Hepatocellular Carcinoma from an Immunologic Perspective

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Abstract
Hepatocellular carcinoma is the third most common cancer worldwide. It is an inflammation-associated cancer. Multiple investigators have demonstrated that analysis of the tumor microenvironment may be used to predict patient outcome, indicating the importance of local immune responses in this disease. In contrast with other types of cancer, in which surgery, radiation, and systemic cytotoxic chemotherapies dominate the treatment options, in hepatocellular carcinoma locoregional treatments are widely applied. Such treatments induce rapid tumor cell death and antitumor immune responses, which may favor or impair the patients’ outcome. Recent immunotherapeutic studies demonstrating promising results include trials evaluating intratumoral injection of an oncolytic virus expressing granulocyte macrophage colony-stimulating factor, glypican-3 targeting treatments, and anti-CTLA4 treatment. Although some of these novel approaches may provide benefit as single agents, there is a clear opportunity in hepatocellular carcinoma to evaluate these in combination with the standard modalities to more effectively harness the immune response. Clin Cancer Res; 19(24); 6678–85. ©2013 AACR.

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No potential conflicts of interest were disclosed.

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Learning Objectives
Upon completion of this activity, the participant should understand the basic principles of immunotherapy for hepatocellular carcinoma (HCC). The participant should also have a better understanding of how ablative therapies could potentially induce immune responses and be combined with immunotherapy in the future to treat patients with advanced HCC.

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Introduction
According to the International Agency for Research on Cancer, hepatocellular carcinoma is the third most common cause of cancer-related death worldwide, with an estimated 692,000 cases per year. Hepatocellular carcinoma typically occurs in the setting of chronic inflammation such as viral hepatitis. Although patients with early disease have a relatively good prognosis with a 5-year survival rate of more than 70%, the majority of patients with hepatocellular carcinoma are diagnosed with late-stage disease resulting in an overall 5-year survival rate of less than 16% (1). Impaired metabolism due to liver cirrhosis limits the use of cytotoxic chemotherapy, and a number of studies indicate intrinsic resistance of tumor cells to commonly used chemotherapeutic reagents in hepatocellular carcinoma (2). Sorafenib treatment has shown modest improvement in survival for patients with advanced hepatocellular carcinoma (3), but no other systemic treatment has shown efficacy at the phase III level in the past 5 years. With the recent approval of ipilimumab for patients with melanoma and sipuleucel-T for patients with prostate cancer, immunotherapy has gained the wider attention of both basic scientists and clinicians interested in solid tumors in general, including hepatocellular carcinoma. Several characteristics relating to both the treatment and biology of hepatocellular carcinoma make it amenable to immunotherapy. In this review, we discuss some of the aspects that are specific to hepatocellular carcinoma and why we believe immunotherapy is an attractive research option for patients...
with this type of cancer, either as an alternative modality or complementary to already existing treatments. We summarize the data currently available relating to how the human immune system potentially promotes hepatocarcinogenesis and how it responds to hepatocellular carcinoma progression. We also review recent promising results from clinical trials using immune-based approaches to treat patients with hepatocellular carcinoma and discuss the future of immunotherapy for the treatment of hepatocellular carcinoma.

**Hepatocellular carcinoma—an inflammation-induced cancer**

Hepatocellular carcinoma can be considered a classical inflammation-induced cancer. Hepatitis B and hepatitis C virus (HBV and HCV) infection are known risk factors for the development of hepatocellular carcinoma. In most cases, patients with chronic viral hepatitis will first develop liver cirrhosis and then hepatocellular carcinoma; however, select patients, for example, those with chronic HBV infection, are at high risk of developing hepatocellular carcinoma even in the absence of liver cirrhosis. On the basis of the pivotal vaccination studies performed in Taiwan, which clearly demonstrated that HBV vaccination decreases the number of children diagnosed with hepatocellular carcinoma (4), global childhood vaccination against HBV has been introduced and may even be considered the first prophylactic cancer vaccine. However, new risk factors are emerging. Obesity, and especially visceral adiposity, can result in non-alcoholic fatty liver disease and nonalcoholic steatohepatitis (NASH). Based on murine studies, local intrahepatic chronic inflammatory processes promote hepatocarcinogenesis in mice with NASH (5), and accumulating human data indicate an increasing role for NASH as a risk factor for hepatocellular carcinoma development (6). With a dramatic increase of obesity in the Western world (7), treating NASH (and thereby inflammatory processes) may move more into focus as a way to prevent hepatocellular carcinoma for this new and rapidly increasing patient population (Fig. 1).

**Spontaneous immune responses and immune suppression in hepatocellular carcinoma**

Spontaneous immune responses, including T-cell responses (8) as well as humoral responses to different tumor-associated antigens (9), have been described in hepatocellular carcinoma. Different immune cell subsets, cytokines, and chemokines have been studied in hepatocellular carcinoma with respect to their relevance for patient outcome. A number of studies have shown that tumor-infiltrating CD4$^+$ regulatory T cells correlate with poor outcome in patients who undergo surgical resection (10, 11). Myeloid-derived suppressor cells (MDSC) represent a different subset of immune suppressor cells. These cells are not only increased in frequency in patients with hepatocellular carcinoma and suppress both T cells and NK cells (12) but have also been shown to induce CD4$^+$ regulatory T cells, thus suggesting a dense interactive network of different immune mechanisms within the tumor microenvironment. A recent study showed an inverse correlation between MDSC frequencies and patient outcome after radiofrequency ablation (RFA) treatment (13). Relevant markers and cells, which either correlate with outcome or are different from healthy control subjects, are summarized in Tables 1 and 2.

Systemic inflammatory responses are routinely described by a combination of serum C-reactive protein levels and albumin concentration [Glasgow Prognostic Score (GPS)]. Inflammation-associated indices have been shown to predict patient outcome in patients with surgically resectable disease (14) as well as in patients with more advanced hepatocellular carcinoma (15). One difficulty with these indices, however, is the confounding fact of coexistent liver dysfunction, which is present in the majority of patients with hepatocellular carcinoma. The GPS may be influenced by liver function, and future prospective studies are clearly needed to verify these results. Gene signature studies have been widely conducted to identify hepatocellular carcinoma subgroups according to their prognosis and, even more importantly,
changing the tumor microenvironment from a Th1 into a Th2-type milieu has been described to predict development of venous metastasis in hepatocellular carcinoma and impaired outcome (18).

### Disease-specific considerations: why hepatocellular carcinoma is an attractive target for immune-based approaches

The clinical management of hepatocellular carcinoma has been summarized elsewhere, but, arguably, it differs from that of most other solid tumors in that a wider array of modalities have more common application compared with other tumors whose management is largely confined to (19) surgical resection, radiation, and chemotherapy. In hepatocellular carcinoma, orthotopic liver transplantation is an alternative to surgical resection and requires lifetime immunosuppression. Local ablative approaches are performed to a much larger extent than in other solid tumors, and these locoregional approaches are used with both curative and palliative intent in hepatocellular carcinoma. RFA is the most commonly applied ablative procedure, but alternatives therapies, cryoablation, photodynamic therapy, percutaneous ethanol or acetic acid injection, laser ablation, and high-intensity frequency ultrasound (HIFU), are also commonly used depending on local expertise. Transarterial chemoembolization (TACE) is a palliative treatment option for those with liver-confined disease not amenable to potentially curative ablation or surgery. The basic principle of all local ablative therapies is to induce tumor cell death through different mechanistic approaches such as physical destruction, radiation, or elimination of vascular supply in combination with chemotherapy. From an immunologic point of view, these type of procedures result in the release of tumor antigens, which are taken up by antigen-presenting cells (mainly dendritic cells) and which have been shown to activate a tumor-specific immune response (20). Ablated tumor tissue has been shown to promote dendritic cell maturation (and resultant T-cell stimulatory properties; refs. 21, 22). This antigen release is potentially significant because, although ablative procedures are very effective in eradicating visible lesions, a tumor-specific immune response may prevent recurrent disease in addition to treating distant metastases. In other words, RFA and TACE have the potential to turn a patient’s tumor into an endogenous vaccine. Indeed, several studies have documented an increase in peripheral antitumor immunity following interventional radiologic procedures, and these are summarized below.

### Immune responses following RFA and TACE

Activation and increased cytolytic activity of tumor-specific CD8+ T-cell responses after RFA have been demonstrated in patients with hepatocellular carcinoma (Fig. 2) and colorectal liver metastases (23). Mizokushi and colleagues evaluated T-cell responses in patients with hepatocellular carcinoma undergoing RFA. These investigators observed immune responses to antigens for which no T-cell response was detected at baseline before RFA and the number of tumor-specific T cells after...
RFA correlated with the prevention of hepatocellular carcinoma recurrence in patients treated with curative intent (24). Zerbini and colleagues found that RFA treatment was followed by a significant increase of patients responsive to tumor antigens derived from both the untreated and the necrotic tumor tissue. Here, T-cell responses to recall antigens were also significantly augmented and phenotypic analysis of circulating T cells and natural killer cells showed an increased expression of activation and cytotoxic surface markers. Ayaru and colleagues evaluated the immune response in 10 patients with hepatocellular carcinoma who were undergoing TACE and described an expansion of α-fetoprotein (AFP)-specific CD4 T-cell responses upon TACE. Patients with increased frequencies of AFP-specific CD4 T cells after treatment also showed more tumor necrosis and an improved clinical outcome (26). In a different study, AFP-specific responses were evaluated in patients after TACE in which patients also received dendritic cells. An increase in frequency of AFP-specific T cells was observed in some patients after ablation. However, tumor recurrence was not completely prevented in patients although they displayed enhanced immune responses (24). Two other small studies investigated tumor-specific immune responses in response to combined TACE and RFA treatment or each individual treatment, and confirmed the finding that ablative therapies induce tumor-specific T-cell responses in individual patients upon ablative therapies (27, 28). Recent studies demonstrated that both cryoablation and HiFU might induce immune responses. A change in CD4:CD8 T-cell ratio in peripheral blood was observed in patients with cancer treated with HiFU (29). In a different study, the association between circulating PD-L1/PD-1 levels and prognosis after cryoablation in patients with HBV-related hepatocellular carcinoma was studied. In this study, upregulation of circulating PD-L1/PD-1 was associated with poor prognosis after cryoablation (30). Although many studies suggest that tumor ablation induced antitumor immune responses, no direct comparison of different ablative approaches has been performed in patients with hepatocellular carcinoma to identify the most immunogenic ablation technique (31).
Sorafenib treatment

Many chemotherapeutics and molecular targeting agents have been shown to affect antitumor immune responses (32). Sorafenib is the only approved and effective drug for the treatment of hepatocellular carcinoma. In mice, sorafenib has been shown to have limited effects on antitumor immunity (33). In vitro studies suggest that low-dose sorafenib may promote effector CD4+ T-cell function by eliminating Treg suppressor function in peripheral blood mononuclear cells obtained from patients with hepatocellular carcinoma (34). Furthermore, it has been reported that sorafenib treatment decreases Th2 and Treg cells in peripheral blood from patients with hepatocellular carcinoma (35).

Immunotherapy in hepatocellular carcinoma

Immunotherapy has been tested in hepatocellular carcinoma for many years. Most studies in the past have either used cytokine or antigen-based approaches (36, 37). Although most of these studies have proven to be safe and able to induce tumor-specific immune responses, most of them have failed to demonstrate clinical efficacy. Here, we summarize recent results from different immune-based approaches in hepatocellular carcinoma. These studies differ from previous immunotherapy studies in a number of respects: (i) the approach taken to activate patients’ immune system; (ii) the target; (iii) the quality of the immune response being activated; and (iv) the more promising results seen.

 Oncolytic viruses

The use of oncolytic virus for the treatment of hepatocellular carcinoma is a very recent development, and promising preliminary data have been presented using this approach in early clinical trials. JX-594 is an oncolytic poxvirus modified by insertion of β-galactosidase, a surrogate marker for detection of viral gene expression and human granulocyte macrophage colony-stimulating factor (GM-CSF) to stimulate antitumor immune responses into the thymidine kinase region. The vaccinia virus replicates in cancer cells harboring activation of the EGF receptor (EGFR)/Ras pathway and depends on cellular TK, the concentration of which is increased by cell-cycle abnormalities in cancer cells. JX-595 induces cell lysis in infected cells followed by the induction of a GM-CSF–enhanced anticancer immunity (38). Although initial studies using a JX-594 prototype were done in patients with melanoma (39), intratumoral application of JX-594 was shown to be safe in a phase I study in hepatocellular carcinoma (40). Results from a second study in 30 patients with advanced hepatocellular carcinoma were recently reported (41). In this trial, patients were randomized for treatment with low-dose (10⁶ PFU) or high-dose (10⁷ PFU) JX-594, and safety, patient outcome, and induction of immunity against both cancer and vaccinia were studied. JX-594 was administered by imaging-guided intratumoral injection on days 1, 15, and 29. The objective response rate was 15%, and an intrahepatic disease control rate of 50% was achieved. It should be noted that disease control rates were equivalent in injected and distant untreated tumors, suggesting the presence of a systemic immune response. In contrast with tumor response rates and immune endpoints, there was a clear dose relationship with regard to overall survival (6.7 months in the low- and 14.1 months in the high-dose group).

Immune monitoring studies demonstrated that at least 11 of 16 patients developed hepatocellular carcinoma-specific antibody responses. Cellular T-cell responses were noted, and a significant decrease in HBV DNA concentrations were noted in patients treated with JX-594 previously (42).

 Glypican 3 targeted therapies

Glypican-3 (GPC3) has emerged as an interesting target for immunotherapy in hepatocellular carcinoma. GPC3 is a member of the glypican family of heparan sulfate proteoglycans that are attached to the cell surface. GPC3 is specifically overexpressed in approximately 80% of hepatocellular carcinoma, and correlates with poor prognosis (43, 44). Different studies suggest that it may be used as a serum marker in patients with hepatocellular carcinoma. Immuno-staining for GPC3 is recommended by international guidelines for pathologic diagnosis of hepatocellular carcinoma, especially as it can be used to distinguish high-grade dysplastic nodules from early hepatocellular carcinoma (45). GPC3 is an attractive target as a tumor antigen because it is not only tumor specific, but also important for cell proliferation (46). Both a peptide-based vaccine approach and anti-GPC3 antibodies are currently in clinical development. Thirty-three patients with advanced hepatocellular carcinoma were vaccinated with two different GPC3-derived peptides depending on their HLA haplotype. Although safety was the primary endpoint of this study and, as expected, vaccination was well tolerated, secondary endpoint analysis demonstrated a partial response in 1 patient and 19 patients with stable disease 2 months after initiation of treatment. GPC-3–specific T-cell responses were observed in 30 patients, and GPC-3–specific CTL frequency after vaccination correlated with overall survival (47). In one case, an autopsy was performed on one of the patients, which revealed central necrosis in most of the intrahepatic tumor following peptide vaccination with an infiltration of GPC-3–reactive CD8-positive T cells in the residual carcinoma, but not within the cirrhotic area (48). A phase II study using this approach in the adjuvant setting after surgical resection is currently ongoing.

In an alternative approach, anti-glypican-3 antibodies have been developed and are currently in preclinical and clinical evaluation for the treatment of patients with hepatocellular carcinoma. GC33 is a novel recombinant fully humanized monoclonal antibody that binds to human glypican-3 (GPC3). Twenty patients were enrolled and treated with GC33 in a pilot trial. Patients tolerated treatment well, and no dose-limiting toxicities were observed. Potential antitumor activity that was associated with the target GPC3 expression was observed in this study. Stable
disease was seen in 4 patients, all of whom had high GPC3 expression. In a second study, the combination of sorafenib with anti-glypican-3 was tested. Using phage-display technology, anti-glypican 3 antibody HN3 was identified. This antibody binds to a unique conformational epitope in the core protein of GPC3 with high affinity ($K_d = 0.6$ nmol/L). In preclinical studies, it was shown that HN3 inhibits proliferation of GPC3-positive hepatocellular carcinoma tumor cells implementing a second and immune-independent mode of action by direct inhibition of cell proliferation via targeting cell-cycle arrest through inactivating yap (49).

### Immune checkpoint inhibitors

Monoclonal antibodies that target the immunoregulatory dampening mechanisms of host responses to tumor-associated antigens have recently gained a lot of interest with the approval of anti-CTLA-4 (ipilimumab) for the treatment of patients with advanced melanoma. CTLA-4 is induced upon antigen stimulation and blocks further T-cell activation. The most important molecular function of CTLA-4 seems to be the inhibition of CD28 costimulation, as evidenced by the CD28-dependent lymophoproliferative and autoimmune syndrome observed in CTLA-4 null mice. Ipilimumab blocks the interaction of CTLA4 with its ligands CD80 and CD86 and thereby promotes T-cell activation (50). In patients with previously treated advanced melanoma, ipilimumab improved median overall survival by almost 4 months and resulted in long-term disease control in a significant minority (51). On the basis of these encouraging results, tremelimumab, a different anti-CTLA4 antibody, was tested in a pilot clinical trial in 20 patients with advanced hepatocellular carcinoma and HCV infection. Tremelimumab was given at a dose of 15 mg/kg i.v. every 90 days and the treatment was tolerated well. Three of 17 evaluable patients developed confirmed partial responses and 10 patients had stable disease as the best response to treatment. HCV-specific T-cell responses were studied in these patients as a surrogate marker for the anti-CTLA4–induced immune responses. HCV-specific T-cell responses were noted in a number of patients, and a decline in viral load was observed in most patients followed for at least 3 months. Three patients had a complete viral response (52). Other immune checkpoint inhibitors such as anti-PD1 are currently under clinical investigation (NC101658878).

### Outlook and Future

Although this report has focused on human studies and it is clearly beyond the scope of this review to summarize data from murine studies, it is obvious that good animal models...
mimicking the patients’ disease are needed to develop future immune-based approaches for the treatment of hepatocellular carcinoma. The fact that 90% of patients with hepatocellular carcinoma also suffer from underlying liver disease, which impairs metabolism of cytotoxic and targeted therapies, in conjunction with recent advances in immunotherapy, clearly calls for the development of new immune-based treatment approaches in hepatocellular carcinoma. The observation of tumor regression at sites distant from the primary site of radiotherapy in a melanoma patient treated with ipilimumab (53) suggests that combination of local tumor treatments with checkpoint inhibitors may induce strong antitumor immune responses leading to clinical responses. In addition, recent observations using checkpoint inhibitors for the treatment of patients with other types of solid cancer clearly call for studies in hepatocellular carcinoma. Because local tumor ablation is routinely used in patients with hepatocellular carcinoma and has been shown to induce immune responses, a rational extension would be to boost this immune response by combination with, for example, a checkpoint inhibitor. These as well as all other immune-based treatment approaches and concepts including but not restricted to dendritic cell–based vaccines and antibody-based and cytokine treatments have the potential to induce delayed but long-lasting antitumor effects in a patient population that is, at present, poorly served by systemic therapies (Fig. 3). Choosing the best response evaluation criteria will be a true challenge. Two different response evaluation tools already exist for hepatocellular carcinoma—RECIST and modified RECIST (19). Changes induced by inflammation inside the tumors and frequently observed delayed responses in other indications have formed the rationale to develop “immune-related response criteria” (54), which will need to be used for patients with hepatocellular carcinoma undergoing immunotherapy trials. Underlying liver cirrhosis with impaired liver function as well as chronic hepatitis infection commonly found in patients with hepatocellular carcinoma will require considerable expertise in the design of new study concepts. Only trials with adequate immune monitoring will advance the field of immunotherapy in hepatocellular carcinoma. Most importantly, investigators need to keep in mind that hepatocellular carcinoma is vastly different from other types of cancer and may require disease-specific considerations in the development of new treatment approaches for patients with hepatocellular carcinoma.

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