



2016 Hepatocellular Carcinoma: Global view

Current status and perspectives of immune-based therapies for hepatocellular carcinoma

Maridi Aerts, Daphné Benteyn, Hans Van Vlierberghe, Kris Thielemans, Hendrik Reynaert

Maridi Aerts, Hendrik Reynaert, University hospital, UZ Brussel, Department of Gastroenterology and Hepatology, 1090 Brussels, Belgium

Daphné Benteyn, Kris Thielemans, Vrije Universiteit Brussel (VUB), Department of Immunology, 1090 Brussels, Belgium

Hans Van Vlierberghe, UZ Gent, Department of Gastroenterology and Hepatology, De Pintelaan, 9000 Ghent, Belgium

Author contributions: Aerts M, Benteyn D and Reynaert H analyzed the literature and wrote the manuscript; Van Vlierberghe H and Thielemans K critically revised the manuscript.; all authors approved the final version of the manuscript.

Supported by Grant from Kankerplan Action 29, Ministry of health, Belgium (to Aerts M); Van Vlierberghe H is senior researcher of the Flemish Fund for Research (FWO).

Conflict-of-interest statement: The authors have no conflict of interest to report.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Hendrik Reynaert, MD, PhD, Professor, University hospital, UZ Brussel, Department of Gastroenterology and Hepatology, Laarbeeklaan 101, 1090 Brussels, Belgium. hendrik.reynaert@uzbrussel.be
Telephone: +32-2-4776811
Fax: +32-2-4776810

Received: June 8, 2015
Peer-review started: June 11, 2015
First decision: July 14, 2015
Revised: August 11, 2015
Accepted: October 26, 2015

Article in press: October 26, 2015
Published online: January 7, 2016

Abstract

Hepatocellular carcinoma (HCC) is a frequent cancer with a high mortality. For early stage cancer there are potentially curative treatments including local ablation, resection and liver transplantation. However, for more advanced stage disease, there is no optimal treatment available. Even in the case of a "curative" treatment, recurrence or development of a new cancer in the precancerous liver is common. Thus, there is an urgent need for novel and effective (adjuvant) therapies to treat HCC and to prevent recurrence after local treatment in patients with HCC. The unique immune response in the liver favors tolerance, which remains a genuine challenge for conventional immunotherapy in patients with HCC. However, even in this "immunotolerant" organ, spontaneous immune responses against tumor antigens have been detected, although they are insufficient to achieve significant tumor death. Local ablation therapy leads to immunogenic tumor cell death by inducing the release of massive amounts of antigens, which enhances spontaneous immune response. New immune therapies such as dendritic cell vaccination and immune checkpoint inhibition are under investigation. Immunotherapy for cancer has made huge progress in the last few years and clinical trials examining the use of immunotherapy to treat hepatocellular carcinoma have shown some success. In this review, we discuss the current status of and offer some perspectives on immunotherapy for hepatocellular carcinoma, which could change disease progression in the near future.

Key words: Hepatocellular carcinoma; Immunotherapy; Dendritic cells; Dendritic cell vaccination; Therapy

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Hepatocellular carcinoma is a frequent cancer with a high mortality. For early stage cancer there are potentially curative treatments including local ablation, resection and liver transplantation. However, recurrence or development of a new tumor after treatment are not uncommon. Moreover, for more advanced stage disease, there is no optimal treatment available. Thus, there is an urgent need for novel and effective therapies for advanced stage hepatocellular carcinoma, and to prevent and to treat recurrence after local treatment of hepatocellular carcinoma.

Aerts M, Benteyn D, Van Vlierberghe H, Thielemans K, Reynaert H. Current status and perspectives of immune-based therapies for hepatocellular carcinoma. *World J Gastroenterol* 2016; 22(1): 253-261 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v22/i1/253.htm> DOI: <http://dx.doi.org/10.3748/wjg.v22.i1.253>

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and fatal cancers in the world. Men are more often affected than women, with 554000 and 228000 new cases per year, respectively. It is the second most common cause of death from cancer worldwide, leading to 746000 deaths in 2012. Viral hepatitis B and C, chronic alcohol consumption and non-alcoholic fatty liver disease are major risk factors^[1].

Once diagnosed, HCC frequently has a dismal prognosis because of the low effectiveness of available treatments. The choice of treatment is selected according to the Barcelona Clinic Liver Cancer staging system, which integrates tumor characteristics and performance status with liver function and links them to evidence-based therapeutic option^[2]. Early and very early stage tumors are potentially curable with surgery (resection or liver transplantation) or local therapies including radiofrequency ablation (RFA) or percutaneous ethanol injection. The 5 year survival rate in these patients ranges from 40% to 70%^[2]. In the absence of liver transplantation, tumor recurrence is observed in 70% of patients after resection or RFA after a time period of 3 years. Unfortunately, less than 30% of HCC patients are eligible for these procedures because most have intermediate or advanced stage disease at diagnosis (large or multifocal tumors, or liver insufficiency, which limits treatment). For intermediate stage tumors, transarterial chemoembolization (TACE) (conventional or drug eluting beads) has become the standard of care^[3]. However, there is certainly room for improvement because the 3-year survival rate is only approximately 60%^[4,5]. In advanced stage disease, the oral multi-targeted kinase inhibitor sorafenib offers

a survival benefit of approximately 3 mo^[6]. Other molecules, such as sunitinib or lenvatinib have not been proven to be superior to sorafenib^[7,8]. Several newer molecules have been shown to confer a survival advantage in a subset of patients^[9]. The mean survival of patients with advanced stage HCC is less than 1 year.

Recurrence rates remain high in very early to intermediate stages despite the availability of potentially curative treatment. There are 2 main reasons responsible for this phenomenon. First, a small tumor that is undetectable using current imaging modalities may exist before treatment and would thus be left untreated; second, a new tumor may occur in the diseased liver, which can be considered a pre-neoplastic organ. The efficacy of combining local treatment with systemic treatment has been studied in several clinical trials. In phase I and II trials, the preliminary results from using combination therapies were promising. However, two large multicenter phase III trials studying the effect of sorafenib after TACE (SPACE study)^[10] or surgical resection/RFA (STORM study)^[11] failed to demonstrate any adjuvant effect of sorafenib on survival. This underlines the need for novel and effective adjuvant therapies to treat patients with HCC and to prevent recurrence after local treatment.

IMMUNE RESPONSE IN THE LIVER AND IMMUNOTHERAPY

The liver is a unique organ in several ways, one of which is its blood supply. Approximately 25% and 75% of the blood enters the liver through the hepatic artery and portal vein, respectively^[12]. After a meal, the percentage of the portal vein supply increases further. The portal vein drains into smaller diameter structures, known as the sinusoids. Vascular resistance is very low in these structures, and portal venous blood, which is loaded with food and microbial antigens from the intestine, flows extremely slowly in the sinusoids. Moreover, liver sinusoidal cells (LSECs) are fenestrated thus facilitating the passage of cells and antigens between the sinusoids and the space of Disse, which is in contact with hepatocytes. With the exception of hepatocytes and biliary cells, the liver hosts a number of non-parenchymal cells including LSECs, hepatic stellate cells, Kupffer cells, dendritic cells (DCs), and lymphocytes. All of these cells play roles in the barrier function of the liver, separating it from the gastrointestinal tract and the rest of the body. Indeed, DCs, LSECs, hepatic stellate cells and Kupffer cells are all able to present antigens to antigen-specific lymphocytes. These cells are resident cells and induce tolerance rather than immunity. For a more detailed discussion of the immunological properties of the liver, we refer the reader to several recent, excellent reviews on this topic^[13-15].

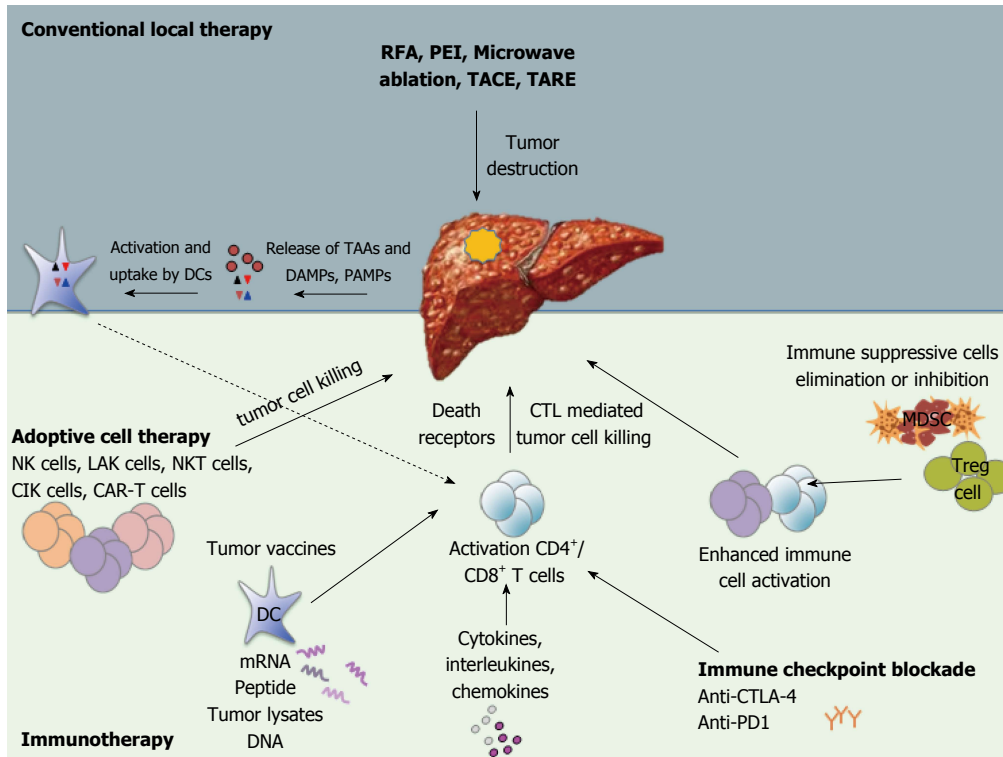


Figure 1 Immune-based therapies for hepatocellular carcinoma. Tumor-specific T cells can be stimulated by several ways including adoptive cell therapy, tumor vaccines, cytokines or by inhibiting immune suppressive mechanisms (immune checkpoint inhibitors, depletion of Tregs or MDSCs). These tumor-specific T cells are able to kill the tumor cells in an antigen-specific way. Conventional therapies (RFA, TACE, PEI, microwave ablation, TARE) can destroy tumor cells, which result in the release of tumor antigens and danger-associated molecular patterns that can activate DCs resulting in the activation of tumor-specific T cells. RFA: Radiofrequency ablation; PEI: Percutaneous ethanol injection; TACE: Transarterial chemoembolization; TARE: Transarterial radioembolization; DAMPs: Danger-associated molecular patterns; PAMPs: Pathogen-associated molecular patterns; DC: Dendritic cell; NK: Natural killer; LAK: Lymphokine-activated killer; NKT cell: Natural killer T cell; CIK: Cytokine-induced killer; CAR: Chimeric antigen receptor; CTL: Cytotoxic T lymphocyte; Treg: Regulatory T cell; MDSC: Myeloid derived suppressor cells; CTLA-4: Cytotoxic T lymphocyte antigen 4; PD-1: Programmed-death 1.

Immunotherapy for cancer is based on harnessing the potential of the immune system to destroy malignant cells. However, immune responses in the liver might be problematic, as the liver mainly induces tolerance. The liver is a distinctive organ with respect to immune function and possesses a unique form of immune regulation: immune tolerance is induced to avoid chronic inflammation caused by antigens present in portal vein blood. This might prevent an adequate immune response against malignant cells. Indeed, it has been shown that an increased quantity of circulating regulatory T (Treg) cells in patients with HCC is associated with a high mortality rate and reduced survival^[16]. Overcoming this immune tolerance is thus an important challenge in the search for an effective immunotherapy against HCC. On the other hand, there have been reports of spontaneous regression of HCC associated with tumor hypoxia or systemic inflammatory response^[17]. Additionally, the regression of HCC has been described following the discontinuation of immunosuppressive therapy^[18]. Therefore, immunotherapy remains an attractive approach for boosting the immune system in patients with HCC to enhance the efficacy of current therapies (Figure 1).

SPONTANEOUS TUMOR-SPECIFIC IMMUNE RESPONSE IN THE LIVER

Immune evasion is a characteristic of cancer and is even more prevalent in organs with high immune tolerance such as the liver. High levels of $CD4^+CD25^+FOXP3^+$ Treg cells in peripheral blood are an independent predictive factor of poor survival after TACE treatment in patients with HCC^[19]. In addition, the presence of high numbers of intrahepatic $CD8^+FoxP3^+$ regulatory T cells was found to be associated with more advanced stages of HCC^[20]. Additionally, there is an increased presence of myeloid-derived suppressor cells is increased in patients with HCC compared to healthy donors^[21].

Even if HCC is not generally considered an "immunogenic" tumor type, immune responses do occur in livers that have been invaded by HCC. In a number of patients, spontaneous immune responses against tumor antigens have been detected, but these were insufficient to achieve a significant therapeutic effect^[22-24]. Moreover, naturally occurring tumor-associated antigen-specific T-cell responses exist in patients with HCC and correlate with patient survival^[25]. Patients whose tumors express multiple tumor-associated antigens (TAAs) and contain TAA-specific

CD8⁺ T-cell lymphocytic infiltrates show longer survival rates and a lower risk of recurrence^[25-27]. A strong CD8⁺ T-cell response against TAAs was shown to improve recurrence-free survival after surgery.

Over the past 15 years, a number of TAAs have been identified in HCC, some of which elicit tumor-specific immune responses^[24]. Most of the identified TAAs are not specific to HCC, but some HCC-specific TAAs are targeted by T cells, which makes them good potential targets for immunotherapy; these include alpha-fetoprotein (AFP), glypican 3 (GPC3), melanoma antigen gene A (MAGE-A), and New York-esophageal squamous cell carcinoma-1 (NY-ESO-1)^[25,28].

AFP, which is expressed in the fetus and repressed after birth, is re-expressed in the majority of HCCs. Even if it has a low sensitivity and specificity as a serum marker, it continues to represent a useful clinical marker for HCC^[29]. In some studies, HCC patients had increased frequencies of circulating AFP-specific CD8⁺ T cells^[23,30].

GPC3 is a cell surface heparin sulfate proteoglycan. GPC3 mRNA expression is low or absent in normal liver tissue, in benign tumor lesions (such as focal nodular hyperplasia) and in a cirrhotic liver^[31]. However, GPC-3 is detected in approximately 80% of HCCs even in the early stages^[27,32]. GPC3-specific cytotoxic T-lymphocytes have a high level of killing activity against HCC tumor cells. A study from Nobuoka *et al.*^[33] showed that GPC3 has strong immunogenicity.

MAGE-A was first described in melanoma, but it has been shown to be widely expressed in various tumors. In cirrhotic patients with HCC, mRNA encoding MAGE-1 was found to be present in 80% of resected HCCs^[34]. MAGE-A and SSX-2 specific CD8⁺ T cells were found to be enriched in HCC, but not in surrounding liver tissue^[35]. In healthy subjects, NY-ESO-1 is only expressed in testes, but it is expressed in several tumors, including HCC. NY-ESO-1-specific CD8⁺ T cells have also been shown to be present in HCC^[36,37].

Flecken *et al.*^[25] studied the frequency and tumor-infiltration capacity of naturally occurring CD8⁺ T-cell responses targeting AFP, GPC3, MAGE-A and NY-ESO-1. They found antigen-specific CD8⁺ T-cell responses directed against all four TAAs in over 50% of the patients. Moreover, survival was significantly increased in patients with TAA-specific CD8⁺ T-cell response, which suggests that immunotherapy may be beneficial for patients with HCC. Unfortunately, they were unable to expand functional TAA-specific CD8⁺ T cells from HCC patients *in vitro*.

ENHANCING SPONTANEOUS IMMUNE RESPONSE

Controlling HCC by harnessing naturally occurring, specific immune responses typically fails because the immune responses are not strong enough to overcome the disease. Tumor-specific CD8⁺ T cells

are dysfunctional, regulatory T cells reduce immune response, and tumor cells have acquired mutations, allowing them to escape the immune response. This phenomenon is called cancer immunoediting^[38]. Because the immune response is inadequate, it is important to stimulate the immune system and avoid immune escape.

Ablative therapies such as TACE, cryoablation and RFA result in immunogenic tumor cell death by inducing the release of massive amounts of antigens together with "danger signals" from tumor cells, such as damage-associated molecular pattern molecules (DAMPs). This release leads to the activation of DCs and *in vivo* auto-immunization or *in situ* vaccination^[39-41]. Increased frequencies of GPC3-specific cytotoxic T cells were observed in patients after RFA and TACE treatment^[33]. Hiroishi *et al.*^[42] studied GPC3, NY-ESO-1- and MAGE-1-specific CD8⁺ T-cell responses before and after ablative (RFA or TACE) treatment for HCC. They observed that the presence of strong TAA-specific CD8⁺ T-cell responses suppressed the recurrence of HCC and that the magnitude of a TAA-specific CD8⁺ T-cell response was a prognostic factor for a prolonged tumor-free interval. Nobuoka *et al.*^[33] reported that RFA induced a GPC3-specific T-cell response. For the first time, they showed that RFA induced a stronger GPC3-specific immune response than surgical resection because RFA destroys tumor tissue and causes local necrosis followed by the release of tumor-associated antigens, whereas surgery removes almost all of tumor-associated antigens.

From these results, it appears that immunotherapy to induce TAA-specific cytotoxic T lymphocytes after local therapy should be considered for clinical application in patients with HCC.

THERAPEUTIC VACCINATION FOR HCC: DENDRITIC CELL VACCINATION

The aim of cancer vaccination is the induction and perpetuation of a tumor-specific immune response by eliciting effector T cells that can specifically decrease tumor load and induce immunological memory to control tumor relapse. Thus, dendritic cell-based therapies aim to either induce new or enhance pre-existing antigen-specific T cells, but they have no direct effect on tumor cells. Instead, these vaccines affect on different cell types of the immune system, which can induce tumor cell death. Once targetable tumor antigens have been identified, they can be used to load professional antigen presenting cells, *i.e.*, DCs. DCs play key roles in both innate and adaptive immunity^[43]. DCs can either be loaded *in vivo* or *ex vivo*. DCs capture antigens and convert them to peptides that are presented on major histocompatibility complex (MHC) molecules, which are recognized by T cells in lymphoid organs. In addition, RFA treatment has been shown to up-regulate tumor antigen expression and

Table 1 Clinical trials of immune therapy in hepatocellular carcinoma

Regimen	Patients, <i>n</i>	Clinical response	Reference
HCC vaccines			
DC's + auto-tumor lysate	31	PR: 12.9%, SD: 54.8%	Lee <i>et al</i> ^[53] , 2005
DC's + 4 AFP peptides	16	No clinical response	Butterfield <i>et al</i> ^[30] , 2007
DC's + HepG2 lysate	25	PR + SD: 28%	Palmer <i>et al</i> ^[52] , 2009
GV 1001 + GM-CSF	40	SD: 45.9%	Greten <i>et al</i> ^[71] , 2010
GPC3 peptides	33	PR: 3%, SD: 57.6%	Sawada <i>et al</i> ^[72] , 2012
DC's	30	PR: 17%, SD: 60%	El Ansary <i>et al</i> ^[49] , 2013
Immune checkpoint inhibitors			
Tremelimumab	21	PR: 17.6%, SD: 58.8%	Sangro <i>et al</i> ^[62] , 2013

PR: Partial response; SD: Stable disease.

MHC presentation in tumor cells in an *in vivo* mouse model^[44]. These results suggest the effectiveness of combining active immunotherapy and conventional therapies, such as RFA, to augment tumor cell recognition by T cells and eventually improve clinical outcome.

However, both infections and tumors can suppress immunity through the release of cytokines, such as interleukin (IL)-6, vascular endothelial growth factor (VEGF) and IL-10, which suppress DC activity. Moreover, tumors may condition local DCs to form suppressive T cells, diminishing immune responses against cancer cells. In humans, immature DCs are capable of inducing antigen-specific regulatory CD8⁺ T cells^[45]. It is therefore imperative to use mature DCs to avoid the immunosuppressive effects of tumor cells^[46] or to counteract the inhibitory mechanisms of tumor cells when using anti-PD1/PD-L1, or anti-CTLA4, treatments (discussed in more detail below). Several strategies have been developed to accomplish this, including *ex vivo* DC activation; the addition of strong activation stimuli; the optimization of administered tumor-associated antigens; and the optimization of the dose, frequency and route of administration of a vaccine. Using these strategies, DC vaccines are capable of initiating strong cytotoxic T-lymphocyte responses against TAAs, and DC vaccination remains a good approach for immunotherapy of HCC^[47,48].

Ex vivo-matured DCs pulsed with tumor lysate were injected intra-dermally into patients with advanced HCC. This therapy was shown to be safe, and there were significantly more AFP-specific CD8⁺ T cells 1 mo after DC injection. There were fewer patients with progressive disease in the vaccinated vs in the non-vaccinated group, and the mean patient survival was prolonged, although not significantly^[49]. In a phase 1 study, 10 patients were treated with radical microwave ablation of HCC followed by 3 courses of mature DC

injection into the inguinal lymph nodes and the infusion of immature DCs into microwave-treated HCC lesions. No grade 3/4 toxicity was observed. The percentage of CD4⁺CD25⁺ regulatory T lymphocytes decreased significantly and the percentage of CD8⁺CD28⁻ effector cells increased significantly by 1 mo after therapy, but this encouraging result disappeared by 6 mo after therapy^[50].

In another phase 1 study, 5 patients were treated with TACE, followed by repeated DC vaccination. The vaccine was prepared by pulsing DCs with cytoplasmic transduction peptide-attached AFP, GPC3 and MAGE-1 recombinant fusion proteins. Mature DCs were injected subcutaneously near the inguinal lymph nodes in combination with the application of Toll-like receptor 7 agonist at the site of injection. In all patients, the vaccine was safe and elicited TAA-specific T cell responses. In one patient, this resulted in stable disease^[51]. Thirty-five patients were included in a phase II clinical trial investigating the safety and efficacy of intravenous vaccination with mature autologous DCs pulsed *ex vivo* with tumor cell lysate. Patients received up to 6 vaccines at 3-wk intervals. The treatment was safe and well tolerated, generating antigen-specific immune responses in some cases; unfortunately, there were very low clinical responses^[52]. In another phase II clinical trial that assessed 31 patients with advanced HCC, DCs were pulsed with autologous tumor lysates. The patients were treated with five courses of DC vaccination intravenously at weekly intervals and in 17 patients this was followed by monthly boost vaccinations. The treatment was safe. Among these 31 patients, 4 had a partial response and 17 had stable disease. Moreover, the patients treated with the boosted therapy had a 1-year survival rate of 63.3% vs 10.7% in patients treated with the initial pulsed therapy alone^[53]. These results are promising, but the overall results of DC vaccination are unsatisfactory and should be improved (Table 1).

One possibility for improvement could be combining vaccination with anti-angiogenic tyrosine kinase inhibitors (TKIs), such as sorafenib. This strategy targets multiple components of the tumor microenvironment and could mediate an anti-tumor response by immunogenic modulation and immune subset conditioning^[54]. Additionally, the vascular changes caused by TKIs affect tumor-infiltrating immune cells, so the combining TKIs with immune therapy could enhance the clinical benefit^[55].

Another area of concern is the recurrence of HCC after liver transplantation^[56]. Indeed, it has been shown that the recurrence rates of HCC after liver transplantation for HCC are high and have a dismal prognosis^[57]. Disease progression was significantly faster in transplanted patients than in patients who underwent surgical resection of HCC, probably due to immunosuppression and reduced host immunity^[58]. Immunosuppression *via* mammalian targets of rapamycin seems to decrease recurrence rates and

to slow progression in case of recurrence^[59]. DC vaccination has not been tested in liver transplant patients, but it has been shown to be safe and promising with regards to immunological responses after allogeneic-hematopoietic cell transplantation^[60].

IMMUNE CHECKPOINT BLOCKADE

The intensity of an immune response results from the balance between stimulatory and inhibitory signals, known as immune checkpoints. These checkpoints are often activated by tumor signals and promote tumor evasion from immunity. Cytotoxic T lymphocyte-associated antigen (CTLA-4) and programmed death 1 (PD-1) are the two most studied immune checkpoints, and inhibitory antibodies against these are already being used in clinical trials. The mechanisms of action of CTLA-4 and PD-1 and their possible roles in treating HCC were recently reviewed^[61]. The CTLA-4 inhibitor, tremelimumab was studied in a phase 1 clinical trial. It was well tolerated, and 76% of the enrolled patients had either a partial response or stable disease, of which 45% were stable for more than 6 mo^[62]. PD-1 was found in liver-infiltrating lymphocytes and its ligands PD-L1 and PD-L2 were shown to be up-regulated in HCC tissue^[63]. Currently, several anti-PD-1 and anti-PD-L1 antibodies are being developed, and their use in clinical studies of HCC is planned.

PERSPECTIVES

Antigen-encoding mRNA is emerging as a particularly promising vaccination tool as it has many advantages to offer. Its advantage over classical vaccination with peptides is that RNA encodes genetic information corresponding to whole antigens. RNA processing by endogenous cell machinery and presentation on MHC complexes are independent of the HLA-subtype of a patient. In addition, RNA does not pose a risk of genomic integration, giving it a favorable safety profile compared to DNA. Due to its transient nature, RNA is only expressed during a controlled period of time and is eventually degraded into natural products. Furthermore, RNA acts as its own adjuvant, prompting co-stimulatory signals, which is advantageous in the context of RNA-based immunotherapy. Even hard-to-modify cells, such as DCs can be modified with mRNA. Two routes for exogenous mRNA delivery into DCs have been applied: either *ex vivo* delivery with subsequent adoptive transfer of transfected DCs or the direct administration of mRNA with subsequent uptake *in vivo*. For the former, DCs derived from patients are cultivated and electroporated with mRNA followed by their restitution into the patient.

In situ modification of DCs by immunization via the direct application of naked mRNA was first described 25 years ago^[64]. Since then, several studies have shown anti-tumor immune responses following the injection of naked mRNA in a variety of mouse

models^[65]. It is assumed that intradermal delivery of mRNA results in its uptake by Langerhans' cells and dermal DCs at the injection site, which are transported to draining lymph nodes. It is moreover assumed that these DCs transfer their antigenic cargo to lymph node-resident CD8⁺ DCs when they arrive in the draining lymph nodes. Direct injection of mRNA into lymph nodes results in the uptake of mRNA and has been shown to be superior to intradermal injection of mRNA with respect to the induction of antigen-specific T cell responses^[65]. Currently, intranodal administration of mRNA is proposed as the optimal route for delivery because lymph nodes harbor a high number of DCs. Intranodal administration creates a microenvironment that favors the induction of potent and sustained immune responses. Lymph node resident dendritic cells up-regulate CD86, and this is required for the efficient activation of naïve T cells and for immunologic memory^[66,67].

Currently, we are performing a phase 1 study investigating the feasibility and safety of the intranodal injection of a TriMix-based mRNA vaccine. The concept was studied in melanoma animal models^[67]. The idea is to use mRNA for the *in vivo* modification of DCs by direct administration into lymph nodes, which harbor a high number of DCs in close contact with T cells. For a detailed description, we refer to our recent review^[68]. In melanoma patients, we previously showed that TriMix mRNA (mRNA encoding CD40 ligand, CD70 and a constitutively active form of TLR4), induced the activation of DCs, resulting in the induction of a T cell attracting and stimulatory environment^[69]. Moreover, the co-administration of tumor antigen mRNA and TriMix resulted in the recruitment of antigen-specific CD4⁺ and CD8⁺ T cells. Simultaneous delivery of TriMix and antigen mRNA significantly enhanced the induction of antigen-specific T cells compared to intranodal delivery of antigen mRNA alone^[67,68]. In the current study, TriMix mRNA and mRNA encoding the target antigens GPC3 and MAGE-C2 mRNA were injected intranodally the same day as RFA treatment of HCC thereby increasing tumor antigen load. The mRNA injection was repeated 3 times at 2-wk intervals. If this intervention appears to be safe, we plan to add immune checkpoint blockers, which could increase the efficacy of the vaccination as we showed in previous melanoma studies^[68]. To avoid systemic autoimmune toxicity caused by anti-CTLA-4 or anti-PD-1 treatment, mRNA encoding factors that have the ability to block CTLA-4 or PD-1 can be used^[70].

CONCLUSION

Hepatocellular carcinoma is a very common cancer with a high mortality, and the current standard treatment for it is unsatisfactory. Tumor recurrence and the development of new cancer after treatment is frequent and remains a major problem. Because immunotherapy not only treats an existing tumor, but

also has the potential to prevent the development of new cancers in the cirrhotic liver, vaccination for HCC remains an attractive treatment option. In this context, the combination of active specific immunotherapy with ablative therapy might be an appealing and feasible approach and may provide better results than individual treatments. Immunotherapy will be most effective during or shortly after ablative therapy, when tumor cells are dying and an active immune response has commenced. This first "priming" by the ablative therapy should be sustained by "booster" immunizations to maintain immune control over the tumor. One difficulty is the immune tolerance of the liver, and the immunosuppressive environment of cancer, which makes *in situ* activation of immune cells problematic. This problem can be overcome by *ex vivo* activation of DCs or by *in vivo* activation of DCs in skin or lymph nodes. The addition of immune checkpoint inhibitors will undoubtedly add significantly to efficacy, but this will probably increase side effects.

REFERENCES

- 1 **Theise ND.** Liver cancer. In: WILD BWSCP, editor. World Cancer Report 2014. World Health Organization, 2014; 576-592
- 2 **European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer.** EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]
- 3 **Sieghart W, Hucce F, Peck-Radosavljevic M.** Transarterial chemoembolization: modalities, indication, and patient selection. *J Hepatol* 2015; **62**: 1187-1195 [PMID: 25681552 DOI: 10.1016/j.jhep.2015.02.010]
- 4 **Burrell M, Reig M, Forner A, Barrufet M, de Lope CR, Tremosini S, Ayuso C, Llovet JM, Real MI, Bruix J.** Survival of patients with hepatocellular carcinoma treated by transarterial chemoembolisation (TACE) using Drug Eluting Beads. Implications for clinical practice and trial design. *J Hepatol* 2012; **56**: 1330-1335 [PMID: 22314428 DOI: 10.1016/j.jhep.2012.01.008]
- 5 **Takayasu K, Arii S, Kudo M, Ichida T, Matsui O, Izumi N, Matsuyama Y, Sakamoto M, Nakashima O, Ku Y, Kokudo N, Makuuchi M.** Superselective transarterial chemoembolization for hepatocellular carcinoma. Validation of treatment algorithm proposed by Japanese guidelines. *J Hepatol* 2012; **56**: 886-892 [PMID: 22173160 DOI: 10.1016/j.jhep.2011.10.021]
- 6 **Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Gretten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J.** Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
- 7 **Cainap C, Qin S, Huang WT, Chung JJ, Pan H, Cheng Y, Kudo M, Kang YK, Chen PJ, Toh HC, Gorbunova V, Eskens FA, Qian J, McKee MD, Ricker JL, Carlson DM, El-Nowiem S.** Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2015; **33**: 172-179 [PMID: 25488963 DOI: 10.1200/jco.2013.54.3298]
- 8 **Cheng AL, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E.** Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J Clin Oncol* 2013; **31**: 4067-4075 [PMID: 24081937 DOI: 10.1200/jco.2012.45.8372]
- 9 **Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, Van Vlierberghe H, Trojan J, Kolligs FT, Weiss A, Miles S, Gasbarrini A, Lencioni M, Cicalese L, Sherman M, Gridelli C, Buggisch P, Gerken G, Schmid RM, Boni C, Personeni N, Hassoun Z, Abbadessa G, Schwartz B, Von Roemeling R, Lamar ME, Chen Y, Porta C.** Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *Lancet Oncol* 2013; **14**: 55-63 [PMID: 23182627 DOI: 10.1016/s1470-2045(12)70490-4]
- 10 **Lencioni R, Llovet JM, Han G, Tak WY, Yang JY, Leberre MA, Niu W, Nicholson K, Meinhardt G, Bruix J.** Sorafenib or placebo in combination with transarterial (TACE) with doxorubicin-eluting beads (DEBDOX) for intermediate-stage hepatocellular carcinoma (HCC): Phase II, randomized, double-blind SPACE trial. *J Clin Oncol* 2012; **30**: LBA154
- 11 **Bruix J, Takayama T, Mazzaferro V.** STORM: a phase III randomized, double-blind, placebo-controlled trial of adjuvant sorafenib after resection or ablation to prevent recurrence of hepatocellular carcinoma (HCC). *J Clin Oncol* 2014; **32** (Suppl): 5s
- 12 **Reynaert H, Urbain D, Geerts A.** Regulation of sinusoidal perfusion in portal hypertension. *Anat Rec* (Hoboken) 2008; **291**: 693-698 [PMID: 18484616 DOI: 10.1002/ar.20669]
- 13 **Racaneli V, Rehmann B.** The liver as an immunological organ. *Hepatology* 2006; **43**: S54-S62 [PMID: 16447271 DOI: 10.1002/hep.21060]
- 14 **Gao B, Jeong WI, Tian Z.** Liver: An organ with predominant innate immunity. *Hepatology* 2008; **47**: 729-736 [PMID: 18167066 DOI: 10.1002/hep.22034]
- 15 **Tiegs G, Lohse AW.** Immune tolerance: what is unique about the liver. *J Autoimmun* 2010; **34**: 1-6 [PMID: 19717280 DOI: 10.1016/j.jaut.2009.08.008]
- 16 **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]
- 17 **Huz JI, Melis M, Sarpel U.** Spontaneous regression of hepatocellular carcinoma is most often associated with tumour hypoxia or a systemic inflammatory response. *HPB* (Oxford) 2012; **14**: 500-505 [PMID: 22762397 DOI: 10.1111/j.1477-2574.2012.00478.x]
- 18 **Kumar A, Le DT.** Hepatocellular Carcinoma Regression After Cessation of Immunosuppressive Therapy. *J Clin Oncol* 2014; Epub ahead of print [PMID: 25245441 DOI: 10.1200/jco.2013.51.4067]
- 19 **Li F, Guo Z, Lizée G, Yu H, Wang H, Si T.** Clinical prognostic value of CD4+CD25+FOXP3+regulatory T cells in peripheral blood of Barcelona Clinic Liver Cancer (BCLC) stage B hepatocellular carcinoma patients. *Clin Chem Lab Med* 2014; **52**: 1357-1365 [PMID: 24646790 DOI: 10.1515/cclm-2013-0878]
- 20 **Yang ZQ, Yang ZY, Zhang LD, Ping-Bie SG, Ma KS, Li XW, Dong JH.** Increased liver-infiltrating CD8+FoxP3+ regulatory T cells are associated with tumor stage in hepatocellular carcinoma patients. *Hum Immunol* 2010; **71**: 1180-1186 [PMID: 20870003 DOI: 10.1016/j.humimm.2010.09.011]
- 21 **Shen P, Wang A, He M, Wang Q, Zheng S.** Increased circulating Lin(-/low) CD33(+) HLA-DR(-) myeloid-derived suppressor cells in hepatocellular carcinoma patients. *Hepatology Res* 2014; **44**: 639-650 [PMID: 23701406 DOI: 10.1111/hepr.12167]
- 22 **Bei R, Budillon A, Reale MG, Capuano G, Pomponi D, Budillon G, Frati L, Muraro R.** Cryptic epitopes on alpha-fetoprotein induce spontaneous immune responses in hepatocellular carcinoma, liver cirrhosis, and chronic hepatitis patients. *Cancer Res* 1999; **59**: 5471-5474 [PMID: 10554020]
- 23 **Thimme R, Neagu M, Boettler T, Neumann-Haefelin C, Kersting N, Geissler M, Makowiec F, Obermaier R, Hopt UT, Blum HE, Spangenberg HC.** Comprehensive analysis of the alpha-fetoprotein-specific CD8+ T cell responses in patients with hepatocellular carcinoma. *Hepatology* 2008; **48**: 1821-1833 [PMID: 19003875 DOI: 10.1002/hep.22535]
- 24 **Mizukoshi E, Nakamoto Y, Arai K, Yamashita T, Sakai A, Sakai Y, Kagaya T, Yamashita T, Honda M, Kaneko S.** Comparative

- analysis of various tumor-associated antigen-specific t-cell responses in patients with hepatocellular carcinoma. *Hepatology* 2011; **53**: 1206-1216 [PMID: 21480325 DOI: 10.1002/hep.24149]
- 25 **Flecken T**, Schmidt N, Hild S, Gostick E, Drognitz O, Zeiser R, Schemmer P, Bruns H, Eiermann T, Price DA, Blum HE, Neumann-Haefelin C, Thimme R. Immunodominance and functional alterations of tumor-associated antigen-specific CD8+ T-cell responses in hepatocellular carcinoma. *Hepatology* 2014; **59**: 1415-1426 [PMID: 24002931 DOI: 10.1002/hep.26731]
- 26 **Wada Y**, Nakashima O, Kutami R, Yamamoto O, Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. *Hepatology* 1998; **27**: 407-414 [PMID: 9462638 DOI: 10.1002/hep.510270214]
- 27 **Liang J**, Ding T, Guo ZW, Yu XJ, Hu YZ, Zheng L, Xu J. Expression pattern of tumour-associated antigens in hepatocellular carcinoma: association with immune infiltration and disease progression. *Br J Cancer* 2013; **109**: 1031-1039 [PMID: 23868000 DOI: 10.1038/bjc.2013.390]
- 28 **Breous E**, Thimme R. Potential of immunotherapy for hepatocellular carcinoma. *J Hepatol* 2011; **54**: 830-834 [PMID: 21145836 DOI: 10.1016/j.jhep.2010.10.013]
- 29 **Hu B**, Tian X, Sun J, Meng X. Evaluation of individual and combined applications of serum biomarkers for diagnosis of hepatocellular carcinoma: a meta-analysis. *Int J Mol Sci* 2013; **14**: 23559-23580 [PMID: 24317431 DOI: 10.3390/ijms141223559]
- 30 **Butterfield LH**, Ribas A, Potter DM, Economou JS. Spontaneous and vaccine induced AFP-specific T cell phenotypes in subjects with AFP-positive hepatocellular cancer. *Cancer Immunol Immunother* 2007; **56**: 1931-1943 [PMID: 17522860 DOI: 10.1007/s00262-007-0337-9]
- 31 **Yamauchi N**, Watanabe A, Hishinuma M, Ohashi K, Midorikawa Y, Morishita Y, Niki T, Shibahara J, Mori M, Makuuchi M, Hippo Y, Kodama T, Iwanari H, Aburatani H, Fukayama M. The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Mod Pathol* 2005; **18**: 1591-1598 [PMID: 15920546 DOI: 10.1038/modpathol.3800436]
- 32 **Shirakawa H**, Suzuki H, Shimomura M, Kojima M, Gotohda N, Takahashi S, Nakagohri T, Konishi M, Kobayashi N, Kinoshita T, Nakatsura T. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. *Cancer Sci* 2009; **100**: 1403-1407 [PMID: 19496787 DOI: 10.1111/j.1349-7006.2009.01206.x]
- 33 **Nobuoka D**, Motomura Y, Shirakawa H, Yoshikawa T, Kuronuma T, Takahashi M, Nakachi K, Ishii H, Furuse J, Gotohda N, Takahashi S, Nakagohri T, Konishi M, Kinoshita T, Komori H, Baba H, Fujiwara T, Nakatsura T. Radiofrequency ablation for hepatocellular carcinoma induces glypican-3 peptide-specific cytotoxic T lymphocytes. *Int J Oncol* 2012; **40**: 63-70 [PMID: 21922136 DOI: 10.3892/ijo.2011.1202]
- 34 **Zerbini A**, Pilli M, Soliani P, Ziegler S, Pelosi G, Orlandini A, Cavallo C, Uggeri J, Scandroglio R, Crafa P, Spagnoli GC, Ferrari C, Missale G. Ex vivo characterization of tumor-derived melanoma antigen encoding gene-specific CD8+ cells in patients with hepatocellular carcinoma. *J Hepatol* 2004; **40**: 102-109 [PMID: 14672620]
- 35 **Bricard G**, Bouzourene H, Martinet O, Rimoldi D, Halkic N, Gillet M, Chaubert P, Macdonald HR, Romero P, Cerottini JC, Speiser DE. Naturally acquired MAGE-A10- and SSX-2-specific CD8+ T cell responses in patients with hepatocellular carcinoma. *J Immunol* 2005; **174**: 1709-1716 [PMID: 15661935]
- 36 **Xu H**, Gu N, Liu ZB, Zheng M, Xiong F, Wang SY, Li N, Lu J. NY-ESO-1 expression in hepatocellular carcinoma: A potential new marker for early recurrence after surgery. *Oncol Lett* 2012; **3**: 39-44 [PMID: 22740853 DOI: 10.3892/ol.2011.441]
- 37 **Korangy F**, Ormandy LA, Bleck JS, Klempnauer J, Wilkens L, Manns MP, Greten TF. Spontaneous tumor-specific humoral and cellular immune responses to NY-ESO-1 in hepatocellular carcinoma. *Clin Cancer Res* 2004; **10**: 4332-4341 [PMID: 15240519 DOI: 10.1158/1078-0432.ccr-04-0181]
- 38 **Dunn GP**, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoeediting. *Immunity* 2004; **21**: 137-148 [PMID: 15308095 DOI: 10.1016/j.immuni.2004.07.017]
- 39 **Ali MY**, Grimm CF, Ritter M, Mohr L, Allgaier HP, Weth R, Bocher WO, Endrulat K, Blum HE, Geissler M. Activation of dendritic cells by local ablation of hepatocellular carcinoma. *J Hepatol* 2005; **43**: 817-822 [PMID: 16087270]
- 40 **Dromi SA**, Walsh MP, Herby S, Traugber B, Xie J, Sharma KV, Sekhar KP, Luk A, Liewehr DJ, Dreher MR, Fry TJ, Wood BJ. Radiofrequency ablation induces antigen-presenting cell infiltration and amplification of weak tumor-induced immunity. *Radiology* 2009; **251**: 58-66 [PMID: 19251937 DOI: 10.1148/radiol.2511072175]
- 41 **Zerbini A**, Pilli M, Penna A, Pelosi G, Schianchi C, Molinari A, Schivazappa S, Zibera C, Fagnoni FF, Ferrari C, Missale G. Radiofrequency thermal ablation of hepatocellular carcinoma liver nodules can activate and enhance tumor-specific T-cell responses. *Cancer Res* 2006; **66**: 1139-1146 [PMID: 16424051 DOI: 10.1158/0008-5472.can-05-2244]
- 42 **Hiroishi K**, Eguchi J, Baba T, Shimazaki T, Ishii S, Hiraide A, Sakaki M, Doi H, Uozumi S, Omori R, Matsumura T, Yanagawa T, Ito T, Imawari M. Strong CD8(+) T-cell responses against tumor-associated antigens prolong the recurrence-free interval after tumor treatment in patients with hepatocellular carcinoma. *J Gastroenterol* 2010; **45**: 451-458 [PMID: 19936602 DOI: 10.1007/s00535-009-0155-2]
- 43 **Steinman RM**, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007; **449**: 419-426 [PMID: 17898760 DOI: 10.1038/nature06175]
- 44 **Gameiro SR**, Higgins JP, Dreher MR, Woods DL, Reddy G, Wood BJ, Guha C, Hodge JW. Combination therapy with local radiofrequency ablation and systemic vaccine enhances antitumor immunity and mediates local and distal tumor regression. *PLoS One* 2013; **8**: e70417 [PMID: 23894654 DOI: 10.1371/journal.pone.0070417]
- 45 **Dhodapkar MV**, Steinman RM. Antigen-bearing immature dendritic cells induce peptide-specific CD8(+) regulatory T cells in vivo in humans. *Blood* 2002; **100**: 174-177 [PMID: 12070024]
- 46 **Schuler G**, Schuler-Thurner B, Steinman RM. The use of dendritic cells in cancer immunotherapy. *Curr Opin Immunol* 2003; **15**: 138-147 [PMID: 12633662]
- 47 **Morris LF**, Ribas A. Therapeutic cancer vaccines. *Surg Oncol Clin N Am* 2007; **16**: 819-31, ix [PMID: 18022546 DOI: 10.1016/j.soc.2007.07.007]
- 48 **Pizzurro GA**, Barrio MM. Dendritic cell-based vaccine efficacy: aiming for hot spots. *Front Immunol* 2015; **6**: 91 [PMID: 25784913 DOI: 10.3389/fimmu.2015.00091]
- 49 **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]
- 50 **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]
- 51 **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]
- 52 **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]
- 53 **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]
- 54 **Kwilas AR**, Donahue RN, Tsang KY, Hodge JW. Immune consequences of tyrosine kinase inhibitors that synergize with cancer immunotherapy. *Cancer Cell Microenviron* 2015; **2**: pii: e677 [PMID:

- 26005708 DOI: 10.14800/ccm.677]
- 55 **Farsaci B**, Donahue RN, Coplin MA, Grenga I, Lepone LM, Molinolo AA, Hodge JW. Immune consequences of decreasing tumor vasculature with antiangiogenic tyrosine kinase inhibitors in combination with therapeutic vaccines. *Cancer Immunol Res* 2014; **2**: 1090-1102 [PMID: 25092771 DOI: 10.1158/2326-6066.cir-14-0076]
- 56 **Rahimi RS**, Trotter JF. Liver transplantation for hepatocellular carcinoma: outcomes and treatment options for recurrence. *Ann Gastroenterol* 2015; **28**: 323-330 [PMID: 26130250]
- 57 **Yokoyama I**, Carr B, Saitsu H, Iwatsuki S, Starzl TE. Accelerated growth rates of recurrent hepatocellular carcinoma after liver transplantation. *Cancer* 1991; **68**: 2095-2100 [PMID: 1655200]
- 58 **Cheng JW**, Shi YH, Fan J, Huang XW, Qiu SJ, Xiao YS, Wang Z, Dai Z, Tang ZY, Zhou J. An immune function assay predicts post-transplant recurrence in patients with hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2011; **137**: 1445-1453 [PMID: 21809031 DOI: 10.1007/s00432-011-1014-0]
- 59 **Duvoux C**, Toso C. mTOR inhibitor therapy: Does it prevent HCC recurrence after liver transplantation? *Transplant Rev (Orlando)* 2015; **29**: 168-174 [PMID: 26071984 DOI: 10.1016/j.tre.2015.02.003]
- 60 **Plantinga M**, de Haar C, Nierkens S, Boelens JJ. Dendritic Cell Therapy in an Allogeneic-Hematopoietic Cell Transplantation Setting: An Effective Strategy toward Better Disease Control? *Front Immunol* 2014; **5**: 218 [PMID: 24904573 DOI: 10.3389/fimmu.2014.00218]
- 61 **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]
- 62 **Sangro B**, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garraza 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]
- 63 **Wang BJ**, Bao JJ, Wang JZ, Wang Y, Jiang M, Xing MY, Zhang WG, Qi JY, Roggendorf M, Lu MJ, Yang DL. Immunostaining of PD-1/PD-Ls in liver tissues of patients with hepatitis and hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 3322-3329 [PMID: 21876620 DOI: 10.3748/wjg.v17.i28.3322]
- 64 **Wolff JA**, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felgner PL. Direct gene transfer into mouse muscle in vivo. *Science* 1990; **247**: 1465-1468 [PMID: 1690918]
- 65 **Van Lint S**, Heirman C, Thielemans K, Breckpot K. mRNA: From a chemical blueprint for protein production to an off-the-shelf therapeutic. *Hum Vaccin Immunother* 2013; **9**: 265-274 [PMID: 23291946]
- 66 **Kreiter S**, Selmi A, Diken M, Koslowski M, Britten CM, Huber C, Türeci O, Sahin U. Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoral immunity. *Cancer Res* 2010; **70**: 9031-9040 [PMID: 21045153 DOI: 10.1158/0008-5472.can-10-0699]
- 67 **Van Lint S**, Goyvaerts C, Maenhout S, Goethals L, Disy A, Benteyn D, Pen J, Bonehill A, Heirman C, Breckpot K, Thielemans K. Preclinical evaluation of TriMix and antigen mRNA-based antitumor therapy. *Cancer Res* 2012; **72**: 1661-1671 [PMID: 22337996 DOI: 10.1158/0008-5472.can-11-2957]
- 68 **Van Lint S**, Wilgenhof S, Heirman C, Corthals J, Breckpot K, Bonehill A, Neyns B, Thielemans K. Optimized dendritic cell-based immunotherapy for melanoma: the TriMix-formula. *Cancer Immunol Immunother* 2014; **63**: 959-967 [PMID: 24878889 DOI: 10.1007/s00262-014-1558-3]
- 69 **Bonehill A**, Tuyaerts S, Van Nuffel AM, Heirman C, Bos TJ, Fostier K, Neyns B, Thielemans K. Enhancing the T-cell stimulatory capacity of human dendritic cells by co-electroporation with CD40L, CD70 and constitutively active TLR4 encoding mRNA. *Mol Ther* 2008; **16**: 1170-1180 [PMID: 18431362 DOI: 10.1038/mt.2008.77]
- 70 **Pruitt SK**, Boczkowski D, de Rosa N, Haley NR, Morse MA, Tyler DS, Dannull J, Nair S. Enhancement of anti-tumor immunity through local modulation of CTLA-4 and GITR by dendritic cells. *Eur J Immunol* 2011; **41**: 3553-3563 [PMID: 22028176 DOI: 10.1002/eji.201141383]
- 71 **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]
- 72 **Sawada Y**, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, Motomura Y, Mizuno S, Ishii H, Nakachi K, Konishi M, Nakagohri T, Takahashi S, Gotohda N, Takayama T, Yamao K, Uesaka K, Furuse J, Kinoshita T, Nakatsura T. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. *Clin Cancer Res* 2012; **18**: 3686-3696 [PMID: 22577059]

P- Reviewer: Lukacs-Kornek V, Nagai H, Rodriguez-Peralvarez M
S- Editor: Yu J **L- Editor:** A **E- Editor:** Wang CH



