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Review

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Cancer immunotherapy for hepatocellular carcinoma

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Abstract

Most hepatocellular carcinomas (HCCs) arise on a background of chronically inflamed liver, and thus are considered typical immunogenic cancers. Although there have been advances in treatment options for HCC, many patients still struggle with a limited chance of survival requiring further innovative approach. Especially for the advanced HCC, many other molecular targeted therapies had been evaluated without success. Based on the immunological mechanisms thought to be acting during HCC development, the effects of diverse immunomodulatory regimens such as therapeutic vaccination, immune checkpoint inhibitors, and adoptive cellular immunotherapy have been investigated. Notably, many strategies have been developed in adoptive cellular immunotherapy, including dendritic cells, cytotoxic T cells, natural killer cells, cytokine-induced killer (CIK) cells, and genetically engineered T cells. In recent clinical trials, adjuvant CIK cell immunotherapy increased progression free survival after curative treatment of HCC. Most recently, new immunomodulatory agents were introduced for oncological treatment, eventually leading to the clinical breakthrough of checkpoint inhibitors targeting cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death-1 (PD-1). To date, very promising published evidence with checkpoint inhibitors in HCC has been reported in the clinical trials with anti-CTLA-4 agent tremelimumab and a large phase II trial with anti-PD-1 agent nivolumab. Further investigations of immuno-oncology potentially popularized the applications of immunotherapy in the various stages of HCCs, and thus immune-based therapies are the promising innovative approach for patients with HCC. Hopefully, the immuno-oncology will bring about a paradigm shift of anti-cancer treatment for HCC.

Keywords: Hepatocellular carcinoma, adoptive immunotherapy, cytotoxic T lymphocyte associated antigen-4, programmed cell death 1 protein

INTRODUCTION

Hepatocellular carcinoma (HCC) ranks fifth as the most common cancer in the world and the second most common cause of cancer-related death, accounting for 70%-85% of primary liver cancers[1-3]. The current standard treatments for HCC offer a fair chance of survival but there are still many patients who struggle with only a limited chance of survival[4]. A majority of patients present with disease too advanced to be treated with curative modalities such as surgical resection, transplantation, or radiofrequency ablation (RFA)[2]. Although the Barcelona Clinic Liver Cancer (BCLC) guideline recommends sorafenib in advanced HCC, which proved a survival benefit of 2.8 months compared to the placebo group[5], many liver cancer centers still select multimodality approaches including transarterial chemoembolization (TACE), radiotherapy (RT), and hepatic arterial infusion chemotherapy (HAIC)[6,7].

Most HCCs arise on a background of chronically inflamed liver, and thus are considered typical immunogenic cancers[8]. Based on the immunological mechanisms thought to be acting during HCC development, the effects of diverse immunomodulatory regimens such as therapeutic vaccination, immune checkpoint inhibitors, and transfer of adoptive cellular immunotherapy, have been investigated[8,9]. In the 21st century, cell-based therapies developed to bolster human anti-tumor immunity represent a growing component of cancer therapeutics[10,11]. Of note, adoptive cellular immunotherapies have employed several types of immune cells, including dendritic cells (DCs), cytotoxic T lymphocytes (CTLs), lymphokine-activated killer (LAK) cells, cytokine-induced killer (CIK) cells, and natural killer (NK) cells[12]. In addition, therapeutic cancer vaccines utilizing tumor antigens with or without DCs have been investigated.

Immune suppressor cells comprising tumor-associated macrophages (TAMs), regulatory T cells (Tregs), or myeloid-derived suppressive cells (MDSCs) in the HCC tumor microenvironment, could disturb the immune surveillance resulting in cancer immune evasion or immune escape[13]. It is well known that the interactions of HCC cells with the immune cells and their factors of immune system play a major role in its progression[14,15]. Inadequate co-stimulation, failure of tumor-associated antigens (TAAs) processing and presentation by antigen-presenting cells (APCs), along with suppression of effector cells are proposed mechanisms that result in weakened immune response in HCC patients[15,16]. To complement these immunosuppressive tumor microenvironment of HCC, previous cancer immunotherapy has aimed so far to enhance immune cell activity to kill the HCC tumor cells.

In this regard, cancer vaccines help the immune system recognize and attack cancer cells[17]. Unlike preventive vaccine, which prevents a development of a certain disease in advance, therapeutic cancer vaccine aims to treat the existing cancer. DCs are professional APCs that serve as a key player for inducing and activating the effector anti-tumor CTLs. There is ample evidence to justify therapeutic DC vaccines in HCC[18]. Decreased function of peripheral blood DCs in patients with HCC is well established[19]. Up to date, although DC vaccines are used in various stages of clinical trials of HCC, unfortunately, no therapeutic cancer vaccine has been approved for HCC[19]. Meanwhile, the failure of these approaches for boosting immune responses by cancer vaccine using peptides or DCs, could be associated with the brake function in immunity (i.e., immune checkpoints)[20]. It is now clear that tumors modulate immune checkpoints as one of the mechanisms to escape anti-cancer immune surveillance.

These immune checkpoints are known to regulate different stages and signaling processes of the immune response[21]. At the initial stage of "priming" of naïve T cell activation, cytotoxic T lymphocyte associated antigen-4 (CTLA-4):B7 binding blocks stimulatory signals, and stops the development of potentially autoreactive T cells[22]. Compared to CTLA-4, the major role of programmed cell death 1 protein (PD-1) and its ligand, PD-L1, is related to regulate previously activated CTLs at the later "effector" stage of immune response[23]. In the tumor microenvironment, antigen-specific T cells induce PD-1 expression on reactive CTLs and upregulate PD-L1 in cancer cells[8,23].

The above immune checkpoint molecules are highly expressed in HCCs that are recognized as immunogenic tumors[24]. Also, the hepatitis B (HBV) and hepatitis C virus (HCV) infections, two major pathogens of HCC, have been shown to interfere with antiviral immunity via the immune checkpoint pathways[25-27]. Blocking these immune checkpoint molecules restores T cell function, which release the brakes on the anti-tumor immune surveillance, allowing the immune system to more effectively detect and kill the HCC tumor cells[2,20,28].

As "cancer immunotherapy comes of age"[29] in this era, the topic of "immuno-oncology in HCC" could be a timely one. In this review, we focus on the human clinical immunotherapy trials in HCC, according to the four major categories: (1) adoptive immunotherapies using CIK, NK and engineered T cells; (2) therapeutic cancer vaccine; (3) immune checkpoint blockades; and (4) combination of immunotherapies with other cancer treatments.

ADOPTIVE CELLULAR IMMUNOTHERAPY

Adoptive cellular immunotherapy is a form of passive immunization in which autologous effector cells are *ex vivo* sensitized and or expanded and then given back to the cancer patients[30]. To date, adoptive immunotherapy is one stone in the pillar of cancer immunotherapy, which relies on the various lymphocytes including tumor-infiltrating lymphocytes (TILs), CD8+ CTLs, CD56+ NK cells, LAK cells, CIK cells, and engineering T cells. As one of main immunotherapeutic strategies, adoptive immunotherapy is widely used in the current cancer clinical trials. A sizable portion of immunotherapy clinical trials for HCCs are adoptive cellular immunotherapies [Table 1][30].

In 1989, regression of tumor size in ten HCC patients was shown after treatment with LAK cells combined with interleukin-2 (IL-2)[31]. Later, two separate, but similar, clinical trials combining adriamycin chemotherapy with LAK cells after hepatoma resection were performed in 1991 and 1995[32,33]. The former study showed a decrease in postoperative recurrence rate of HCC[32]. However, in the latter study in 1995, there was no statistically significant difference between the two groups in the survival rate[33].

Another source of adjuvant immunotherapy is TILs[34]. TILs acquired from patients with hepatic malignancies, activated by IL-2 and anti-CD3 antibody and labeled with indium-111 were found to move to the tumor sites preferentially[34]. This might augment the antitumor effects of adoptive immunotherapy. In 1997, TILs isolated from resected tumors of 12 patients with HCC were activated and expanded *in vitro* by IL-2, and then infused to the patients[35]. In this study, TIL infusion as an adjuvant immunotherapy for HCC patients significantly decreased recurrence rate at 6 and 12 months compared to the control group.

Another promising cellular immunotherapy as the adjuvant setting for HCC involves CIK cell immunotherapies. Also, the recent clinical trials from many Asian-pacific countries reported that adjuvant CIK cell immunotherapy increased progression free survival (PFS) after curative treatment for HCC[30,36,37].

Adoptive immunotherapy using CIK cells

CIK cells are heterogeneous cell population consisting of CD8+ CTLs, CD56+ NK cells and both CD3+CD56+ NK like T (NKT) cells that were first discovered in the 1990s[11,37]. CIK cells display both anti-tumor ability of antigen specific CD8+ CTLs and non-major histocompatibility complex (MHC) restricted cancer cell killing capacity of NK cells [Figure 1][38]. Earlier clinical studies have shown a potent antitumor activity of CIK cells against various types of tumors[36].

In 2000, CIK cell immunotherapy is demonstrated to be a safe and feasible treatment that can lower recurrence rate and improve PFS after curative resection of HCC[37]. In this randomized trial, CIK cells were infused 5 times during the first 6 postoperative months. During the median follow-up of 4.4 years, recurrence rate reduced remarkably by 18% in the CIK cell treatment group (59%, 45/76) compared with that

Table 1. Selected clinical trials with adoptive cellular immunotherapy for HCC

Registered No.	Recruitment status	Start year	Phase	Immunotherapy	Included patients of HCC
NCT00161187	Completed	2001	I	Therapeutic allogeneic lymphocytes: irradiated lymphocytes from a donor	Unresectable or metastatic disease
NCT01828762	Completed	2005	I	Autologous immune killer cell	Locally advanced or metastatic HCC
NCT00699816	Completed	2008	III	Immuncell-LC	Stage I/II, after curative treatment
NCT01749865	Completed	2008	III	CIK	After radical resection
NCT00769106	Completed	2008	III	CIK	After radical resection
NCT01024530	Unknown	2009	II/III	Autologous immune killer cells with TACE	BCLC stage B/C
NCT01212341	Completed	2010	I	MG4101: allogeneic NK cells	Solid tumors
NCT01147380	Completed	2010	I	Liver NK cell inoculation with liver transplantation	Liver transplant recipient
NCT01174121	Recruiting	2010	II	Autologous TILs and IL-2 with cyclophosphamide, fludarabine and pembrolizumab	Metastatic HCC who has received sorafenib
NCT01218867	Completed	2010	I/II	Anti-VEGFR2 CAR CD8 and PBL with cyclophosphamide, IL-2 and fludarabine	Metastatic cancer
NCT01462903	Unknown	2011	I	Autologous TILs and IL-2	Metastatic HCC after primary operation, radiotherapy and chemotherapy
NCT01758679	Recruiting	2012	IV	CIK and Licartin	Postoperative HCC
NCT01801852	Recruiting	2013	I	Autologous NKT cell infusion	Refractory to conventional treatment
NCT01897610	Recruiting	2013	II	Immuncell-LC with sorafenib	Stage III/IV
NCT02008929	Recruiting	2014	II	MG4101: allogeneic NK cell	After curative resection
NCT01914263	Recruiting	2014	I	Cord blood-derived CIKs	After radical resection
NCT02587689	Recruiting	2015	I/II	Anti-MUC1 CAR T cells	MUC1+ malignancies
NCT02959151	Recruiting	2015	I/II	GPC3-CAR T cell	HCC with GPC3 high expression
NCT02725996	Not yet recruiting	2016	II	Autologous NK cells	Stage I/II, after curative treatment
NCT02856815	Not yet recruiting	2016	II	Immuncell-LC	BCLC stage B, tumor removal has been confirmed after TACE
NCT02715362	Recruiting	2016	I/II	GPC3-CAR T cells with transcatheter arterial infusion (TAI)	Persistent cancer after at least one prior standard of care chemotherapy
NCT02839954	Recruiting	2016	I/II	Anti-MUC1 CAR-pNK cells	MUC1+ malignancies
NCT02959151	Recruiting	2016	I/II	GPC3-CAR T cell	HCC with GPC3 expression
NCT02854839	Recruiting	2016	IIA	MG4101: allogeneic NK cells	Complete remission after TACE
NCT03175679	Recruiting	2017	I	iNKT cells and IL-2 with 5-fluorouracil	Relapsed/advanced HCC, BCLC stage C
NCT03199807	Not yet recruiting	2017	IB/II	Personalized new antigen reactive immune cells (NRT), radiotherapy	Advanced HCC, unresectable and no chemotherapy before
NCT03130712	Recruiting	2017	I/II	GPC3-CAR T cells intratumor injection	Advanced HCC, persistent cancer after at least one prior standard of chemotherapy or surgery
NCT03132792	Recruiting	2017	I	Autologous genetically modified $AFP^{c332}T$ cells: genetically changed T cells that target alpha-fetoprotein	Positive for HLA-A*02:01 or HLA-A*02:642 allele
NCT03302403	Not yet recruiting	2017	N/A	Autologous T cells transduced with CAR recognizing CD19, BCMA, GPC3 and Claudin18.2	Advanced HCC with previous ablation or resection in the last 4 to 12 weeks
NCT02905188	Not yet recruiting	2018	I	GPC3-CAR T cells with fludarabine and cytoxan	BCLC stage A/B/C
NCT03441100	Not yet recruiting	2018	I	IMA202 Product (CAR T cell) with fludarabine and cyclophosphamide	HCC not amenable to treatments with curative intent

HCC: hepatocellular carcinoma; CIK: cytokine induced killer; TACE: transarterial chemoembolization; BCLC: Barcelona clinic liver cancer; NK: natural killer; TIL: tumor infiltrating lymphocyte; IL-2: interleukin-2; VEGFR: vascular endothelial growth factor receptor; CAR: chimeric antigen receptor; PBL: peripheral blood lymphocyte; NKT: natural killer T; MUC1: mucin1; GPC3: glypican-3; AFP: alpha-fetoprotein; HLA: human leukocyte antigen; BCMA: B-cell maturation antigen; N/A: not applicable

in the control group (77%, 57/74). Moreover, the PFS was significantly improved in the CIK treatment group ($P = 0.01$). All of the adverse events (AEs) were grade I or II and self-limiting. AEs associated with treatment were fever (47%), headache (4%), nausea (4%), dizziness (1%), itching (1%) and tachycardia (1%).

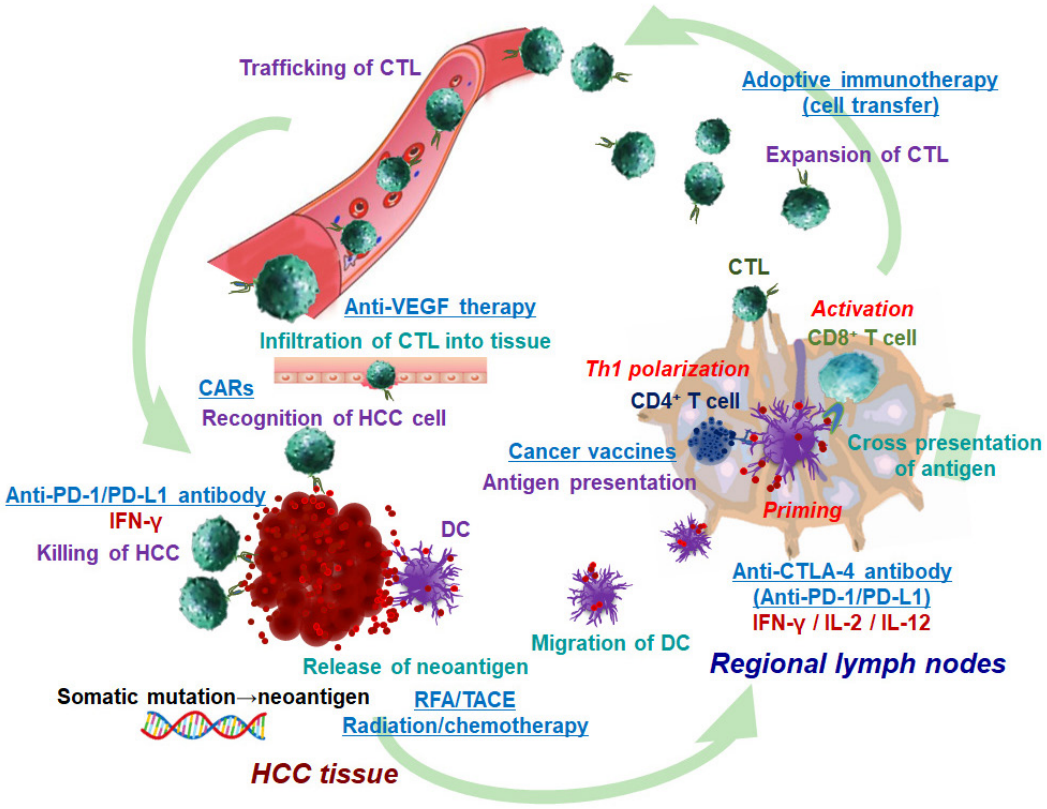

Figure 1. Cancer-immunity cycle and targets of immune therapies. Hepatocellular carcinoma (HCC) cells produce various tumor-associated antigens (TAAs) and neoantigens; the latter derive cancer-specific somatic mutations. The initial steps of anti-tumor immune response include uptake of TAAs and neoantigens by dendric cells (DCs). After that, the DCs migrate into regional lymph nodes and present processed antigen to CD4+ T cells. Antigen recognition leads to proliferation of CD4+ T cells and induction of interferon (IFN)-γ in the presence of IL-12 and type I IFN (Th1 polarization). The cross-presentation of antigenic peptide to CD8+ T cells by DCs facilitates the development of antigen-specific CD8+ cytotoxic T lymphocytes (CTLs). After the trafficking of CTLs to HCC tissues, the antigen-specific CTLs exert anti-tumor effecter function through release of humoral factors, such as granzyme B and perforin, and interaction with death receptors on tumor cells. Locoregional therapies and systemic chemotherapies should enhance the release of neoantigens and TAAs through HCC cell death. Cancer vaccines can promote the antigen presentation; anti-CTLA-4 antibody mainly acts in priming phase and facilitates the Th1 polarization and activation of CD8+ T cells. Adoptive immune therapies (immune cell transfer) increase the peripheral anti-tumor immune cells; chimeric antigen receptor (CAR) T cells can more directly targets cancer cells compared to conventional adoptive immunotherapies. Anti-vascular endothelial growth factor (VEGF) potentially induce infiltration of T cell into tumor tissues. Anti-PD-1/PD-L1 antibodies block the co-inhibitory signal of CD8+ T cells and induce cancer cell killing. CTLA-4: cytotoxic T lymphocyte associated antigen-4; PD-1: programmed cell death 1 protein; PD-L1: PD-1 ligand

In 2004, the influence of autologous CIK cells was investigated in terms of phenotypes of CIK effector cells, peripheral T lymphocyte subsets and DC subsets in 13 HCC patients who had liver cirrhosis and chronic HBV infection[39]. Peripheral blood mononuclear cells (PBMCs) were collected by a blood cell separator, and then expanded by priming them with interferon-gamma (IFN-γ), monoclonal antibody against CD3 and IL-2. After two weeks of *in vitro* incubation, the percentages of CD8+ CTL and CD3+CD56+ NKT cells increased significantly from 33.5% and 7.7% to 36.6% and 18.9%, respectively. CIK cell therapy increased the proportions of type I DC and type II DC from 0.59% and 0.26% to 0.85% and 0.43%, respectively (all $P < 0.01$). These results indicated that autologous CIK cells could efficiently improve the immunological status in HCC patients.

In 2009, a randomized trial was conducted to investigate the impact of postoperative adjuvant CIK immunotherapy on the prognosis[40]. In 127 HCC patients who underwent radical hepatic resection, CIK cell therapy significantly increased the disease-free survival rate compared with the control group. However, the overall survival (OS) was not significantly different[40].

In 2010, the impact of adjuvant CIK therapy after TACE combined with sequential RFA on tumor recurrence was demonstrated in relation to serum AFP level[41]. After curative TACE plus RFA therapy, 83 patients with AFP level less than 37.5 ng/mL (1.5 times the normal range) were randomly assigned for CIK immunotherapy or for best supportive treatments. CIK cell infusions were given either intravenously or via common hepatic arteries every week for at least 4 times. During the follow-up of 12 months, AFP levels in the CIK group but not in the control group gradually decreased from the baseline levels, and those reduced levels were maintained. Furthermore, the reduced AFP levels of the CIK group were lower than the AFP levels of the control group with statistical significance both in 1 month ($P < 0.05$) and in 3 months ($P < 0.05$) after treatment. The 1-year recurrence rate was 7.1% for the CIK study group and 23.1% for the control group ($P = 0.04$). In addition, the authors showed that HBV DNA titer decreased after CIK cell therapy. They concluded that the adjuvant CIK cell therapy can reduce the serum AFP and HBV DNA levels and decrease the 1-year recurrence rate of patients with HCC after curative TACE plus RFA[41].

The most recent clinical trial, reported in 2015, demonstrated that adjuvant CIK cell immunotherapy after curative treatment for HCC increased not only the PFS but also the OS[36]. In this study, 230 patients with HCC who were treated by surgical resection, RFA, or percutaneous ethanol injection were included. Patients were assigned randomly to receive adjuvant CIK cell immunotherapy 16 times during 60 weeks or no adjuvant therapy. The median time of PFS was 44.0 months in the CIK cell therapy group and 30.0 months in the control group ($P = 0.01$). Hazard ratios (HR) of all-cause death (0.21; 95% CI, 0.06-0.75; $P = 0.008$) and HR of cancer-related death (0.19; 95% CI, 0.04-0.87; $P = 0.02$) were significantly lower in the CIK cell immunotherapy group compared with the control group. This study proved that adjuvant immunotherapy with activated CIK cells increase PFS as well as OS of HCC patients after the curative treatments including surgery and RFA[36]. However, the efficacy of CIK immunotherapy for HCC needs to be further validated, by extending the sample size and follow up duration of the HCC research cohort.

NK cell based immunotherapy

Human NK cell, recognized as a CD3-CD56+ lymphocyte, is a very important part of innate immune system. It provides surveillance toward tumor cells eliminating those when detected. Thus, NK cell was suggested to be used for cancer therapy[12]. NK cells are characterized by an inborn receptor diversity which allows NK cells to recognize and to respond to different pathogens including virus-infected cells and neoplastic cells without prior sensitization or acquired receptor rearrangement[17]. It is well known that NK cells can be long-lived, remember past exposures, and interact with MHC class I molecules to acquire full function. NK cell function is tightly regulated by signals from natural cytotoxicity receptors, CD16 receptor for antibody-dependent cellular cytotoxicity (ADCC), C-type lectins, and killer cell immunoglobulin-like receptors (KIR).

Recently, there have been advances in *ex vivo* techniques of NK cell activation and expansion[17]. Autologous cytokine-stimulated NK cell therapy has been tried with multiple tumors such as renal cell carcinoma, glioblastoma and myeloma[42]. On the other hand, allogeneic NK cell therapy is particularly beneficial because it can enhance the anti-cancer efficacy of NK cells via donor-recipient incompatibility in terms of KIRs on donor NK cells and MHC class I on recipient tissues[43]. Thus, the use of allogeneic NK cell therapy is being actively investigated in hematologic malignancies with or without hematopoietic stem cell transplantation[12]. In these settings, HLA-haploidentical NK cells have been used mostly.

In HCC patients, impaired functions of DC and NK cell were observed in relation to elevated level of serum MHC class I-related chain A (MICA), an inhibitory ligand for NKG2D[44]. Increase of Tregs and MDSCs were also known to contribute to the functional impairment of NK cells and in turn the reduced anti-tumor immune response[30,45]. In contrast, increased number of NK cells in peripheral blood and tumor tissues accompanied by an upregulation of related chemokines was an immune-gene signature which determines a long-term survival in resectable HCC[46].

A group in the University of Miami suggested that NK cells extracted from donor liver graft perfusate could be used as a source of a treatment to reduce recurrence rate after liver transplantation (LT)[47]. When the NK cells acquired from donor graft was activated with IL-2, activation markers and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which is critical for NK cell mediated cancer cell death, were greatly upregulated. The authors concluded that the adoptive transfer of IL-2 stimulated NK cells from deceased donor liver graft could be a promising treatment for LT patients with HCC[47]. Moreover, the cytokines and chemokines released by activated NK cells may stimulate both innate and adaptive immune responses toward cancer.

In this regard, a phase I clinical trial was conducted to evaluate the feasibility and safety of the adoptive transfer of activated NK cells extracted from cadaveric donor liver graft perfusate after LT (NCT01147380). According to preliminary results posted on the website clinicaltrials.gov, there seemed to be no side effects or serious adverse events. There are also ongoing clinical trials on adoptive NK cell therapy. A phase II clinical trial (NCT02008929) initiated in August 2014 aims to evaluate the safety and efficacy of *ex vivo* expanded allogeneic NK cells (MG4101) as a secondary treatment after curative liver resection on advanced HCC patients with a high risk of recurrence. Adoptive cell transfer of allogeneic NK cells that came from a totally unrelated donor had been demonstrated to be safe without any significant side effects [Figure 1][30]. Notably, another multi-center, open label, phase IIA clinical trial (NCT02854839) with a purpose to evaluate the safety and efficacy of allogeneic NK cell (MG4101) therapy for intermediate-stage HCC patients after TACE started in September, 2016.

Adoptive immunotherapy using genetically engineered T cell receptor or chimeric antigen receptors

The emergence of immuno-oncology as the first broadly successful strategy for metastatic cancer will require clinicians to integrate this new pillar of medicine with the pillars of already established therapeutic methods such as chemotherapy, RT and targeted small molecule compounds[48]. Chimeric antigen receptor (CAR) T cell therapy combines adoptive cellular immunotherapy with targeted molecular therapy, and it has proven that engineered immune cells can serve as a powerful new class of cancer therapeutics [Figure 1]. Adoptive immunotherapy retargeting T cells to CD19 via CAR is an investigational treatment capable of inducing complete tumor regression of B-cell malignancies[49]. The major hurdle in developing CAR T cell therapy is the on-target off-tumor toxicity as was shown in a metastatic colon cancer patient who died 5 days after infusion of ErbB2 targeting CAR T cell[50]. Expression of ErbB2 on lung epithelium even with a low level brought a detrimental result. Therefore, finding a target antigen which is effective enough for cancer-killing and at the same time safe enough for the normal tissue is a key requirement in the development of CAR T cell therapy for HCC[51].

It has been demonstrated that HBV antigens can serve as a tumor specific antigen in HBV related HCC and can be targeted by adoptively transferred HBV-specific T cell receptor (TCR) redirected T cells in preclinical models[52,53]. Recently, Qasim *et al.*[54] have reported the clinical results of immunotherapy for HCC metastases with autologous TCR redirected T cells, targeting HBV surface antigen (HBsAg) in a liver transplant patient. Autologous T cells genetically modified to express an HBsAg specific TCR were infused with no immediate infusion-related toxicities despite the patient's frail condition. The authors confirmed that HBV antigens were expressed in metastatic lesions of HCC and demonstrated that tumor cells were recognized *in vivo* by the engineered T lymphocytes. Furthermore, the engineered T cells successfully survived, expanded, and mediated a reduction in HBsAg levels without exacerbation of liver inflammation or other toxicity. Although the clinical efficacy in this patient was not established with end-stage metastatic HCC, these results confirm the feasibility of autologous CAR T cell immunotherapy targeting HBsAg in HBV associated HCC[54].

In 2010, Food and Drug Administration (FDA) approved a phase I/II study of CAR T cell immunotherapy targeting vascular endothelial growth factor receptor (VEGFR)-2 (NCT01218867), where HCC patients without hepatitis B and C were included[55]. The result of this study is still awaited. Recently, phase I/II

Table 2. Selected clinical trials with therapeutic cancer vaccine immunotherapy for HCC

Registered No.	Recruitment status	Start year	Phase	Immunotherapy	Included patients of HCC
NCT00004604	Completed	1997	I	CEA RNA-pulsed DC cancer vaccine	Metastatic adenocarcinoma expressing CEA that has failed conventional therapy
NCT00019331	Completed	1997	II	Ras peptides and IL-2 or GM-CSF	Solid tumors potentially expressing mutant Ras
NCT00005629	Completed	1999	I/II	AFP gene HCC vaccine	HLA-A*0201 positive, serum AFP levels > 2 times above the upper limit of normality
NCT00022334	Completed	2001	I/II	AFP peptide-pulsed autologous DC	HLA-A*0201 positive, HCC with a serum AFP determination > 30 ng/mL
NCT00028496	Completed	2001	I	Recombinant fowlpox-CEA(6D)/ TRICOM vaccine, sargramostim and recombinant fowlpox GM-CSF vaccine adjuvant	Failed standard curative options and no standard palliative options required within the next 8weeks
NCT00027534	Completed	2002	I	TRICOM-CEA(6D)	Histologically confirmed advanced or metastatic malignancy expressing CEA
NCT00629759	Completed	2006	I	JX-594: recombinant vaccinia virus (TK-deletion plus GM-CSF)	Progressing HCC
NCT00610389	Unknown	2008	II	DC with PEG-IFN alfa and GM-CSF	HCC not amenable of curative treatment with Child´s stage A or B
NCT01266707	Unknown	2010	I	VEGFR1 and VEGFR2 specific epitope vaccine	Unresectable or treatment-resistant HCC
NCT01828762	Completed	2012	N/A	DC incubated with irradiated autologous tumor stem cells in GM-CSF	BCLC stage A/B, after resection and TACE
NCT01974661	Completed	2013	I	COMBIG-DC (ilixadencel): allogenic dendrite-cell based therapeutic vaccine	BCLC stage B/C, not eligible for curative treatment or TACE
NCT02232490	Recruiting	2014	III	Hepcortespenlisimut-L (V5)	HCC with AFP serum test higher or equal to 30 IU/mL
NCT02409524	Recruiting	2016	II	AlloVax, AlloStim and CRCL	Unresectable HCC with minimum 90 days of sorafenib treatment
NCT03203005	Recruiting	2017	I/II	A new cancer vaccine called IMA970A combined with CV8102 with cyclophosphamide	BLCL stage 0/A/B following any standard treatment

HCC: hepatocellular carcinoma; CEA: carcinoembryonic antigen; RNA: ribonucleic acid; DC: dendritic cell; GM-CSF: granulocyte-macrophage colony-stimulating factor; AFP: alpha-fetoprotein; HLA: human leukocyte antigen; TK: thymidine kinase; TRICOM: triad of costimulatory molecules (B7-1, ICAM-1 and LFA-3); PEG-IFN: pegylated interferon; VEGFR: vascular endothelial growth factor receptor; BCLC: Barcelona clinic liver cancer; TACE: transarterial chemoembolization; COMBIG: combined toll-like receptor interferon-gamma; CRCL: chaperone rich cell lysate; N/A: not applicable

clinical trials with CAR T cells targeting glypian-3 (GPC3), alpha-fetoprotein (AFP), and mucin 1 (MUC1) are being conducted [Table 1][56,57]. Moreover, a CAR NK cell immunotherapy targeting MUC1 is being conducted (NCT02839954)[58].

Taken together, adoptive cellular immunotherapy in HCC is a safe and feasible treatment. However, its efficacy in preventing recurrence and prolonging survival in advanced HCC patients remains controversial[59]. Indeed, cellular immunotherapy seems to be more effective in patients with low burden of micrometastases[36]. The current situation lacking sufficiently effective cellular immunotherapy for advanced stages of HCC calls for further improvement in immunotherapeutic strategies and additional approaches with immune checkpoints modulators.

THERAPEUTIC CANCER VACCINES

Therapeutic cancer vaccine is an important part of cancer immunotherapy. Vaccination with cancer antigens or peptides is believed to help the immune system to recognize cancer cells and attack them more easily [Tables 2 and 3]. In therapeutic cancer vaccine, DC is an important component. As professional APCs, DCs serve as an essential link between innate and adaptive immune systems[17]. Two functional states of DC are described, as immature or mature DCs. Several factors can induce maturation of DCs. Mature DCs are specialized APCs, which express high levels of surface MHC I and MHC II class, as well as the appropriate

Table 3. Current trials on combinational immunotherapy strategies in HCC

Registered No.	Recruitment status	Start year	Phase	Immunotherapy	Included patients of HCC
NCT01522820	Completed	2012	I	DEC-205/NY-ESO-1 fusion protein CDX-1401 with sirolimus	After resection or TACE
NCT01853618	Completed	2013	I/II	Tremelimumab with TACE, RFA, SBRT or Cryoablation	BCLC stage B/C
NCT01821482	Recruiting	2013	II	DC-CIK	After complete resection or TACE
NCT02562755	Recruiting	2015	III	Pexastimogene devacirepvec (Pexa Vec) with sorafenib	Advanced HCC (BLCL-C or AASLD-B)
NCT02487017	Recruiting	2015	II	DC-CIK with TACE	After TACE treatment
NCT02432963	Active, not recruiting	2015	I	Modified vaccinia virus Ankara vaccine expressing p53 and pembrolizumab	Advanced HCC, confirmed p53 involvement, failed to or refusal to standard therapy
NCT02821754	Recruiting	2016	II	Durvalumab and tremelimumab with RFA, cryotherapy or TACE	Multiple HCC technically amenable to ablative therapy
NCT02837029	Recruiting	2016	I	Nivolumab with Yttrium Y 90 glass microspheres	Stage III/IV
NCT02795429	Recruiting	2016	I/II	PDR001 with or without INC280	Advanced, recurrent or metastatic HCC
NCT02886897	Recruiting	2016	I/II	DC-CIK and anti-PD-1 antibody	Advanced HCC
NCT03259867	Recruiting	2017	IIA	Nivolumab or pembrolizumab with trans-arterial tirapazamine embolization	Advanced HCC (BCLC-C), progressive disease (PD) on, intolerant of or refusing sorafenib
NCT03380130	Recruiting	2017	II	Nivolumab with selective internal radiation therapy	Candidates for locoregional therapy using selective internal radiation-spheres
NCT03277352	Recruiting	2017	I/II	INCAGN01876, pembrolizumab and epacadostat	Locally advanced or metastatic disease
NCT03241173	Recruiting	2017	I/II	INCAGN01949, nivolumab and/or ipilimumab	Locally advanced or metastatic disease
NCT03126110	Recruiting	2017	I/II	INCAGN01876, nivolumab and/or ipilimumab	Locally advanced or metastatic disease
NCT03095781	Recruiting	2017	I	Hsp90 inhibitor XL888 and pembrolizumab	Stage IV or locally advanced unresectable gastrointestinal adenocarcinomas
NCT03203005	Recruiting	2017	I/II	A new cancer vaccine called IMA970A and CV8102 with cyclophosphamide	BLCL stage 0/A/B following any standard treatment
NCT03067493	Recruiting	2017	II	Neo-MASCT (antigen-pulsed DC, autologous specific cytotoxic T-cells)	Primary HCC with previous RFA or resection
NCT03071094	Recruiting	2017	I/IIA	Pexastimogene devacirepvec (Pexa Vec) and nivolumab	Advanced HCC per EASL-EORTC
NCT03482102	Recruiting	2018	II	Tremelimumab and durvalumab with radiation	Locally advanced/unresectable or metastatic disease
NCT03439891	Recruiting	2018	II	Nivolumab with sorafenib	Unresectable, locally advanced and/or metastatic HCC
NCT03511222	Not yet recruiting	2018	I	Vorolanib and pembrolizumab	A solid tumor that can be treated with either pembrolizumab or nivolumab as part of standard of care

HCC: hepatocellular carcinoma; GM-CSF: granulocyte-macrophage colony-stimulating factor; BCLC: Barcelona clinic liver cancer; PD-1: programmed cell death 1 protein; TACE: transarterial chemoembolization; RFA: radiofrequency ablation; AASLD: American association for the study of liver diseases; SBRT: stereotactic body radiotherapy; DC: dendritic cells; CIK: cytokine-induced killer; Hsp: heat shock protein; Neo-MASCT: neoantigen multiple target antigen stimulating cell therapy; EASL: European association for the study of the liver; EORTC: European organisation for research and treatment of cancer

costimulatory molecules required for T-cell activation. One of the most important functions of mature DCs is the rapid production of high amounts of type I IFN, especially in response to virus-derived nucleic acids through activation of Toll-like receptors (TLRs), both TLRs 7 and 9.

Although immunotherapy is not recommended for the clinical management of HCC patients under current guidelines, several different immunotherapy vaccine strategies have been investigated in the last decade for HCC[15]. Moreover, significantly lower numbers of CD83+ DCs (mature and activated DCs) have been found in liver tissue of patients with HCC compared with liver cirrhosis patients[60].

Many of the HCC clinical studies on therapeutic cancer vaccines have focused on AFP-based vaccinations since the majority of human HCCs overexpress AFP[15]. CD8+ T cell epitopes derived from AFP peptides

were used to carry on the first HCC vaccine clinical trial. AFP positive HCC patients received three biweekly intradermal injections of the AFP peptides. All of the patients (n = 6) developed the AFP-specific T cell responses, clearly proving the immunogenicity of AFP even in the environment of high circulating levels of AFP in HCC patients[61]. Subsequently, the authors conducted another phase I/II trial. This time, they immunized AFP positive HCC patients with autologous DCs *ex vivo* pulsed by AFP epitopes. DCs were prepared from PBMCs cultured with granulocyte-macrophage colony-stimulating factor and IL-4 for 7 days[62]. In this study, AFP-specific T cell response and increased IFN-γ production were shown. Despite this immune response, clinical response was not observed. The authors found the reason for it in a subsequent study that CD4+ T cell help was lacking, which resulted in non-functional AFP-specific CD8+ T cells[63]. Unfortunately, a limited number of clinical trials for HCC have been conducted based on therapeutic vaccine immunotherapy.

Meanwhile, the bioactivity and beneficial effects of DC infusion were evaluated in HCC patients following trans-catheter hepatic arterial embolization (TAE). In this study, tumor recurrence was not completely prevented in patients with TAE and DC infusion than in those with TAE alone. However, TAE with DC infusion enhanced the tumor-specific immune responses more effectively than TAE alone. The authors demonstrated that combination therapy using TAE together with DC infusion is safe for patients with cirrhosis and HCC[64].

In another phase II study, the safety and efficacy of vaccination with mature autologous DCs pulsed with a liver tumor cell line lysate (HepG2) have been investigated in patients with advanced HCC and not suitable for radical or loco-regional therapies[65]. The authors showed that autologous DC vaccination in patients with HCC is safe and well tolerated with evidence of antitumor efficacy with generation of antigen-specific immune responses in some cases. More recent study, reported in 2013, also showed similar results. The safety and efficacy of the autologous pulsed DC vaccine was compared to supportive treatment in advanced HCC patients. They showed that autologous DC vaccination in advanced HCC patients was safe and well tolerated. Additionally, both CD8+ CTL and serum IFN-γ were elevated after DC vaccine[66].

Actually, to date, no vaccine has been approved so far for HCC treatment[19]. Further investigations and improvements of therapeutic cancer vaccines will be required to achieve better efficacies in HCC patients.

IMMUNE CHECKPOINT BLOCKADES IN HCC

During the last decade, new immuno-oncological treatments were introduced for diverse cancers, eventually leading to the clinical breakthrough of immune checkpoint blockades targeting CTLA-4, PD-1, PD-L1 and PD-L2[67,68]. Under physiological conditions these checkpoint molecules resolve T cell activation to maintain inflammatory homeostasis, also limit collateral tissue damage and prevent unwanted auto-immunity, as observed in response to chronic viral hepatitis[26,27]. Meta-analysis data on solid tumors have suggested that overexpression of PD-L1 in tumor cells, as well as in APCs of tumor microenvironment, is associated with poor prognosis in patients with malignant tumors including HCC[21,69]. The subsequent PD-1/PD-L1 interaction results in T-cell exhaustion and immune evasion by cancer cells[70]. The inhibitory effects of the PD-1/PD-L1 pathway on T cell-mediated antitumor immunity are commonly reported regarding HCC carcinogenesis, and the PD-L1 is over-activated in HCC[9,71]. Also the PD-1/PD-L1 interaction is known to be associated with persistent HBV and HCV viremia, or the progression of HCC, by suppressing specific T-cell immunity and thereby inducing immune tolerance or immune escape of cancer cells[8,27].

Notably, immune checkpoint inhibitors have proven effective in patients who are refractory to tyrosine kinase inhibitors (TKIs) such as sorafenib, and recently several blocking antibodies targeting PD-1 or CTLA-4 have shown promising results in advanced HCC patients who received previous treatment with sorafenib[20,28,72]. Compared to TKIs, immunotherapy has several advantages for the treatment of cancer, as its effects are

not hampered by common mutations or neoantigen heterogeneity of tumor cells[28,73]. Therefore, immuno-oncology agent is effective regardless of the response to prior therapies, and also a durable response can be expected due to adaptive immunity to the cancer cells[74]. However, the profile of AEs is completely different from those of other cytotoxic and molecular targeting agents[28]. The tolerability of immuno-oncology agents generally depends on the severity of immune-related AEs (irAEs), although the majority of irAEs are mild and manageable[20,75].

Different clinical trials are currently underway to investigate the safety and efficacy of checkpoint inhibitors for HCC immunotherapy as in monotherapy or in combination [Table 3][28].

The PD-1/PD-L1 pathway

Higher intra-tumoral expression of PD-1/PD-L1 had been associated with significantly poorer PFS and OS after hepatectomy as well as postoperative recurrence in HCC[76]. It was shown that PD-1 immune checkpoint inhibitor therapies have a strong therapeutic effect on patients with high levels of PD-L1 expression[77]. This could be due to the ability of the PD-1/PD-L1 pathway to act as an anti-apoptotic receptor on cancer cells [Figure 1][23,69].

To date, two kinds of anti-PD-1 (nivolumab and pembrolizumab) and anti-PD-L1 (durvalumab, avelumab) antibodies have been applied for clinical trials in HCC and nivolumab, pembrolizumab, and avelumab are in development as monotherapy[20,28]. Two phase III studies are currently ongoing: a comparison of nivolumab and sorafenib in the first line setting for advanced HCC (CheckMate 459), and a comparison of pembrolizumab and a placebo in the second line setting for patients with advanced HCC who progressed on sorafenib (KEYNOTE 240)[20,78].

Nivolumab, a fully human IgG4 anti-PD-1 monoclonal antibody, was granted accelerated approval from U.S. FDA on September 2017 for treatment of HCC patients who were previously treated with sorafenib. Approval was based on findings in a phase I/II, open-label, non-comparative, dose escalation and expansion trial (CheckMate 040) consisting of patients with HCC and Child-Pugh A cirrhosis[78]. Between November 2012 and August 2016, 262 eligible patients were treated (48 patients in the dose-escalation phase and 214 in the dose-expansion phase). At the American Society of Clinical Oncology (ASCO) meeting in 2015, results of the dose-escalation trial of CheckMate 040 were presented; 68% of patients had drug-related AEs, the complete response (CR) rate was 5%, and the partial response (PR) rate 14%. The safety profile of nivolumab is generally consistent with what was previously-reported in other tumor types. Twelve (25%) of 48 patients in the dose-escalation phase had grade 3/4 treatment-related AEs. Autoimmune disease and hepatic dysfunction, which were the AEs of initial concern, were not observed[20]. In the 2017 ASCO meeting, final results of the phase I/II CheckMate 040 study with nivolumab in advanced HCC showed favorable results with objective response rate (ORR) 20% and disease control rate (DCR) 64%[78]. The OS rate of the fixed dose of 3 mg/kg nivolumab group at 12 months was 62%. Considering that a high proportion (66%) progressed on sorafenib treatment, these outcomes appear to be extremely good. In addition, nivolumab was effective regardless of prior sorafenib administration and viral status, indicating that nivolumab could be effective even in cases refractory to sorafenib[20,28,78]. However, the ORR of HBV-positive cases was lower (14%) compared to non-HBV cases (20%-23%)[28,78]. There was no significant association between PD-L1 expression in HCC and the response to nivolumab[20,78].

Another anti-PD-1 antibody, pembrolizumab, was associated with PR and prolongation of survival in a patient with progressive metastatic HCC while being treated with sorafenib[79]. The randomized, placebo-controlled phase III KEYNOTE 240 study (NCT02702401) to compare the efficacy and safety of the pembrolizumab with best supportive care for the treatment of advanced HCC after failure to sorafenib is ongoing. Recently, findings from the KEYNOTE 224 study (NCT02702414), open-label phase II trial

investigating pembrolizumab monotherapy in patients with advanced HCC who were previously treated with sorafenib, were presented at the 2018 Gastrointestinal Cancers Symposium. Results showed the ORR of 16.3% (95% CI, 9.8%-24.9%; *n* = 17/104) with CR of 1% (95% CI, 0.0%-5.2%) and PR of 15.4% (95% CI, 9.1%-23.8%). The DCR was 61.5% (95% CI, 51.5%-70.9%; *n* = 64/104) and median PFS time was 4.8 months (95% CI, 3.4-6.6 months), with a 6-month PFS rate of 43% and 6-month OS rate of 78%.

Furthermore, a clinical trial of monotherapy agents targeting PD-L1, such as avelumab, has also been conducted in advanced HCC patients (NCT03389126)[28].

The CTLA-4 pathway

Tremelimumab is an IgG2 type anti-CTLA-4 antibody that was evaluated in a phase II clinical trial (NCT01008358) investigating the tremelimumab monotherapy in 21 patients with HCV-related HCC[80]. This study with tremelimumab in HCV infected HCC patients has shown a good safety profile along with a promising PR rate of 17.6% and a time-to-progression (TTP) of 6.5 months[80]. In this CTLA-4 trial, a transient complete virologic response or decrease in HCV viral load was also observed in most patients with the DCR of 76.4%[28,80]. The trial demonstrated efficacy of tremelimumab monotherapy in HCC patients and the anti-tumoral and antiviral effects that warrant further investigation.

Notably, the feasibility of combined locoregional therapies and tremelimumab administration was investigated in patients with liver cirrhosis and HCC[81]. The use of tremelimumab plus RFA, cryoablation, or TACE in patients with BCLC B or C HCC was associated with ORR of 26.3% in areas outside of the ablation zone, and median TTP was 7.4 months. The combination of tremelimumab with local tumor ablation is a smart synergistic mechanism, because, in patients responding to local ablative therapy, prolonged TTP gives time for immunotherapy to unfold[72]. In addition, local tumor ablation releases TAA from apoptotic or necrotic HCC tissue, which in turn accelerates tumor specific APCs and CTLs activation, resulting in immunological synergy evolving from the combination of both treatment modalities [Figure 1]. Also, in this study, 12 of 14 patients with quantifiable HCV experienced a marked reduction in viral load, especially in the patients with PR. Studies have shown that tremelimumab in combination with tumor ablation is a potential new treatment for patients with advanced HCC[81]. Particularly, the positive antiviral immune responses may act as a surrogate for disease control in HCC immunotherapy[72].

In summary, immune checkpoint blockade therapy (anti-PD-1 and anti-CTLA-4) had a favorable safety profile in patients with HCC[20]. It can be used safely in patients with HBV and HCV infection, and its high ORR was a great achievement compared to the rates achievable with other types of immunotherapy[28].

Other immune checkpoint pathways

Although anti-PD-1/PD-L1 antibody is a promising agent for the treatment of HCC, a considerable percentage of HCC patients could not attain satisfactory tumor control, likely due to the immune suppressive cellular components, humoral mediators, and diverse inhibitory checkpoint molecules[28,82]. Their crosstalk becomes more complex during tumor progression. Also, the continuous production of cytokines and chemokines in the inflamed liver and solid immunosuppressive stroma of HCC could induce the production of many types of suppressive checkpoint molecules[83,84].

Cellular components including MDSCs, TAMs, Tregs and type 2 helper T cells might facilitate the immune evasion of HCC tumor cells[20,83]. MDSCs also produce transforming growth factor (TGF)-β and IL-10 that lead to the suppression of CD56+ NK cell and CD8+ CTL activities[85]. TGF-β from MDSCs induces the expression of T-cell immunoglobulin and mucin-containing protein-3 (TIM-3) on TAMs, which is associated with galectin-9 and further facilitates the M2 polarization of macrophages in tumors[86]. Galectin-9, which

is a ligand of TIM-3, also induces Treg stimulation and T cell exhaustion[83]. The TIM-3/galectin-9 signaling pathway reportedly mediates T-cell dysfunction in HBV-associated HCC, which might explain the poor ORR of HBV-associated HCC compared with that of non-HBV-associated HCC during the anti-PD-1 antibody administration[78,83,87].

Galectin-3 interacts with lymphocyte activation gene-3 (LAG-3) and inhibits CD8+ T cell and NK cell functions[83]. LAG-3 expression on TILs, along with PD-L1 on tumor cells, is also reported in HCC[88]. As in the PD-1/PD-L1 in HCC tumor, the galectin-3/LAG-3 expression is also associated with a poor prognosis in HCC patients[89]. Another report also showed up-regulated LAG-3 expression and impaired effector function of CD8+ CTLs in HBV-positive HCC patients[90]. Taken together, TIM-3, LAG-3, and PD-1 act synergistically and facilitate the HCC immune evasion resulting in worse prognosis[88]. According to these findings, TIM-3 and LAG-3, checkpoint molecules expressed on the effector T cell, could mediate resistance to the PD-1/PD-L1 blockade[86,88]. Given the fact that there are multiple players in the establishment of immune escape in HCC, anti-PD-1/PD-L1 therapy is being paired with agents targeting TIM-3 (NCT03099109) and LAG-3 (NCT01968109), respectively[83].

HCC IMMUNOTHERAPIES IN COMBINATION WITH OTHER CANCER TREATMENTS

While the present adoptive immunotherapy has been restricted to the patients with small tumor burdens so far, treatments using these engineered immune cells have generated some remarkable responses in patients with advanced cancer by combinational immunotherapy[91]. Ongoing investigations of adoptive immunotherapy combined with traditional HCC treatments, including surgery, locoregional interventions, and systemic chemotherapy, may achieve the best objective responses in various stages of HCCs[92,93]. In 2013, a retrospective study was conducted in 174 HCC patients from January 1999 to April 2012. Among them, 85 patients were given CIK cell infusion after treatment with TACE and RFA alone. The results demonstrated that CIK cell infusion significantly prolonged the PFS in patients compared to TACE or RFA monotherapy[94]. A different approach is pretreatment of HCC with TACE, RFA, or RT to induce inflammation of cancer cells, thereby creating conditions that favor tumor neoantigen generation prior to the initiation of immunotherapy[84].

Although the efficacy of immune checkpoint inhibitors in HCC is promising, the majority of the patients remain refractory, due to the immunosuppressive mechanisms of HCC comprising multiple humoral mediators and suppressive checkpoint molecules[82,83]. To enhance the anti-tumor activity, several studies on combined immune checkpoint blockades are being conducted [Table 3]. The most relevant combination is a CTLA-4 and PD-1/PD-L1 blockade[28]. The rationale of this strategy is based on the idea that if CD8+ CTL do not exist in cancer tissue, blockade of the PD-1/PD-L1 pathway cannot be expected to be efficacious. Therefore, blocking CTLA-4 may be an effective strategy to increase the number of activated effector T cells that infiltrate the tumor tissue[20]. Durvalumab, a monoclonal antibody to PD-L1, is currently evaluated in combination with an anti-CTLA-4 antibody (tremelimumab) for sorafenib-experienced HCC patients in a phase II trial (NCT02519348)[83]. Another anti-CTLA-4 antibody, ipilimumab, is also being analyzed in combination with the anti-PD-1 antibody, nivolumab, for evaluation of the safety and efficacy in HCC patients (NCT01658878, NCT03222076)[20].

Given the fact that molecular target agents could collectively block the signaling from various growth factors and affect immune effectors and the vasculature, the combination of TKIs and immune checkpoint inhibitors could reactivate the immune response to HCC[28,84]. Several early phase studies are currently underway to explore the safety and tolerability of TKIs such as sorafenib (NCT03211416, NCT01658878, NCT02988440), lenvatinib (NCT03418922, NCT03006926), cabozantinib (NCT03299946, NCT01658878), axitinib (NCT03289533), and capmatinib (NCT02795429) in combination with immune checkpoint

inhibitors[83]. Of note, current clinical trials are focusing on how immunosuppressive conditions in HCC might be overcome using immune checkpoint inhibitors in combination with different types of immune checkpoint blockades, TKIs, and other conventional treatments[83]. To improve the HCC immunotherapy strategies as well as immune stimulatory approaches, identification of TAAs and neoantigens specific to HCC and testing the potential benefits of combinatorial immunotherapies will achieve the most beneficial effect for HCC patients[59].

CONCLUSIONS AND FUTURE PERSPECTIVES

The journal *Science* selected cancer immunotherapy as its "Breakthrough of the Year" in 2013, and especially the use of immune checkpoint blockade in cancer therapy is making a paradigm shift in cancer treatment[20,95]. Of note, immunotherapy has the potential to achieve complete, long-lasting remissions and cancer cures, representing the most promising new cancer treatment approach with few side effects[74]. Although disease progression is sometimes observed immediately after initiation of immunotherapy, some responders require longer duration of immunotherapy to achieve tumor response[20]. Therefore, the biomarkers of immunotherapy to predict response are urgently needed, both from the perspective of the effective use of medical resources and to prevent adverse effects caused by unnecessary treatment[84]. There are several highly promising candidate predictors of the cancer immunotherapy: PD-L1 expression in tumor tissue, TAA related mutanome analyses including next-generation sequencing, and the immunome analyses, which employ T cell repertoire analysis and proteomic analysis[96]. Also, additional questions still remain regarding the most effective combination of therapeutic modalities and biomarkers to predict long term treatment outcomes in HCC immuno-oncology.

Notably, to date, very promising published evidence with checkpoint inhibitors in HCC has been reported in the clinical trials of anti-CTLA-4 agent tremelimumab and a large phase II trial with anti-PD-1 agent nivolumab[56,78,80]. Further investigations of immuno-oncology potentially spread the applications of immunotherapy in the various stages of HCCs, and thus immune-based therapies will bring about a paradigm shift of anti-cancer treatment for HCC. We hope the immunotherapy will play a key role in HCC treatment in the near future.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Lee JH, Nishida N

Performed data acquisition, as well as provided administrative, technical, and material support: Oh SY, Kim JY

Availability of data and materials

Not applicable.

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Conflicts of interest

The authors declare there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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