-Bergkamen H, Galle PR. IFN-alpha-induced apoptosis in hepatocellular carcinoma involves promyelocytic leukemia protein and TRAIL independently of p53. *Cancer Res* 2009; **69**: 855-862 [PMID: 19141642 DOI: 10.1158/0008-5472.CAN-08-2831]

13 **Li P**, Du Q, Cao Z, Guo Z, Evankovich J, Yan W, Chang Y, Shao L, Stolz DB, Tsung A, Geller DA. Interferon-γ induces autophagy with growth inhibition and cell death in human hepatocellular carcinoma (HCC) cells through interferon-regulatory factor-1 (IRF-1). *Cancer Lett* 2012; **314**: 213-222 [PMID: 22056812 DOI: 10.1016/j.canlet.2011.09.031]

14 **Obora A**, Shiratori Y, Okuno M, Adachi S, Takano Y, Matsushima-Nishiwaki R, Yasuda I, Yamada Y, Akita K, Sano T, Shimada J, Kojima S, Okano Y, Friedman SL, Moriwaki H. Synergistic induction of apoptosis by acyclic retinoid and interferon-beta in human hepatocellular carcinoma cells. *Hepatology* 2002; **36**: 1115-1124 [PMID: 12395321 DOI: 10.1053/jhep.2002.36369]

15 **Damdinsuren B**, Nagano H, Wada H, Kondo M, Ota H, Nakamura M, Noda T, Natsag J, Yamamoto H, Doki Y, Umeshita K, Dono K, Nakamori S, Sakon M, Monden M. Stronger growth-inhibitory effect of interferon (IFN)-beta compared to IFN-alpha is mediated by IFN signaling pathway in hepatocellular carcinoma cells. *Int J Oncol* 2007; **30**: 201-208 [PMID: 17143530 DOI: 10.1016/j.canlet.2011.09.031.]

16 **Lai CL**, Lau JY, Wu PC, Ngan H, Chung HT, Mitchell SJ, Corbett TJ, Chow AW, Lin HJ. Recombinant interferon-alpha in inoperable hepatocellular carcinoma: a randomized controlled trial. *Hepatology* 1993; **17**: 389-394 [PMID: 8383088]

17 **Llovet JM**, Sala M, Castells L, Suarez Y, Vilana R, Bianchi L, Ayuso C, Vargas V, Rodés J, Bruix J. Randomized controlled trial of interferon treatment for advanced hepatocellular carcinoma. *Hepatology* 2000; **31**: 54-58 [PMID: 10613728 DOI: 10.1002/hep.510310111]

18 **Chen LT**, Chen MF, Li LA, Lee PH, Jeng LB, Lin DY, Wu CC, Mok KT, Chen CL, Lee WC, Chau GY, Chen YS, Lui WY, Hsiao CF, Whang-Peng J, Chen PJ. Long-term results of a randomized, observation-controlled, phase III trial of adjuvant interferon Alfa-2b in hepatocellular carcinoma after curative resection. *Ann Surg* 2012; **255**: 8-17 [PMID: 22104564 DOI: 10.1097/SLA.0b013e3182363ff9]

19 **Yeo W**, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ. A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005; **97**: 1532-1538 [PMID: 16234567 DOI: 10.1093/jnci/dji315]

20 **Kasai K**, Ushio A, Kasai Y, Sawara K, Miyamoto Y, Oikawa K, Kuroda H, Takikawa Y, Suzuki K. Therapeutic efficacy of combination therapy with intra-arterial 5-fluorouracil and systemic pegylated interferon α-2b for advanced hepatocellular carcinoma with portal venous invasion. *Cancer* 2012; **118**: 3302-3310 [PMID: 22072099 DOI: 10.1002/cncr.26648]

21 **Lee D**, Chung YH, Kim JA, Park WH, Jin YJ, Shim JH, Ryu SH, Jang MK, Yu E, Lee YJ. Safety and efficacy of adjuvant pegylated interferon therapy for metastatic tumor antigen 1-positive hepatocellular carcinoma. *Cancer* 2013; **119**: 2239-2246 [PMID: 23564564 DOI: 10.1002/cncr.28082]

22 **Li M**, Lu C, Cheng J, Zhang J, Cao C, Xu J, Xu J, Pan H, Zhong B, Tucker S, Wang D. Combination therapy with transarterial chemoembolization and interferon-alpha compared with transarterial chemoembolization alone for hepatitis B virus related unresectable hepatocellular carcinoma. *J Gastroenterol Hepatol* 2009; **24**: 1437-1444 [PMID: 19486255 DOI: 10.1111/j.1440-1746.2009.05863.x]

23 **Shen YC**, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. *J Hepatol* 2010; **52**: 889-894 [PMID: 20395009 DOI: 10.1016/j.jhep.2009.12.041]

24 **Wang J**, He XD, Yao N, Liang WJ, Zhang YC. A meta-analysis of adjuvant therapy after potentially curative treatment for hepatocellular carcinoma. *Can J Gastroenterol* 2013; **27**: 351-363 [PMID: 23781519]

25 **Wang L**, Jia D, Duan F, Sun Z, Liu X, Zhou L, Sun L, Ren S, Ruan Y, Gu J. Combined anti-tumor effects of IFN-α and sorafenib on hepatocellular carcinoma in vitro and in vivo. *Biochem Biophys Res Commun* 2012; **422**: 687-692 [PMID: 22634008 DOI: 10.1016/j.bbrc.2012.05.056]

26 **Perrillo R**. Benefits and risks of interferon therapy for hepatitis B. *Hepatology* 2009; **49**: S103-S111 [PMID: 19399806 DOI: 10.1002/hep.22956]

27 **Xu G**, Yang F, Ding CL, Wang J, Zhao P, Wang W, Ren H. MiR-221 accentuates IFN׳s anti-HCV effect by downregulating SOCS1 and SOCS3. *Virology* 2014; **462-463**: 343-350 [PMID: 25019494 DOI: 10.1016/j.virol.2014.06.024]

28 **Lygidakis NJ**, Kosmidis P, Ziras N, Parissis J, Kyparidou E. Combined transarterial targeting locoregional immunotherapy-chemotherapy for patients with unresectable hepatocellular carcinoma: a new alternative for an old problem. *J Interferon Cytokine Res* 1995; **15**: 467-472 [PMID: 7544232 DOI: 10.1089/jir.1995.15.467]

29 **Sangro B**, Mazzolini G, Ruiz J, Herraiz M, Quiroga J, Herrero I, Benito A, Larrache J, Pueyo J, Subtil JC, Olagüe C, Sola J, Sádaba B, Lacasa C, Melero I, Qian C, Prieto J. Phase I trial of intratumoral injection of an adenovirus encoding interleukin-12 for advanced digestive tumors. *J Clin Oncol* 2004; **22**: 1389-1397 [PMID: 15084613 DOI: 10.1200/JCO.2004.04.059]

30 **Zhao JJ**, Pan QZ, Pan K, Weng DS, Wang QJ, Li JJ, Lv L, Wang DD, Zheng HX, Jiang SS, Zhang XF, Xia JC. Interleukin-37 mediates the antitumor activity in hepatocellular carcinoma: role for CD57+ NK cells. *Sci Rep* 2014; **4**: 5177 [PMID: 24898887 DOI: 10.1038/srep05177]

31 **Yuan L**, Zhao H, Zhang L, Liu X. The efficacy of combination therapy using adeno-associated virus-mediated co-expression of apoptin and interleukin-24 on hepatocellular carcinoma. *Tumour Biol* 2013; **34**: 3027-3034 [PMID: 23907578 DOI: 10.1007/s13277-013-0867-z]

32 **Marra F**, Tacke F. Roles for chemokines in liver disease. *Gastroenterology* 2014; **147**: 577-594.e1 [PMID: 25066692 DOI: 10.1053/j.gastro.2014.06.043]

33 **Ghanem I**, Riveiro ME, Paradis V, Faivre S, de Parga PM, Raymond E. Insights on the CXCL12-CXCR4 axis in hepatocellular carcinoma carcinogenesis. *Am J Transl Res* 2014; **6**: 340-352 [PMID: 25075251]

34 **Du D**, Liu Y, Qian H, Zhang B, Tang X, Zhang T, Liu W. The effects of the CCR6/CCL20 biological axis on the invasion and metastasis of hepatocellular carcinoma. *Int J Mol Sci* 2014; **15**: 6441-6452 [PMID: 24743888 DOI: 10.3390/ijms15046441]

35 **Liu Y**, Poon RT, Hughes J, Feng X, Yu WC, Fan ST. Chemokine receptors support infiltration of lymphocyte subpopulations in human hepatocellular carcinoma. *Clin Immunol* 2005; **114**: 174-182 [PMID: 15639651 DOI: 10.1016/j.clim.2004.10.006]

36 **Chew V**, Chen J, Lee D, Loh E, Lee J, Lim KH, Weber A, Slankamenac K, Poon RT, Yang H, Ooi LL, Toh HC, Heikenwalder M, Ng IO, Nardin A, Abastado JP. Chemokine-driven lymphocyte infiltration: an early intratumoural event determining long-term survival in resectable hepatocellular carcinoma. *Gut* 2012; **61**: 427-438 [PMID: 21930732 DOI: 10.1136/gutjnl-2011-300509]

37 **Hato T**, Goyal L, Greten TF, Duda DG, Zhu AX. Immune checkpoint blockade in hepatocellular carcinoma: current progress and future directions. *Hepatology* 2014; **60**: 1776-1782 [PMID: 24912948 DOI: 10.1002/hep.27246]

38 **Grohmann U**, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, Candeloro P, Belladonna ML, Bianchi R, Fioretti MC, Puccetti P. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat Immunol* 2002; **3**: 1097-1101 [PMID: 12368911 DOI: 10.1038/ni846]

39 **Schneider H**, Downey J, Smith A, Zinselmeyer BH, Rush C, Brewer JM, Wei B, Hogg N, Garside P, Rudd CE. Reversal of the TCR stop signal by CTLA-4. *Science* 2006; **313**: 1972-1975 [PMID: 16931720 DOI: 10.1126/science.1131078]

40 **Sangro B**, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, Barrera P, Riezu-Boj JI, Larrea E, Alfaro C, Sarobe P, Lasarte JJ, Pérez-Gracia JL, Melero I, 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 [PMID: 23466307 DOI: 10.1016/j.jhep.2013.02.022]

41 **Okazaki T**, Maeda A, Nishimura H, Kurosaki T, Honjo T. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. *Proc Natl Acad Sci USA* 2001; **98**: 13866-13871 [PMID: 11698646 DOI: 10.1073/pnas.231486598]

42 **Manzotti CN**, Liu MK, Burke F, Dussably L, Zheng Y, Sansom DM. Integration of CD28 and CTLA-4 function results in differential responses of T cells to CD80 and CD86. *Eur J Immunol* 2006; **36**: 1413-1422 [PMID: 16708397 DOI: 10.1002/eji.200535170]

43 **Francisco LM**, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med* 2009; **206**: 3015-3029 [PMID: 20008522 DOI: 10.1084/jem.20090847]

44 **Hamid O**, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, Dronca R, Gangadhar TC, Patnaik A, Zarour H, Joshua AM, Gergich K, Elassaiss-Schaap J, Algazi A, Mateus C, Boasberg P, Tumeh PC, Chmielowski B, Ebbinghaus SW, Li XN, Kang SP, Ribas A. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 2013; **369**: 134-144 [PMID: 23724846 DOI: 10.1056/NEJMoa1305133]

45 **Chang MH**. Cancer prevention by vaccination against hepatitis B. *Recent Results Cancer Res* 2009; **181**: 85-94 [PMID: 19213561]

46 **Zhao YJ,** Ju Q, Li GC. Tumor markers for hepatocellular carcinoma. *Mol Clin Oncol* 2013; **1**: 593-598 [PMID: 24649215 DOI: 10.3892/mco.2013.119]

47 **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, 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 [PMID: 15501973 DOI: 10.1158/1078-0432.CCR-04-0502]

48 **Butterfield LH**, Ribas A, Meng WS, Dissette VB, Amarnani S, Vu HT, Seja E, Todd K, Glaspy JA, McBride WH, 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 [PMID: 14676113]

49 **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, 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 [PMID: 16675560 DOI: 10.1158/1078-0432.CCR-05-2267]

50 **Hong Y**, Peng Y, Guo ZS, Guevara-Patino J, Pang J, Butterfield LH, Mivechi NF, Munn DH, Bartlett DL, He Y. Epitope-optimized alpha-fetoprotein genetic vaccines prevent carcinogen-induced murine autochthonous hepatocellular carcinoma. *Hepatology* 2014; **59**: 1448-1458 [PMID: 24122861 DOI: 10.1002/hep.26893]

51 **Chen Y**, Yang D, Li S, Gao Y, Jiang R, Deng L, Frankel FR, Sun B. Development of a Listeria monocytogenes-based vaccine against hepatocellular carcinoma. *Oncogene* 2012; **31**: 2140-2152 [PMID: 21927025 DOI: 10.1038/onc.2011.395]

52 **Zhang Y**, Xu J, Zhao R, Liu J, Wu J. Inhibition effects on liver tumors of BALB/c mice bearing H22 cells by immunization with a recombinant immunogen of GnRH linked to heat shock protein 65. *Vaccine* 2007; **25**: 6911-6921 [PMID: 17728021 DOI: 10.1016/j.vaccine.2007.07.034]

53 **Saini N**, Srinivasan R, Chawla Y, Sharma S, Chakraborti A, Rajwanshi A. Telomerase activity, telomere length and human telomerase reverse transcriptase expression in hepatocellular carcinoma is independent of hepatitis virus status. *Liver Int* 2009; **29**: 1162-1170 [PMID: 19627485 DOI: 10.1111/j.1478-3231.2009.02082.x]

54 **Shimada M**, Hasegawa H, Gion T, Utsunomiya T, Shirabe K, Takenaka K, Otsuka T, Maehara Y, Sugimachi K. The role of telomerase activity in hepatocellular carcinoma. *Am J Gastroenterol* 2000; **95**: 748-752 [PMID: 10710069 DOI: 10.1111/j.1572-0241.2000.01855.x]

55 **Greten TF**, Forner A, Korangy F, N'Kontchou G, Barget N, Ayuso C, Ormandy LA, Manns MP, Beaugrand M, Bruix J. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. *BMC Cancer* 2010; **10**: 209 [PMID: 20478057 DOI: 10.1186/1471-2407-10-209]

56 **Nobuoka D**, Yoshikawa T, Sawada Y, Fujiwara T, Nakatsura T. Peptide vaccines for hepatocellular carcinoma. *Hum Vaccin Immunother* 2013; **9**: 210-212 [PMID: 23442593 DOI: 10.4161/hv.22473]

57 **Lan YH**, Li YG, Liang ZW, Chen M, Peng ML, Tang L, Hu HD, Ren H. A DNA vaccine against chimeric AFP enhanced by HSP70 suppresses growth of hepatocellular carcinoma. *Cancer Immunol Immunother* 2007; **56**: 1009-1016 [PMID: 17186291 DOI: 10.1007/s00262-006-0254-3]

58 **Li SQ**, Lin J, Qi CY, Fu SJ, Xiao WK, Peng BG, Liang LJ. GPC3 DNA vaccine elicits potent cellular antitumor immunity against HCC in mice. *Hepatogastroenterology* 2014; **61**: 278-284 [PMID: 24901124]

59 **Morozov AV**, Morozov VA, Astakhova TM, Timofeev AV, Karpov VL. [DNA vaccine encoding alpha-fetoprotein with fused ornithine decarboxylase degradation signal significantly suppresses hepatocellular carcinoma growth in mice]. *Mol Biol (Mosk)* 2012; **46**: 434-451 [PMID: 22888633]

60 **Butterfield LH**, Economou JS, Gamblin TC, Geller DA. Alpha fetoprotein DNA prime and adenovirus boost immunization of two hepatocellular cancer patients. *J Transl Med* 2014; **12**: 86 [PMID: 24708667 DOI: 10.1186/1479-5876-12-86]

61 **Kakumu S**, Ito S, Ishikawa T, Mita Y, Tagaya T, Fukuzawa Y, Yoshioka K. Decreased function of peripheral blood dendritic cells in patients with hepatocellular carcinoma with hepatitis B and C virus infection. *J Gastroenterol Hepatol* 2000; **15**: 431-436 [PMID: 10824889 DOI: 10.1046/j.1440-1746.2000.02161.x]

62 **Ormandy LA**, Farber A, Cantz T, Petrykowska S, Wedemeyer H, Horning M, Lehner F, Manns MP, Korangy F, Greten TF. Direct ex vivo analysis of dendritic cells in patients with hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 3275-3282 [PMID: 16718852 DOI: 10.3748/wjg.v12.i20.3275]

63 **Ninomiya T**, Akbar SM, Masumoto T, Horiike N, Onji M. Dendritic cells with immature phenotype and defective function in the peripheral blood from patients with hepatocellular carcinoma. *J Hepatol* 1999; **31**: 323-331 [PMID: 10453947 DOI: 10.1016/S0168-8278(99)80231-1]

64 **Cai XY**, Gao Q, Qiu SJ, Ye SL, Wu ZQ, Fan J, Tang ZY. Dendritic cell infiltration and prognosis of human hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2006; **132**: 293-301 [PMID: 16421755 DOI: 10.1007/s00432-006-0075-y]

65 **Tang TJ**, Vukosavljevic D, Janssen HL, Binda RS, Mancham S, Tilanus HW, Ijzermans JN, Drexhage H, Kwekkeboom J. Aberrant composition of the dendritic cell population in hepatic lymph nodes of patients with hepatocellular carcinoma. *Hum Pathol* 2006; **37**: 332-338 [PMID: 16613328 DOI: 10.1016/j.humpath.2005.11.007]

66 **Gabrilovich D**. Mechanisms and functional significance of tumour-induced dendritic-cell defects. *Nat Rev Immunol* 2004; **4**: 941-952 [PMID: 15573129 DOI: 10.1038/nri1498]

67 **Sun JC**, Pan K, Chen MS, Wang QJ, Wang H, Ma HQ, Li YQ, Liang XT, Li JJ, Zhao JJ, Chen YB, Pang XH, Liu WL, Cao Y, Guan XY, Lian QZ, Xia JC. Dendritic cells-mediated CTLs targeting hepatocellular carcinoma stem cells. *Cancer Biol Ther* 2010; **10**: 368-375 [PMID: 20581468]

68 **Bray SM**, Vujanovic L, Butterfield LH. Dendritic cell-based vaccines positively impact natural killer and regulatory T cells in hepatocellular carcinoma patients. *Clin Dev Immunol* 2011; **2011**: 249281 [PMID: 21969837 DOI: 10.1155/2011/249281]

69 **Cao DY**, Yang JY, Yue SQ, Tao KS, Song ZS, Wang DS, Yang YL, Dou KF. Comparative analysis of DC fused with allogeneic hepatocellular carcinoma cell line HepG2 and autologous tumor cells as potential cancer vaccines against hepatocellular carcinoma. *Cell Immunol* 2009; **259**: 13-20 [PMID: 19545862 DOI: 10.1016/j.cellimm.2009.05.007]

70 **Zhang HM**, Zhang LW, Liu WC, Cheng J, Si XM, Ren J. Comparative analysis of DC fused with tumor cells or transfected with tumor total RNA as potential cancer vaccines against hepatocellular carcinoma. *Cytotherapy* 2006; **8**: 580-588 [PMID: 17148035 DOI: 10.1080/14653240600991353]

71 **Homma S**, Komita H, Sagawa Y, Ohno T, Toda G. Antitumour activity mediated by CD4+ cytotoxic T lymphocytes against MHC class II-negative mouse hepatocellular carcinoma induced by dendritic cell vaccine and interleukin-12. *Immunology* 2005; **115**: 451-461 [PMID: 16011514 DOI: 10.1111/j.1365-2567.2005.02179.x]

72 **Iwashita Y**, Tahara K, Goto S, Sasaki A, Kai S, Seike M, Chen CL, Kawano K, Kitano S. A phase I study of autologous dendritic cell-based immunotherapy for patients with unresectable primary liver cancer. *Cancer Immunol Immunother* 2003; **52**: 155-161 [PMID: 12649744]

73 **Ladhams A**, Schmidt C, Sing G, Butterworth L, Fielding G, Tesar P, Strong R, Leggett B, Powell L, Maddern G, Ellem K, Cooksley G. Treatment of non-resectable hepatocellular carcinoma with autologous tumor-pulsed dendritic cells. *J Gastroenterol Hepatol* 2002; **17**: 889-896 [PMID: 12164965 DOI: 10.1046/j.1440-1746.2002.02817.x]

74 **Lee WC**, Wang HC, Hung CF, Huang PF, Lia CR, Chen MF. Vaccination of advanced hepatocellular carcinoma patients with tumor lysate-pulsed dendritic cells: a clinical trial. *J Immunother* 2005; **28**: 496-504 [PMID: 16113606 DOI: 10.1097/01.cji.0000171291.72039.e2]

75 **El Ansary M**, Mogawer S, Elhamid SA, Alwakil S, Aboelkasem F, Sabaawy HE, Abdelhalim O. Immunotherapy by autologous dendritic cell vaccine in patients with advanced HCC. *J Cancer Res Clin Oncol* 2013; **139**: 39-48 [PMID: 22886490 DOI: 10.1007/s00432-012-1298-8]

76 **Palmer DH**, Midgley RS, Mirza N, Torr EE, Ahmed F, Steele JC, Steven NM, Kerr DJ, Young LS, Adams DH. A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. *Hepatology* 2009; **49**: 124-132 [PMID: 18980227 DOI: 10.1002/hep.22626]

77 **Tada F**, Abe M, Hirooka M, Ikeda Y, Hiasa Y, Lee Y, Jung NC, Lee WB, Lee HS, Bae YS, Onji M. Phase I/II study of immunotherapy using tumor antigen-pulsed dendritic cells in patients with hepatocellular carcinoma. *Int J Oncol* 2012; **41**: 1601-1609 [PMID: 22971679 DOI: 10.3892/ijo.2012.1626]

78 **Chi KH**, Liu SJ, Li CP, Kuo HP, Wang YS, Chao Y, Hsieh SL. Combination of conformal radiotherapy and intratumoral injection of adoptive dendritic cell immunotherapy in refractory hepatoma. *J Immunother* 2005; **28**: 129-135 [PMID: 15725956]

79 **Nakamoto Y**, Mizukoshi E, Tsuji H, Sakai Y, Kitahara M, Arai K, Yamashita T, Yokoyama K, Mukaida N, Matsushima K, Matsui O, Kaneko S. Combined therapy of transcatheter hepatic arterial embolization with intratumoral dendritic cell infusion for hepatocellular carcinoma: clinical safety. *Clin Exp Immunol* 2007; **147**: 296-305 [PMID: 17223971 DOI: 10.1111/j.1365-2249.2006.03290.x]

80 **Mizukoshi E**, Nakamoto Y, Arai K, Yamashita T, Mukaida N, Matsushima K, Matsui O, Kaneko S. Enhancement of tumor-specific T-cell responses by transcatheter arterial embolization with dendritic cell infusion for hepatocellular carcinoma. *Int J Cancer* 2010; **126**: 2164-2174 [PMID: 19739081 DOI: 10.1002/ijc.24882]

81 **Une Y**, Kawata A, Uchino J. [Adopted immunochemotherapy using IL-2 and spleen LAK cell--randomized study]. *Nihon Geka Gakkai Zasshi* 1991; **92**: 1330-1333 [PMID: 1658591]

82 **Kawata A**, Une Y, Hosokawa M, Wakizaka Y, Namieno T, Uchino J, Kobayashi H. Adjuvant chemoimmunotherapy for hepatocellular carcinoma patients. Adriamycin, interleukin-2, and lymphokine-activated killer cells versus adriamycin alone. *Am J Clin Oncol* 1995; **18**: 257-262 [PMID: 7747715]

83 **Haruta I**, Yamauchi K, Aruga A, Komatsu T, Takasaki K, Hayashi N, Hanyu F. Analytical study of the clinical response to two distinct adoptive immunotherapies for advanced hepatocellular carcinoma: comparison between LAK cell and CTL therapy. *J Immunother Emphasis Tumor Immunol* 1996; **19**: 218-223 [PMID: 8811496]

84 **Introna M**, Golay J, Rambaldi A. Cytokine Induced Killer (CIK) cells for the treatment of haematological neoplasms. *Immunol Lett* 2013; **155**: 27-30 [PMID: 24084446 DOI: 10.1016/j.imlet.2013.09.017]

85 **Linn YC**, Hui KM. Cytokine-induced killer cells: NK-like T cells with cytotolytic specificity against leukemia. *Leuk Lymphoma* 2003; **44**: 1457-1462 [PMID: 14565644 DOI: 10.3109/10428190309178764]

86 **Schmidt-Wolf IG**, Negrin RS, Kiem HP, Blume KG, Weissman IL. Use of a SCID mouse/human lymphoma model to evaluate cytokine-induced killer cells with potent antitumor cell activity. *J Exp Med* 1991; **174**: 139-149 [PMID: 1711560]

87 **Zoll B**, Lefterova P, Csipai M, Finke S, Trojaneck B, Ebert O, Micka B, Roigk K, Fehlinger M, Schmidt-Wolf GD, Huhn D, Schmidt-Wolf IG. Generation of cytokine-induced killer cells using exogenous interleukin-2, -7 or -12. *Cancer Immunol Immunother* 1998; **47**: 221-226 [PMID: 9875675]

88 **Alvarnas JC**, Linn YC, Hope EG, Negrin RS. Expansion of cytotoxic CD3+ CD56+ cells from peripheral blood progenitor cells of patients undergoing autologous hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2001; **7**: 216-222 [PMID: 11349808 DOI: 10.1053/bbmt.2001.v7.pm11349808]

89 **Jiang J**, Wu C, Lu B. Cytokine-induced killer cells promote antitumor immunity. *J Transl Med* 2013; **11**: 83 [PMID: 23536996 DOI: 10.1186/1479-5876-11-83]

90 **Nishimura R**, Baker J, Beilhack A, Zeiser R, Olson JA, Sega EI, Karimi M, Negrin RS. In vivo trafficking and survival of cytokine-induced killer cells resulting in minimal GVHD with retention of antitumor activity. *Blood* 2008; **112**: 2563-2574 [PMID: 18565854 DOI: 10.1182/blood-2007-06-092817]

91 **Mesiano G**, Todorovic M, Gammaitoni L, Leuci V, Giraudo Diego L, Carnevale-Schianca F, Fagioli F, Piacibello W, Aglietta M, Sangiolo D. Cytokine-induced killer (CIK) cells as feasible and effective adoptive immunotherapy for the treatment of solid tumors. *Expert Opin Biol Ther* 2012; **12**: 673-684 [PMID: 22500889 DOI: 10.1517/14712598.2012.675323]

92 **Sangiolo D**. Cytokine induced killer cells as promising immunotherapy for solid tumors. *J Cancer* 2011; **2**: 363-368 [PMID: 21716717 DOI: 10.7150/jca.2.363]

93 **Sangiolo D**, Mesiano G, Carnevale-Schianca F, Piacibello W, Aglietta M, Cignetti A. Cytokine induced killer cells as adoptive immunotherapy strategy to augment graft versus tumor after hematopoietic cell transplantation. *Expert Opin Biol Ther* 2009; **9**: 831-840 [PMID: 19463075 DOI: 10.1517/14712590903005552]

94 **Ma Y**, Zhang Z, Tang L, Xu YC, Xie ZM, Gu XF, Wang HX. Cytokine-induced killer cells in the treatment of patients with solid carcinomas: a systematic review and pooled analysis. *Cytotherapy* 2012; **14**: 483-493 [PMID: 22277010 DOI: 10.3109/14653249.2011.649185]

95 **Weng DS**, Zhou J, Zhou QM, Zhao M, Wang QJ, Huang LX, Li YQ, Chen SP, Wu PH, Xia JC. Minimally invasive treatment combined with cytokine-induced killer cells therapy lower the short-term recurrence rates of hepatocellular carcinomas. *J Immunother* 2008; **31**: 63-71 [PMID: 18157013 DOI: 10.1097/CJI.0b013e31815a121b]

96 **Hui D,** Qiang L, Jian W, Ti Z, Da-Lu K. A randomized, controlled trial of postoperative adjuvant cytokine-induced killer cells immunotherapy after radical resection of hepatocellular carcinoma. *Digestive and Liver Disease* 2009; **41:** 36-41 [DOI: 10.1016/j.dld.2008.04.007]

97 **Hao MZ**, Lin HL, Chen Q, Ye YB, Chen QZ, Chen MS. Efficacy of transcatheter arterial chemoembolization combined with cytokine-induced killer cell therapy on hepatocellular carcinoma: a comparative study. *Chin J Cancer* 2010; **29**: 172-177 [PMID: 20109346]

98 **Zhou P**, Liang P, Dong B, Yu X, Han Z, Xu Y. Phase Ⅰ clinical study of combination therapy with microwave ablation and cellular immunotherapy in hepatocellular carcinoma. *Cancer Biol Ther* 2011; **11**: 450-456 [PMID: 21258206 DOI: 10.4161/cbt.11.5.14669]

99 **Huang ZM**, Li W, Li S, Gao F, Zhou QM, Wu FM, He N, Pan CC, Xia JC, Wu PH, Zhao M. Cytokine-induced killer cells in combination with transcatheter arterial chemoembolization and radiofrequency ablation for hepatocellular carcinoma patients. *J Immunother* 2013; **36**: 287-293 [PMID: 23719239 DOI: 10.1097/CJI.0b013e3182948452]

100 **Pan K**, Li YQ, Wang W, Xu L, Zhang YJ, Zheng HX, Zhao JJ, Qiu HJ, Weng DS, Li JJ, Wang QJ, Huang LX, He J, Chen SP, Ke ML, Wu PH, Chen MS, Li SP, Xia JC, Zeng YX. The efficacy of cytokine-induced killer cell infusion as an adjuvant therapy for postoperative hepatocellular carcinoma patients. *Ann Surg Oncol* 2013; **20**: 4305-4311 [PMID: 23892527 DOI: 10.1245/s10434-013-3144-x]

101 **Wang FS**, Liu MX, Zhang B, Shi M, Lei ZY, Sun WB, Du QY, Chen JM. Antitumor activities of human autologous cytokine-induced killer (CIK) cells against hepatocellular carcinoma cells in vitro and in vivo. *World J Gastroenterol* 2002; **8**: 464-468 [PMID: 12046071]

102 **Ma Y**, Xu YC, Tang L, Zhang Z, Wang J, Wang HX. Cytokine-induced killer (CIK) cell therapy for patients with hepatocellular carcinoma: efficacy and safety. *Exp Hematol Oncol* 2012; **1**: 11 [PMID: 23210562]

103 **Li X**, Dai D, Song X, Liu J, Zhu L, Xu W. A meta-analysis of cytokine-induced killer cells therapy in combination with minimally invasive treatment for hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol* 2014; **38**: 583-591 [PMID: 24924902 DOI: 10.1016/j.clinre.2014.04.010]

104 **Jinushi M**, Takehara T, Tatsumi T, Hiramatsu N, Sakamori R, Yamaguchi S, Hayashi N. Impairment of natural killer cell and dendritic cell functions by the soluble form of MHC class I-related chain A in advanced human hepatocellular carcinomas. *J Hepatol* 2005; **43**: 1013-1020 [PMID: 16168521 DOI: 10.1016/j.jhep.2005.05.026]

105 **Smyth MJ**, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. *Nat Rev Cancer* 2002; **2**: 850-861 [PMID: 12415255 DOI: 10.1038/nrc928]

106 **Ishiyama K**, Ohdan H, Ohira M, Mitsuta H, Arihiro K, Asahara T. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. *Hepatology* 2006; **43**: 362-372 [PMID: 16440347 DOI: 10.1002/hep.21035]

107 **Cai L**, Zhang Z, Zhou L, Wang H, Fu J, Zhang S, Shi M, Zhang H, Yang Y, Wu H, Tien P, Wang FS. Functional impairment in circulating and intrahepatic NK cells and relative mechanism in hepatocellular carcinoma patients. *Clin Immunol* 2008; **129**: 428-437 [PMID: 18824414 DOI: 10.1016/j.clim.2008.08.012]

108 **Hoechst B**, Voigtlaender T, Ormandy L, Gamrekelashvili J, Zhao F, Wedemeyer H, Lehner F, Manns MP, Greten TF, Korangy F. Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. *Hepatology* 2009; **50**: 799-807 [PMID: 19551844 DOI: 10.1002/hep.23054]

109 **Subleski JJ**, Hall VL, Back TC, Ortaldo JR, Wiltrout RH. Enhanced antitumor response by divergent modulation of natural killer and natural killer T cells in the liver. *Cancer Res* 2006; **66**: 11005-11012 [PMID: 17108139 DOI: 10.1158/0008-5472.CAN-06-0811]

110 **Tsuchiyama T**, Nakamoto Y, Sakai Y, Marukawa Y, Kitahara M, Mukaida N, Kaneko S. Prolonged, NK cell-mediated antitumor effects of suicide gene therapy combined with monocyte chemoattractant protein-1 against hepatocellular carcinoma. *J Immunol* 2007; **178**: 574-583 [PMID: 17182598]

111 **Zerbini A**, Pilli M, Laccabue D, Pelosi G, Molinari A, Negri E, Cerioni S, Fagnoni F, Soliani P, Ferrari C, Missale G. Radiofrequency thermal ablation for hepatocellular carcinoma stimulates autologous NK-cell response. *Gastroenterology* 2010; **138**: 1931-1942 [PMID: 20060829 DOI: 10.1053/j.gastro.2009.12.051]

112 **Bendelac A**, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* 2007; **25**: 297-336 [PMID: 17150027 DOI: 10.1146/annurev.immunol.25.022106.141711]

113 **Kenna T**, Golden-Mason L, Porcelli SA, Koezuka Y, Hegarty JE, O'Farrelly C, Doherty DG. NKT cells from normal and tumor-bearing human livers are phenotypically and functionally distinct from murine NKT cells. *J Immunol* 2003; **171**: 1775-1779 [PMID: 12902477 DOI: 10.4049/jimmunol.171.4.1775]

114 **Berzofsky JA**, Terabe M. NKT cells in tumor immunity: opposing subsets define a new immunoregulatory axis. *J Immunol* 2008; **180**: 3627-3635 [PMID: 18322166 DOI: 10.4049/jimmunol.180.6.3627]

115 **Smyth MJ**, Crowe NY, Hayakawa Y, Takeda K, Yagita H, Godfrey DI. NKT cells - conductors of tumor immunity? *Curr Opin Immunol* 2002; **14**: 165-171 [PMID: 11869887 DOI: 10.1016/S0952-7915(02)00316-3]

116 **Godfrey DI**, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? *Nat Rev Immunol* 2004; **4**: 231-237 [PMID: 15039760 DOI: 10.1038/nri1309]

117 **Sadelain M**, Brentjens R, Rivière I. The promise and potential pitfalls of chimeric antigen receptors. *Curr Opin Immunol* 2009; **21**: 215-223 [PMID: 19327974 DOI: 10.1016/j.coi.2009.02.009]

118 **Gilham DE**, Debets R, Pule M, Hawkins RE, Abken H. CAR-T cells and solid tumors: tuning T cells to challenge an inveterate foe. *Trends Mol Med* 2012; **18**: 377-384 [PMID: 22613370 DOI: 10.1016/j.molmed.2012.04.009]

119 **Curran KJ**, Pegram HJ, Brentjens RJ. Chimeric antigen receptors for T cell immunotherapy: current understanding and future directions. *J Gene Med* 2012; **14**: 405-415 [PMID: 22262649 DOI: 10.1002/jgm.2604]

120 **Lipowska-Bhalla G**, Gilham DE, Hawkins RE, Rothwell DG. Targeted immunotherapy of cancer with CAR T cells: achievements and challenges. *Cancer Immunol Immunother* 2012; **61**: 953-962 [PMID: 22527245 DOI: 10.1007/s00262-012-1254-0]

121 **Dotti G**, Savoldo B, Brenner M. Fifteen years of gene therapy based on chimeric antigen receptors: "are we nearly there yet?". *Hum Gene Ther* 2009; **20**: 1229-1239 [PMID: 19702437 DOI: 10.1089/hum.2009.142]

122 **Savoldo B**, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, Kamble RT, Bollard CM, Gee AP, Mei Z, Liu H, Grilley B, Rooney CM, Heslop HE, Brenner MK, Dotti G. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *J Clin Invest* 2011; **121**: 1822-1826 [PMID: 21540550 DOI: 10.1172/JCI46110]

123 **Morgan RA**, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010; **18**: 843-851 [PMID: 20179677 DOI: 10.1038/mt.2010.24]

124 **Kowolik CM**, Topp MS, Gonzalez S, Pfeiffer T, Olivares S, Gonzalez N, Smith DD, Forman SJ, Jensen MC, Cooper LJ. CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. *Cancer Res* 2006; **66**: 10995-11004 [PMID: 17108138 DOI: 10.1158/0008-5472.CAN-06-0160]

125 **Porter DL**, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 2011; **365**: 725-733 [PMID: 21830940 DOI: 10.1056/NEJMoa1103849]

126 **Grupp SA**, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, Milone MC, Levine BL, June CH. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013; **368**: 1509-1518 [PMID: 23527958 DOI: 10.1056/NEJMoa1215134]

127 **Pizzitola I**, Anjos-Afonso F, Rouault-Pierre K, Lassailly F, Tettamanti S, Spinelli O, Biondi A, Biagi E, Bonnet D. Chimeric antigen receptors against CD33/CD123 antigens efficiently target primary acute myeloid leukemia cells in vivo. *Leukemia* 2014; **28**: 1596-1605 [PMID: 24504024 DOI: 10.1038/leu.2014.62]

128 **June C**, Rosenberg SA, Sadelain M, Weber JS. T-cell therapy at the threshold. *Nat Biotechnol* 2012; **30**: 611-614 [PMID: 22781680 DOI: 10.1038/nbt.2305]

129 **Gehring AJ**, Xue SA, Ho ZZ, Teoh D, Ruedl C, Chia A, Koh S, Lim SG, Maini MK, Stauss H, Bertoletti A. Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. *J Hepatol* 2011; **55**: 103-110 [PMID: 21145860 DOI: 10.1016/j.jhep.2010.10.025]

130 **Kershaw MH**, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, White DE, Wunderlich JR, Canevari S, Rogers-Freezer L, Chen CC, Yang JC, Rosenberg SA, Hwu P. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res* 2006; **12**: 6106-6115 [PMID: 17062687 DOI: 10.1158/1078-0432.CCR-06-1183]

131 **Lamers CH**, Sleijfer S, Vulto AG, Kruit WH, Kliffen M, Debets R, Gratama JW, Stoter G, Oosterwijk E. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J Clin Oncol* 2006; **24**: e20-e22 [PMID: 16648493 DOI: 10.1200/JCO.2006.05.9964]

132 **Lamers CH**, Langeveld SC, Groot-van Ruijven CM, Debets R, Sleijfer S, Gratama JW. Gene-modified T cells for adoptive immunotherapy of renal cell cancer maintain transgene-specific immune functions in vivo. *Cancer Immunol Immunother* 2007; **56**: 1875-1883 [PMID: 17479266 DOI: 10.1007/s00262-007-0330-3]

133 **Lamers CH**, Sleijfer S, van Steenbergen S, van Elzakker P, van Krimpen B, Groot C, Vulto A, den Bakker M, Oosterwijk E, Debets R, Gratama JW. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. *Mol Ther* 2013; **21**: 904-912 [PMID: 23423337 DOI: 10.1038/mt.2013.17]

134 **Brentjens R**, Yeh R, Bernal Y, Riviere I, Sadelain M. Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. *Mol Ther* 2010; **18**: 666-668 [PMID: 20357779 DOI: 10.1038/mt.2010.31]

135 **Disis ML.** Immune regulation of cancer. *J Clin Oncol* 2010; **28:** 4531-4538

136 **Whiteside TL.** Immune responses to malignancies. *J Allergy Clin Immun* 2010; **125** (Supplement 2): S272-S283 [DOI: 10.1016/j.jaci.2009.09.045]

137 **Sakaguchi S**, Powrie F. Emerging challenges in regulatory T cell function and biology. *Science* 2007; **317**: 627-629 [PMID: 17673654 DOI: 10.1126/science.1142331]

138 **Zhou J**, Ding T, Pan W, Zhu LY, Li L, 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 [PMID: 19569243 DOI: 10.1002/ijc.24556]

139 **Kobayashi N**, Hiraoka N, Yamagami W, Ojima H, Kanai Y, Kosuge T, Nakajima A, Hirohashi S. FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. *Clin Cancer Res* 2007; **13**: 902-911 [PMID: 17289884 DOI: 10.1158/1078-0432.CCR-06-2363]

140 **Gao Q**, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, Xu Y, Li YW, Tang ZY. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007; **25**: 2586-2593 [PMID: 17577038 DOI: 10.1200/JCO.2006.09.4565]

141 **Huang Y**, Wang FM, Wang T, Wang YJ, Zhu ZY, Gao YT, Du Z. Tumor-infiltrating FoxP3+ Tregs and CD8+ T cells affect the prognosis of hepatocellular carcinoma patients. *Digestion* 2012; **86**: 329-337 [PMID: 23207161 DOI: 10.1159/000342801]

142 **Shimizu J**, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 1999; **163**: 5211-5218 [PMID: 10553041]

143 **Cany J**, Tran L, Gauttier V, Judor JP, Vassaux G, Ferry N, Conchon S. Immunotherapy of hepatocellular carcinoma: is there a place for regulatory T-lymphocyte depletion? *Immunotherapy* 2011; **3**: 32-34 [PMID: 21524167 DOI: 10.2217/imt.11.29]

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**Figure 1** **Principles of immunotherapy on hepatocellular carcinoma.** NK: Natural killer; LAK: Lymphokine-activated killer; NKT: Natural killer T; CIK: Chemokine-induced killer; CAR-T: Chimeric antigen receptor-T; HBV: Hepatitis B virus; MDSC: Myeloid-derived suppressor cells; Treg: Regulatory T; IL-2: Interleukin 2; IFN: Interferon; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; HCC: Hepatocellular carcinoma.

**Table 1** **Information of clinical trials of checkpoint blockade on hepatocellular carcinoma after 2008**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Interventions** | **Design** | **Start year** | **Main inclusion criteria** | | **Primary outcomes** | **Registered No.** | **Status** |
| CP-675,206: anti-CTLA-4 antibody | Phase II | 2008 | Unresectable disease not amenable to loco regional treatment, HCV chronic infection | Tumor response | | NCT01008358 | Completed |
| CT-011 (Pidilizumab): anti-PD-1 antibody | Phase I/II | 2009 | HCC not eligible for surgery, TACE, or other systematic treatments | Safety and tolerability | | NCT00966251 | Terminated because of slow accrual |
| Nivolumab: anti-PD-1 antibody | Phase I | 2012 | Advanced HCC, failed in previous one line therapy | Adverse events | | NCT01658878 | Recruiting |
| Tremlimumab: anti-CTLA-4 antibody, combined with TACE or RFA | Phase I | 2013 | Not amenable to curative resection, transplantation or ablation | Safety and feasibility | | NCT01853618 | Recruiting |

HCV: Hepatitis C virus; RFA: Radiofrequency ablation; TACE: Transarterial chemoembolization.

**Table 2** **Information of clinical trials of tumor vaccine on hepatocellular carcinoma after 2008**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Interventions** | **Design** | **Start year** | **Main inclusion criteria** | **Primary outcomes** | **Registered No.** | **Status** |
| AFP + GM-CSF plasmid prime and AFP adenoviral vector boost | Phase I/II | 2008 | Locoregionally treated HCC | Dose, toxicity, and immunological response rate | NCT00669136 | Terminated because of poor accrual |
| DC loaded with autologous tumor | Phase II | 2008 | Metastatic HCC, available of tumor tissue | 2-mo response rate | NCT00610389 | Unknown |
| DC loaded with specific peptides of AFP | Phase I/II | 2009 | Patients with previous treatment, AFP ≥ 40 ng/mL, HLA A 0201 group | Adverse events | NCT01128803 | Terminated |
| DEC-205-NY-ESO-1 fusion protein vaccine | Phase I | 2012 | After resection and TACE for HCC | Adverse events | NCT01522820 | Recruiting |
| COMBIG-DC: allogeneic DC cancer vaccine | Phase I | 2013 | Not eligible for curative treatment or TACE, BCLC stage B and C | Adverse events | NCT01974661 | Recruiting |
| In-situ therapeutic cancer vaccine | Phase I | 2013 | Refractory HCC, not eligible for or failed any treatment, AFP ＞ 30 | Safety | NCT01923233 | Recruiting |
| V5 therapeutic vaccine | Phase III | 2014 | Advanced HCC | Changes in plasma AFP | NCT02232490 | Not yet recruiting |

AFP: α-fetoprotein; DC: Dendritic cell; GM-CSF: Granulocyte macrophage colony-stimulating factor; HLA: Human leukocyte antigen; TACE: Transarterial chemoembolization.

**Table 3** **Information of clinical trials of adoptive therapy on hepatocellular carcinoma after 2008**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Interventions** | **Design** | **Start year** | **Main inclusion criteria** | **Primary outcomes** | **Registered No.** | **Status** |
| Immuncell-LC: activated T lymphocyte | Phase III | 2008 | Stage I and II, complete resection within 12 weeks | Efficacy and safety | NCT00699816 | Completed |
| CIK | Phase III | 2008 | After radical resection of HCC, no prior anti-cancer therapy | Time to recurrence | NCT01749865 | Recruiting |
| CIK | Phase III | 2008 | After radical resection, no prior anti-cancer treatment | Time to recurrence | NCT00769106 | Recruiting |
| *Ex vivo* expanded autologous immune killer cell, combined with TACE | Phase II/III | 2009 | Never receive TACE treatment, BCLC stage B and C | 2-yr reduction of tumor cells | NCT01024530 | Unknown |
| NK cells, combined with liver transplantation | Phase I | 2010 | After liver transplantation for HCC | Side effect | NCT01147380 | Ongoing, but not recruiting |
| Young TIL | Phase II | 2010 | Metastatic HCC with at least one lesion resectable | Tumor regression rate | NCT01174121 | Recruiting |
| Autologous tumor infiltrating lymphocytes, combined with IL-2 | Phase I | 2011 | Metastatic HCC | Safety and tolerability | NCT01462903 | Unknown |
| CIK, combined with Licartin | Phase IV | 2012 | Postoperative patients | 1-yr PFS | NCT01758679 | Recruiting |
| Dendritic and cytokine-induced killer cells | Phase II | 2013 | After complete resection or TACE | PFS | NCT01821482 | Not yet recruiting |
| CTL induced by DC loaded with multiple antigens | Phase I | 2013 | Complete tumor resection within 8 weeks | 2-yr PFS and adverse events | NCT02026362 | Recruiting |
| DC incubated with irradiated autologous tumor stem cells + GM-CSF | Phase I | 2013 | Candidates for HCC resection | Vital signs, physical examinations and adverse events | NCT01828762 | Completed |
| Cord blood-derived CIK | Phase I | 2013 | After radical resection | Adverse events | NCT01914263 | Not yet recruiting |
| Autologous NKT cells | Phase I | 2013 | Advanced HCC, refractory to standard treatments | Adverse events | NCT01801852 | Recruiting |
| Immuncell-LC, combined with Nexavar | Phase II | 2013 | Stage III and IV, receiving or ready for Nexavar treatment | 2-yr PFS | NCT01897610 | Recruiting |
| MG4101: *ex vivo* expanded allogeneic NK cell | Phase II | 2013 | Stage III, after curative resection | 1-yr DFS | NCT02008929 | Recruiting |

CIK: Cytokine-induced killer; CTL: Cytolytic T lymphocyte; DC: Dendritic cell; DFS: Disease-free survival; NK: Natural killer; NKT: Natural killer T; OS: Overall survival; PFS: Progression-free survival; TACE: Transarterial chemoembolization; TIL: Tumor infiltrating lymphocyte.