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
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%[3]. 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 linifanib 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.

Reurrence 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].
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 peripheral blood is an independent predictive factor of poor survival after TACE treatment in patients with HCC. 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. Additionally, there is an increased presence of myeloid-derived suppressor cells in patients with HCC compared to healthy donors.

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. 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. Additionally, there is an increased presence of myeloid-derived suppressor cells is increased in patients with HCC compared to healthy donors.

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. Moreover, naturally occurring tumor-associated antigen-specific T-cell responses exist in patients with HCC and correlate with patient survival. 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)[25,26].

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[22,30].

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[29]. 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 immunoeediting[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
Patients, No clinical PR: 17%, SD: Palmer 33 PR: 17.6%, SD: 30 PR: 12.9%, SD: 57x75
study, 10 patients were treated with radical microwave prolonged, although not significantly vaccinated group, and the mean patient survival was progressive disease in the vaccinated including PR: Partial response; SD: Stable disease.

...capable of initiating strong cytotoxic T-lymphocyte a vaccine. Using these strategies, DC vaccines are the dose, frequency and route of administration of tumor-associated antigens; and the optimization of activation stimuli; the optimization of administered including strategies have been developed to accomplish this, or to counteract the inhibitory mechanisms of tumor GPC3 peptides DC's + HepG2 lysate 25 PR + SD: 28% Palmer et al [39], 2009 GV 1001 + GM-CSF 40 SD: 45.9% Greten et al [38], 2010 GPC3 peptides 33 PR: 3%, SD: 57.6% Sawada et al [37], 2012 DC's 30 PR: 17%, SD: 60% El Ansary et al [36], 2013 Immune checkpoint inhibitors Tremelimumab 21 PR: 17.6%, SD: 58.8% Sangro et al [35], 2013

Table 1 Clinical trials of immune therapy in hepatocellular carcinoma

| Regimen                        | Patients, n | Clinical response | Reference          |
|--------------------------------|-------------|-------------------|--------------------|
| HCC vaccines                   |             |                   |                    |
| DC's + auto-tumor lysate       | 31          | PR: 12.9% SD: 54.8%| Lee et al [34], 2005|
| DC's + 4 AFP peptides          | 16          | No clinical response | Butterfield et al [33], 2007 |
| DC's + HepG2 lysate            | 25          | PR + SD: 28%      | Palmer et al [39], 2009 |
| GV 1001 + GM-CSF               | 40          | SD: 45.9%         | Greten et al [38], 2010 |
| GPC3 peptides                  | 33          | PR: 3%, SD: 57.6% | Sawada et al [37], 2012 |
| DC's                           | 30          | PR: 17%, SD: 60%  | El Ansary et al [36], 2013 |
| Immune checkpoint inhibitors   |             |                   |                    |
| Tremelimumab                   | 21          | PR: 17.6% SD: 58.8%| Sangro et al [35], 2013 |

PR: Partial response; SD: Stable disease.

...capable of inducing antigen-specific regulatory CD8\(^+\) T cells. It is therefore imperative to use mature DCs to avoid the immunosuppressive effects of tumor cells 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 TAA, 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 [39]. 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\textsuperscript{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\textsuperscript{60}.  

\section*{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\textsuperscript{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\textsuperscript{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\textsuperscript{63}. Currently, several anti-PD-1 and anti-PD-L1 antibodies are being developed, and their use in clinical studies of HCC is planned.

\section*{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 \textit{ex vivo} delivery with subsequent adoptive transfer of transfected DCs or the direct administration of mRNA with subsequent uptake \textit{in vivo}. For the former, DCs derived from patients are cultivated and electroporated with mRNA followed by their restitution into the patient.

\textit{In situ} modification of DCs by immunization via the direct application of naked mRNA was first described 25 years ago\textsuperscript{64}. Since then, several studies have shown anti-tumor immune responses following the injection of naked mRNA in a variety of mouse models\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{67}. The idea is to use mRNA for the \textit{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{70}.

\section*{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, Hucke 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 Burrel M, Reig M, Forner A, Barrufet M, de Lope CR, Trensomi S, Ayuso C, Llovet JM, Reig M, Brúix 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, Arri S, Kudo M, Ichida T, Matsu O, Iizumi N, Matsuyama Y, Sakamoto M, Nakashima O, KU Y, Kokudo N, Makuchii 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, Grendel T, Gale PR, Sievert JB, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Vouiotis D, Cessation of Immunosuppressive Therapy. J Clin Oncol 2014; 32: 378-390 [PMID: 24646790 DOI: 10.1515/cclm-2013-0878]

7 Caini P, Qin S, Huang WT, Chung JJ, Pan H, Cheng Y, Kudo M, Kaig YK, Chen PJ, Toh HC, Gorbonova V, Esken FA, Qian J, McKee MD, Ricker JL, Carlson DM, El-Noowiem S. Linifanib (BIB3220): Phase II, randomized, double-blind SPACE trial. J Clin Oncol 2012; 30: LBA154

8 Cheng AL, Kim CJ, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzzalone S, Yang L, Leuchga MJ, Raymond E. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. J Clin Oncol 2015; 33: 172-179 [PMID: 25488963 DOI: 10.1200/jco.2013.54.3298]

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, Sherma M, Gridelli C, Uuggling P, Gerkens G, Schmid RM, Honi C, Personini N, Hassoun Z, Abbabessa G, Schwartz B, Von Roemeling R, Lamar ME, Chen Y, Porta C. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomized, 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, Leberer MA, Niw W, Nicholson K, Reinhardt G, Brox J. Sorafenib or placebo in combination with transarterial (TACE) with doxorubicin-eluting beads (DEBOB) for intermediate-stage hepatocellular carcinoma (HCC): Phase II, randomized, double-blind SPACE trial. J Clin Oncol 2012; 30: 1187-1195 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

11 Broux 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 Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology 2006; 43: 554-562 [PMID: 16447271 DOI: 10.1002/hep.21060]

14 Gao B, Jiong WJ, Tian Z. Liver: An organ with predominant innate immunity. Hepatology 2008; 47: 729-736 [PMID: 18160766 DOI: 10.1002/hep.22034]

15 Tigs 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 Hu J, Melis M, Sarpei 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; 32: 378-390 [PMID: 22262937 DOI: 10.11151;1477-2574.2012.00478.x]

19 Li F, Guo Z, Liézé G, Yu H, Wang H, ST. Clinical prognostic value of CD4+CD25+FOPX3+ 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 LK, Ping-Bie SG, Ma KS, Li XW, Dong JH. Increased liver-infiltrating CD8+FOPX+ 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. Hepatol Res 2014; 44: 639-650 [PMID: 23701406 DOI: 10.1111/hep.12167]

22 Bei R, Budillon A, Reale MG, Capuano G, Pompolini D, Budillon G, Frati L, Muraro R. Cryptic epitopes on alpha-feto-protein 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, Hopf UT, Blum HE, Spangenberg HC. Comprehensive analysis of the alpha-feto-protein-specific CD8+ T cell responses in patients with hepatocellular carcinoma. Hepatology 2008; 48: 1821-1833 [PMID: 19003875 DOI: 10.1002/hep.22355]

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: 21480235 DOI: 10.1002/hep.24149]

**Fiecken T**, Schmidt N, Hild S, Gostick E, Drooght O, Zeiser R, Schiemann P, Bruns H, Eiermann T, Pree 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]

**Wada Y**, Nakashima O, Kutani R, Yamamoto O, Kojiro M. Clinicopathological study on hepatocellular carcinoma with lymphocytic infiltration. *Hepatology* 1998; 27: 407-414 [PMID: 9462638 DOI: 10.1002/hep.1010270124]

**Li J**, Ding T, Guo ZW, Xu 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]

**Breaux 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]

**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: 24137431 DOI: 10.3390/ijms141223559]

**Butterfield LH**, Ribas A, Potter DM, Economou JS. Spontaneous and vaccine induced AFP-specific T cell phenotypes in subjects with AFP-positive hepatocellular cancer. *Hepatology* 2007; 56: 1931-1943 [PMID: 17522860 DOI: 10.1001/s0026-007-0337-9]

**Yamauchi N**, Watanabe A, Ishimura M, Ohashi K, Miodorikawa 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]

**Shirakawa H**, Suzuki H, Shimomura M, Kojima M, Gotokha N, Takahashi S, Nakagohi T, Komishi 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]

**Nobukusa D**, Motomura Y, Shirakawa H, Yoshikawa T, Kuronuma T, Takahashi M, Nakahi K, Iishi H, Furuse J, Gotokha N, Takakashi S, Nakagohi T, Komishi M, Kanbara H, Aburatani H, Fujishita T, Nakatsura T. Radiofrequency ablation for hepatocellular carcinoma induces glypican-3-specific peptide cytotoxic T lymphocytes. *Int J Oncol* 2012; 40: 63-70 [PMID: 21922136 DOI: 10.3892/ijo.2011.1202]

**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]

**Hirokishi K**, Eguchi J, Baba T, Shimazaki T, Iishi 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]

**Steinman RM**, Blanchereau J. Taking dendritic cells into medicine. *Nature* 2007; 449: 419-426 [PMID: 17898760 DOI: 10.1038/nature06175]

**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: 23846545 DOI: 10.1371/journal.pone.0070417]

**Dhodapkar MV**, Steinman RM. Antigen-bearing immature dendritic cells induce peptide-specific CD8(+) regulatory T cells in vivo. *Blood* 2002; 100: 174-177 [PMID: 12070024]

**Schuler G**, Schultzer-Thurner B, Steinman RM. The use of dendritic cells in cancer immunotherapy. *Curr Opin Immunol* 2003; 15: 138-147 [PMID: 12633662]

**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]

**Pizzurro GA**, Barrio MM. Dendritic cell-based vaccine efficacy: aiming for hot spots. *Front Immunol* 2015; 6: 91 [PMID: 25784913 DOI: 10.3389/fimmu.2015.00991]

**El Ansary M**, Mogawer S, Elhamid SA, Alwaki S, Aboulkass F, Sabawby 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]

**Zhou P**, Liang P, Dang 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: 21283206]

**Tada F**, Abe M, Hirooka M, Ikeda Y, Hisaia Y, Lee Y, Jung NC, Lee WB, Lee HS, Bae YS, Onji M. Phase 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]

**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: 18890227 DOI: 10.1002/hep.22626]

**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]

**Kwilas AR**, Donahue RN, Tsang KY, Hodge JW. Immune consequences of tyrosine kinase inhibitors that synergize with cancer immunotherapy. *Cancer Cell Microenviron* 2015; 2: e777 [PMID: 26057301]
Immune therapy for HCC

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]

Rahimi RS, Trotter JF. Liver transplantation for hepatocellular carcinoma: outcomes and treatment options for recurrence. Ann Gastroenterol 2015; 28: 323-330 [PMID: 26130250]

Yokoyama I, Carr B, Saito H, Iwatsuki S, Starzl TE. Accelerated growth rates of recurrent hepatocellular carcinoma after liver transplantation. Cancer 1991; 68: 2095-2100 [PMID: 16552060]

Cheng JW, Shi YH, Fan J, Huang XW, Qu 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]

Duvoux C, Toso C. mTOR inhibitor therapy: Does it prevent HCC recurrence after liver transplantation? Transplant Rev (Orlando) 2015; 29: 168-174 [PMID: 26071964 DOI: 10.1016/j.trre.2015.02.003]

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]

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]

Sangro B, Gomez-Martin C, de la Mata M, Iturraiaegui M, Garralda E, Barrera P, Raczu-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]

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]

Wolf JA, Malone RW, Williams P, Chong W, Acadi G, Jani A, Felgner PL. Direct gene transfer into mouse muscle in vivo. Science 1990; 247: 1465-1468 [PMID: 1690918]

VanLint 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]

Kreiter S, Selmi A, Diken M, Koslowski M, Britten CM, Huber C, Türeci O, Sahin U. Intronadal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumor immunity. Cancer Res 2010; 70: 9031-9040 [PMID: 21045153 DOI: 10.1158/0008-5472.can-10-0699]

VanLint S, Goyvaerts C, Maenhout S, Goethals L, Disy A, Bentley 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]

VanLint S, Wilgenhof S, Heirman C, Cortiñas J, Breckpot K, Bonehill A, Neys 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/s00262-014-1558-2]

Bonehill A, Tuyaerts S, Van Nuffel AM, Heirman C, Bos TJ, Fostier K, Neys 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]

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]

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]

Sawada Y, Yoshikawa T, Nohuoka D, Shirakawa H, Karuizawa T, Motonura 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, Nakatsuru 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