Immunotherapy in Hepatocellular Carcinoma: Primed to Make a Difference?

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Advanced hepatocellular carcinoma (HCC) carries a dismal prognosis and the current treatment is limited to sorafenib, an agent with modest benefit. Preclinical data have indicated that several immunologic mechanisms are at play to promote HCC development and growth while impairing effective antitumor immune surveillance. Several novel approaches geared toward manipulating the immune response to HCC have suggested a therapeutic benefit in early-stage clinical trials, indicating a real potential to augment tumor-specific immunity and improve outcomes in patients with this disease. In the current study, the authors reviewed the barriers to an effective immune response against HCC and contemporary clinical investigations that may be “primed” to alter the natural history of HCC.


KEYWORDS: checkpoint inhibitors, hepatocellular carcinoma, immune evasion, immunotherapy.

INTRODUCTION

After decades of research, the hope of effective immunotherapy became a reality with the development of immune checkpoint inhibitors.1 This elegant approach leverages the immune system, which has the capability to recognize a diverse array of both foreign and tumor-derived antigens, to exact a tumor-specific response capable of treating malignancy. With the recent stall in drug development in hepatocellular carcinoma (HCC),2 the focus is shifting from antiangiogenic therapy to novel modalities to improve survival for this deadly disease. Several factors make HCC an attractive target for immunotherapy. Chronic inflammation, which is associated with HCC risk factors including hepatitis B virus (HBV), hepatitis C virus (HCV), and metabolic disorders such as nonalcoholic fatty liver disease, promotes an immunosuppressive environment and T-cell exhaustion. HCC also effectively evades the immune response via several mechanisms, not limited to aberrant expression of immune checkpoint molecules. Furthermore, it is clear that HCC-specific antigens are recognized by the immune system and contemporary clinical studies have indicated that manipulating the immune response can be deleterious to HCC. To answer the question regarding whether immunotherapy is “primed” to make a difference in HCC, this review will focus on the current understanding of HCC immunobiology and methods with which to exploit the immune system as an effective therapy for HCC.

The Liver in Immunity and Tolerance

Because of its dual vasculature, the liver receives nutrients and pathogen-derived molecules (ie, lipopolysaccharides) from the portal vein as well oxygenated blood from the systemic circulation via the hepatic artery. This unique macroscopic anatomy leads to enormous exposure to gut-borne pathogens as well as exogenous nonpathogenic molecules. As such, the immunologic composition of the liver (Fig. 1), which contains the largest concentration of immune effectors in the body, is elegantly designed to play a central role in host defense and, more so, the maintenance of self-tolerance.3,4

Several observations have indicated that in normal physiologic conditions, the immunologic milieu of the liver promotes a tolerogenic environment. Liver sinusoidal endothelial cells (LSECs), the fenestrated cellular barrier between the sinusoidal blood and hepatocytes, have antigen-presenting cell (APC) function, expressing toll-like receptors and the major histocompatibility complex (MHC) class I and II molecules.5 It is interesting to note that LSECs express high levels of the inhibitory molecule program death receptor ligand 1 (PD-L1) and low levels of the costimulatory molecules CD80 and CD86, thereby limiting their ability to effectively activate CD4-positive (CD4+) and CD8 + T lymphocytes.6,7

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Furthermore, in the presence of bacterial particles such as lipopolysaccharides and its mediators (ie, interleukin 10 [IL-10], transforming growth factor-beta [TGF-β], and prostaglandins), MHC expression is downregulated in LSECs.\(^5\) Finally, through direct cell contact, LSECs reduce the ability of dendritic cells (DCs) to activate T cells.\(^10\) Thus, LSECs prevent an exuberant immune response to the constant supply of bacterial-derived particles from the gut but also decrease immune surveillance in normal liver tissue. Kupffer cells (KCs), which are stationary macrophages in the liver sinusoids, mimic LSECs in promoting tolerance in that KCs express low levels of MHC molecules, are poor stimulators of adaptive immunity, produce inhibitory cytokines such as IL-10 and prostaglandins, and preferentially expand inhibitory forkhead box P3 (FoxP3) and CD25 regulatory T cells (Tregs).\(^11,12\) Liver-derived DCs also appear to be less potent at stimulating T cells compared with their counterparts from other organs.\(^13\) These conditions create an environment that will support T-cell proliferation but not T-cell activation. This, of course, is a critical feature for the induction of self-tolerance but may represent a barrier for the development of immunity against tumors of the liver.

**The Immune Response in HCC**

**Chronic inflammation and T-cell exhaustion**

For effective T-cell activation or “priming” to eradicate pathogens or cancer, a foreign antigen or cancer neoantigen must be presented within the context of the MHC molecule to the cognate CD4/CD8 T-cell receptor.\(^14\) This primary signal alone will not activate T cells, and a second stimulatory signal is required for T-cell activation.\(^15\) Several costimulatory receptors and their corresponding ligand on APCs have now been identified and include CD28-CD80/86, ICOS-B7RP1, CD137-CD137L, OX40-OX40L, and CD27-CD70.\(^15\) Coinhibitory receptors dampen or modulate T-lymphocyte activation in normal physiologic circumstances. These receptors, also expressed on T cells, and their corresponding ligands include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)-CD80/86, programmed death 1 (PD-1)-PD-L1, killer cell immunoglobulin (Ig)-like receptors (KIR)-MHC class I and II molecules, lymphocyte-activation gene 3 (LAG3)-MHC class I and II molecules, and T-cell immunoglobulin domain and mucin domain 3 (TIM-3)-galectin 9.\(^9\) For example, the binding of the T-cell coinhibitory receptor PD-1 to its ligands PD-L1 or PD-L2 blocks T-cell activation.\(^16\) To sustain productive priming, a third and final signal via other molecules, such as proinflammatory cytokines (IL-12 and interferon-gamma [IFN-γ]), are required to promote T-cell effector proliferation and survival.\(^17\) Optimal T-cell priming results from the conglomerate effect of these multiple stimulatory and inhibitory signals. Impairment of these signals (eg, by chronic inflammation or malignancy) leads to T-cell deletion; anergy; or, in the right context, T-cell “exhaustion.”

Exhausted T-cells have the capacity to recognize both foreign and cancer neoantigens; however, they exist in a chronic hyporesponsive state, expressing high levels of coinhibitory receptors (ie, CTLA-4 and PD-1) and low effector cytokines, with impaired cytotoxicity necessary for an immune response, reviewed in detail by Jiang et al.\(^18\) Chronically inflamed livers, as associated with viral hepatitis, autoimmune hepatitis, and nonalcoholic fatty liver disease, create a microenvironment that favors T-cell exhaustion.\(^19\) The extent of hepatic inflammation correlates positively with the expression of PD-1 on lymphocytes and PD-L1 expression on intrahepatic KCs, LSECs, and tumor-derived leukocytes. Cytotoxic CD8 + T cells, derived from livers that are chronically infected with HBV/HCV, have an exhausted phenotype and are less adept at delivering a cytotoxic immune response capable of controlling infection.\(^20-23\) In HBV murine models, treatment with an anti-PD-1 or an anti-PD-L1 antibody leads to resurgence of T-cell-mediated immunity and clearance of HBV in vivo.\(^21,24\) In addition to PD-1 overexpression, other coinhibitory molecules such as CTLA-4\(^25,26\) and TIM-3\(^27\) are heavily expressed on T cells in patients with viral hepatitis. In contrast to chronic HBV, HCV-infected livers appear to exhibit a more profound CD8 + T-cell exhaustion phenotype, manifested by high PD-1 and CTLA-4 levels and low expression of CD28 and CD127.\(^26\) In vitro dual CTLA-4 and PD-1 blockade, but not individual blockade, is required to reverse HCV-specific effector T-cell dysfunction.\(^28\) This is an important observation, because it suggests that HCV-derived HCC may be less sensitive to immune checkpoint inhibitor monotherapy.

Other factors that contribute to the immunosuppressive phenotype of chronic inflammation include heightened levels of IL-10 and TGF-β,\(^29,30\) increased frequency of Tregs,\(^31-34\) and impaired antigen presentation.\(^35\) Taken together, these data support the hypothesis that chronic inflammation, associated with known HCC risk factors, leads to T-cell exhaustion and an immunosuppressive environment, which fosters HCC growth and progression.

**Mechanisms of immune evasion in HCC**

Multiple mechanisms of immune escape and evasion have been identified in HCC. Evasion pathways are complex...
and not fully established; however, such mechanisms include perturbations in antigen presentation and immune effector function, alterations in immune checkpoint molecules, and disarray of cytokine profiles.

Hepatomas variably express MHC class I molecules and have low levels of the costimulatory molecules CD80 and CD86.\textsuperscript{36,37} Furthermore, functional studies have indicated that HCCs lack the antigen-processing machinery necessary to effectively present tumor neoantigens, allowing escape from cytotoxic CD8\textsuperscript{+} T-lymphocyte killing.\textsuperscript{38} MHC class II peptide presentation is also altered in the tumor microenvironment. Han et al identified a novel subset of CD14/CTLA-4-positive regulatory DCs that significantly suppress effector T-cell response via IL-10 and indoleamine-2,3-dioxygenase production.\textsuperscript{39} Effective CD4/CD8 \textsuperscript{+} T-lymphocyte response is also dampened by several immune inhibitory cell populations that are abundant in the peritumoral tissue, including Tregs,\textsuperscript{40,41} myeloid-derived suppressor cells (MDSCs),\textsuperscript{40,42} tumor-associated monocytes,\textsuperscript{43} and invariant natural killer T cells (which provide a regulatory function through T-helper 2 cytokine production and inhibit expansion of tumor antigen-specific CD8 \textsuperscript{+} T cells).\textsuperscript{44} HCCs also are infiltrated by hepatic stellate cells, which are also immunologically active; express a component of suppressive cytokines; and, through PD-L1, induce T-cell apoptosis.\textsuperscript{45} Finally, effector CD4 \textsuperscript{+} T cells and cytotoxic CD8 \textsuperscript{+} T cells from HCCs are present at low frequencies and exhibit functional impairments, with the hallmarks of T-cell exhaustion.\textsuperscript{40,46} Many of these alterations adversely affect patient outcome, suggesting that their reversal may have therapeutic benefit. In fact, depletion of Tregs, exhausted CD4 \textsuperscript{+} helper T cells, and MDSC ex vivo can restore the production of granzyme B by CD8 \textsuperscript{+} T cells and IFN-\gamma-producing CD4 \textsuperscript{+} lymphocytes.\textsuperscript{40}

Immune checkpoint molecules are dysregulated in HCC, most notably PD-1, PD-L1, CTLA-4, TIM-3,
KIR, and LAG3. Although all these molecules can potentially be leveraged for therapeutic benefit, the PD-1 and PD-L1 axis is the most topical. PD-L1 is heavily expressed in HCC and surrounding APCs (LSECs, KCs, and tumor-associated monocytes).\(^4^3,4^7,4^8\) Retrospective studies have indicated that PD-L1 expression, as assessed by flow cytometry, Western blot analysis, and immunohistochemical staining, ranges from 45% to 100% in HCC samples.\(^4^4,4^7,4^9,5^0\) In a cohort of 240 patients with surgically resected HCC, tumoral PD-L1 expression was associated with aggressive clinicopathologic features and a statistically significantly shorter disease-free survival.\(^4^9\) As discussed above, certain HCC etiologic factors enrich for PD-L1 expression and it will be interesting to formally assess how expression changes across HCC subtypes.\(^2^6,5^0\) Therapeutically, the preclinical xenograft model of Kuang et al indicated that PD-L1 blockade can suppress HCC tumor growth.\(^4^3\) An important question is whether such monotherapy will be enough to generate a clinically significant response, given the profound immunosuppressive phenotype of chronic inflammation and malignancy.

Disarray of cytokine profiles (ie, increased IL-4, IL-5, IL-8, and IL-10 secretion and relative suppression of IL-1, tumor necrosis factor, and IFN-\(\gamma\)) in the HCC microenvironment results in blunting of the normally protective T-helper 1 immune response needed to effectively combat malignancy.\(^2^9\) This cytokine signature is associated with a poor prognosis and aggressive disease characteristics. High levels of circulating TGF-\(\beta\) also portend inferior survival in patients with HCC. Although the tumor-promoting effects of TGF-\(\beta\) include enhanced neovascularization, the promotion of metastasis, and induction of fibrosis, there is clear evidence that this molecule is immunosuppressive.\(^5^1\) TGF-\(\beta\) induces Treg polarization and differentiation,\(^3^4,5^2\) and is a key negative regulator of CD8+ T cells, promoting T-cell exhaustion.\(^5^3\)

Tumor-associated antigens and protective immune responses in HCC
Rare clinical anecdotes have indicated that spontaneous remission and tumor shrinkage occur in HCC.\(^5^4\) One explanation for these extraordinary observations, despite an overall immunosuppressive environment, is that some individuals are able to exact a concerted HCC-specific immune response. Larger retrospective studies have suggested that certain subsets of patients are able to mount a protective immunity to HCC.\(^4^6,4^9,5^5\) For example, Gao et al demonstrated that the ratio of activated cytotoxic CD8- T cells to inhibitory Tregs in tumor-infiltrating lymphocytes (TILs) is an independent favorable prognostic factor for disease-free and overall survival (OS) after surgical resection of HCC.\(^4^6\) Several human HCC-specific antigens, including alphafetoprotein (AFP),\(^5^6\) glypican 3,\(^5^7\) New York esophageal squamous cell carcinoma 1 (NY-ESO-1),\(^5^8\) melanoma antigen gene A (MAGE-A),\(^3^9\) Wilms tumor 1,\(^6^0\) and human telomerase reverse transcriptase,\(^6^1,6^2\) also have been identified at low levels in patients with HCC. These antigens lead to functional cytotoxic T-cell response against HCC.\(^5^6,5^8,6^1-6^3\) Flecken et al demonstrated that the 51.6% of patients with HCC who developed a CD8+ cytotoxic T-cell response to in vitro antigen stimulation (AFP, MAGE-A, glypican 3, and NY-ESO-1) had an associated improvement in survival.\(^6^4\) Thus, there is ample evidence that HCC is a potentially immunogenic tumor, rendering it an attractive target for immunotherapy.

Clinical Investigations
Cancer vaccines
Peptide-based and DNA-based vaccines with or without DC infusions have been applied in the clinical setting for patients with advanced HCC.\(^6^5-7^2\) The concept of using specific tumor-derived epitopes to prime an HCC immune-mediated response is fascinating, but clinical trials have been disappointing, with low response rates and marginal progression-free survival reported,\(^6^7-6^9\) or have been tested in too few patients to draw any firm conclusions.\(^7^1,7^2\) That being said, several key observations can be made that indicate continued exploration is warranted. First, antigen-specific cytotoxic immune responses can be detected in sera from patients with HCC, and this finding is correlated with a survival advantage.\(^6^8\) Second, there are anecdotal AFP and/or radiographic responses suggesting meaningful clinical activity.\(^6^5,6^6,6^8,7^1\) Last, these approaches are relatively innocuous, with few adverse events. The critical question is whether vaccination strategies can be improved on. An obvious pairing would be with immune checkpoint inhibitors.\(^1\) Other considerations might include the application of multiantigen vaccines,\(^6^4\) or with the advent of genomic medicine, using precise cancer neoantigens found in individual patient tumors to prime an immune response.\(^7^3\)

Interferons and other cytokines
IFN has been studied extensively in patients with HCC as an adjuvant therapy after surgical resection, in the advanced setting, and at a variety of doses, schedules, and combinations over the last several decades.\(^7^4-8^4\) The data are somewhat conflicting, but overall are lackluster, with high rates of toxicity reported. Recent studies manipulating the TGF-\(\beta\) pathway appear to be more promising.\(^5^1\)
Galunisertib, a TGF-β receptor 1 kinase inhibitor, was recently tested in a phase 2 study in 109 patients with HCC. The median OS for the cohort was 36 weeks, but it is interesting to note that, in a subset of AFP responders (24% of patients), the median OS was 96 weeks. Galunisertib is currently being tested in combination with sorafenib and ramucirumab (ClinicalTrials.gov identifier NCT01246986) as well as checkpoint blockade inhibitors (ClinicalTrials.gov identifier NCT02423343).

**Oncolytic viruses**

Oncolytic viruses lead to tumor eradication in 2 ways: selective direct viral replication within tumor cells leading to lysis and activation of cell-mediated, tumor-specific immunity. JX-594 (Pexa-Vec; Jennerex Biotherapeutics Inc, San Francisco, Calif) is derived from a strain of vaccinia that has been engineered to target cancer cells. A randomized phase 2 dose-ranging study was initiated to evaluate the safety and antitumor efficacy of JX-594 when administered at a high dose versus a low dose in patients with advanced HCC (30 patients). All patients in the study experienced flu-like symptoms consisting of fever, chills, rigors, nausea, or vomiting within 24 hours of the administration of JX-594. Other notable toxicities included pustulosis, anorexia, lymphopenia, and mild reversible transaminitis. Four patients responded to treatment based on modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria (1 complete response and 3 partial responses). Furthermore, the OS was significantly longer in the high-dose arm compared with the low-dose arm (median of 14.1 months vs 6.7 months; \( P = .020 \)). In contrast, a phase 2b clinical trial in patients with HCC who failed sorafenib therapy (129 patients) was recently completed and did not achieve the primary endpoint of prolonging OS in patients treated with JX-594 when compared with those treated with best supportive care in this last-line, poor-prognosis patient population. As has been the experience with other immunotherapies, these study results suggest that patients with less advanced disease may be more likely to benefit from an oncolytic immunotherapy. A phase 3 study of JX-594 in combination with sorafenib versus sorafenib alone as first-line treatment in patients with HCC is planned.

**Immune checkpoint inhibitors**

Checkpoint inhibitors have revolutionized cancer care and have been proven to lead to durable tumor shrinkage in a subset of patients that has translated into significant OS advantages over standard therapy in several cancer types. To our knowledge to date, interference with the CTLA-4-CD80/86 and PD-1/PD-L1 axis has shown promise in patients with HCC and many clinical trials using this approach have been reported or currently are underway (Table 1).

** CTLA-4 blockade**

Tremelimumab is a fully human IgG2 monoclonal antibody directed against CTLA-4 on activated T cells. A phase 2 study of tremelimumab in patients with advanced HCC with HCV-related cirrhosis was recently reported and demonstrated promising activity. The study population was comprised of 20 patients with a high burden of disease and impaired liver function (57% of patients with Barcelona Clinic Liver Cancer stage C disease, 43% of patients with Child-Pugh B disease, 29% with portal vein invasion, and 29% with an AFP level ≥400 UI/mL), with the majority of patients failing prior sorafenib. Of 17 evaluable patients, 3 (17.6%) achieved a partial response and 10 patients (58.8%) had stable disease as the best tumor response. The disease control rate was 76.4%, and clinical benefit was >12 months in approximately one-third of patients. The median time to disease progression was 6.5 months, which is favorable compared with historical controls for this population. One area of concern was the relatively high rate of grade 3 and grade 4 (Common Terminology Criteria for Adverse Events version 4) transaminitis (45%), although this adverse event was reversible, did not progress to liver failure, and was not clearly immune mediated in that no patient needed immunosuppression for recovery. Importantly, results of studies of tremelimumab, clinical anecdotes, and a pilot study in patients with viral hepatitis have indicated that immune checkpoint blockade is safe in the setting of chronic viral infection, and may have antiviral activity. Sangro et al demonstrated a decrease of >200-fold in serum HCV viral load at day 210 in 12 patients treated with tremelimumab; in 3 patients there was a transient complete viral response. These results suggest an antitumor and antiviral effect and that immune checkpoint blockade would be of great usefulness in patients with virally mediated HCC.

**PD-1 and PD-L1 blockade**

Emerging results for several studies investigating PD-1 and PD-L1 blockade in patients with advanced HCC as monotherapy are encouraging. Segal et al reported the preliminary results of MEDI4736, a human IgG1 monoclonal antibody to PD-L1. MEDI4736 was found to be tolerable, with lower rates of hepatotoxicity than observed with CTLA-4 blockade in patients with HCC. Of 19 evaluable patients, there were no responders according to
REICST (version 1.1), although 21% of patients achieved disease control at 12 weeks. Additional patients have been enrolled to this study and more data are required before firm conclusions can be made regarding efficacy.

Nivolumab, a fully human IgG4 monoclonal antibody to PD-1, was tested in an HCC-specific phase 1/2 trial that was reported at the 2015 annual meeting of the American Society of Clinical Oncology.\textsuperscript{90} In this study, 47 patients with HCC of varying etiologies (51% uninfected, 25.5% with HCV, and 23.4% with HBV) with excellent hepatic function (98% with a Child-Pugh score of A) who were pretreated with sorafenib (68%) received varying doses of nivolumab. Treatment was tolerable, with only 1 patient discontinuing the study for drug-related hepatitis, and rates of grade 3 transaminitis were relatively low (11% elevation in aspartate aminotransferase and 9% elevation in alanine aminotransferase). Efficacy was encouraging, with 2 complete responses noted and an overall objective response rate of 19% by RECIST (version 1.1). Some responses were quite durable (>12 months). It is important to note that tumor shrinkage also occurred in patients with non-HBV/HCV-mediated HCC, indicating that viral infection is not necessary for response to these agents. The OS rate at 12 months was 62% (95% confidence interval, 42%-76%). As observed with CTLA-4 blockade, anecdotal response of HCV viral infection was also observed in this study.\textsuperscript{90}

**New Novel Checkpoint Inhibitors, Combination Strategies, and Future Directions**

The results presented above argue for the continued development of checkpoint blockade monotherapy in patients with advanced HCC and several pivotal and proof-of-concept studies with the current agents are being planned. Beyond CTLA-4 and PD-1/PD-L1 blockade, preclinical data have indicated that other immune inhibitory checkpoints such as LAG3,\textsuperscript{96} TIM-3,\textsuperscript{97} and KIR\textsuperscript{98} can be blocked, thereby enhancing T-cell-mediated tumor killing. Alternatively, agonists to stimulatory molecules such as CD137\textsuperscript{99} and OX40\textsuperscript{100} have shown activity in an HCC model system. To our knowledge to date, these agents have not been tested clinically in patients with HCC.

These data have also indicated that despite the moderate preliminary activity of anti-CTLA-4 and anti-PD-1/PD-L1 blockade, preclinical data have indicated that other immune inhibitory checkpoints such as LAG3,\textsuperscript{96} TIM-3,\textsuperscript{97} and KIR\textsuperscript{98} can be blocked, thereby enhancing T-cell-mediated tumor killing. Alternatively, agonists to stimulatory molecules such as CD137\textsuperscript{99} and OX40\textsuperscript{100} have shown activity in an HCC model system. To our knowledge to date, these agents have not been tested clinically in patients with HCC.

**TABLE 1. Status of Immune Checkpoint Inhibitors in Hepatocellular Carcinoma**

<table>
<thead>
<tr>
<th>ClinicalTrials.gov Identifier</th>
<th>Agent</th>
<th>Target</th>
<th>Design</th>
<th>Status</th>
<th>Sample Size</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01008358\textsuperscript{88}</td>
<td>Tremelimumab</td>
<td>CTLA-4</td>
<td>II</td>
<td>Completed</td>
<td>20</td>
<td>ORR: 17.6%; DCR: 76%; TTP: 6.5 mo; HCV virologic response</td>
</tr>
<tr>
<td>NCT01853618\textsuperscript{89}</td>
<td>Tremelimumab plus TACE/RFA/SBRT</td>
<td>CTLA-4</td>
<td>I/pilot</td>
<td>Accruing</td>
<td>TACE: 8 RFA: 10</td>
<td>Feasible; ORR: 40% non-RFA/TACE targets; HCV virologic response</td>
</tr>
<tr>
<td>NCT00966251</td>
<td>CT-011</td>
<td>PD-1</td>
<td>I</td>
<td>Terminated</td>
<td>-</td>
<td>Poor accrual</td>
</tr>
<tr>
<td>NCT01658878\textsuperscript{90}</td>
<td>Nivolumab</td>
<td>PD-1</td>
<td>I/II</td>
<td>Accruing</td>
<td>47</td>
<td>ORR: 19%; 12-mo OS: 62%; HCV virologic response</td>
</tr>
<tr>
<td>NCT01938612\textsuperscript{91}</td>
<td>MEDI4736</td>
<td>PD-L1</td>
<td>I/II</td>
<td>Accruing</td>
<td>20</td>
<td>12-mo DCR: 21%</td>
</tr>
<tr>
<td>NCT01714739</td>
<td>Nivolumab plus lirilumab</td>
<td>PD-1 KIR</td>
<td>Ib/II</td>
<td>Accruing</td>
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<td>Pending</td>
</tr>
<tr>
<td>NCT02423343</td>
<td>Nivolumab plus galunisertib</td>
<td>PD-1 TGFR1</td>
<td>Ib/II</td>
<td>Open not accruing</td>
<td>-</td>
<td>Pending</td>
</tr>
</tbody>
</table>

Abbreviations: CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DCR, disease control rate; HCV, hepatitis C virus; KIR, killer cell immunoglobulin-like receptor; ORR, overall response rate; OS, overall survival; PD-1, program cell death receptor 1; PD-L1 programmed cell death receptor 1 ligand; RFA, radiofrequency ablation; SBRT, stereotactic body radiotherapy; TACE, transarterial chemoembolization; TGFR1, transforming growth factor beta receptor 1; TTP, time to disease progression.

\*Sample size at the time of data lock or preliminary publication.
One attractive hypothesis is that multitargeted tyrosine kinase inhibitors, such as sorafenib, have host immunoregulatory properties, which have the potential to aid in the clearance or suppression of neoplastic cells. Emerging data have indicated that sorafenib impacts the immune synapse by modulation of multiple effectors, including cytotoxic and regulatory T cells, and natural killer cells, and as such pairing sorafenib with an immune checkpoint blockade is logical. Chen et al have demonstrated that sorafenib-induced hypoxia upregulates expression of PD-L1 and stromal-derived factor 1α on tumor and surrounding stroma, thereby leading to an influx of an immunosuppressive cell infiltrate. Combination treatment with sorafenib and a C-X-C chemokine receptor type 4 inhibitor (ie, the stromal-derived factor 1α receptor) can reverse this finding and inhibit HCC growth in vivo. It is interesting to note that the addition of a PD-L1 antibody to this combination leads to greater antitumor activity and induces a cytotoxic CD8+ T-cell response. Thus, pairing antiangiogenics with checkpoint blockade is warranted in this disease.

An area of clear interest is combining locoregional therapies, a standard of care in patients with HCC, with immune checkpoint blockade at earlier stages of the disease. Local tumor destruction via ablation, chemoembolization, or radioembolization has the potential to differentially alter and enhance tumor-specific antigen presentation. It has been established that the hypoxia response, a consequence of regional therapy, modulates the expression of critical immunotherapy targets, including CD137, OX40, and PD-L1, in the tumor microenvironment. Noman et al demonstrated that hypoxia leads to hypoxia-inducible factor 1α-dependent upregulation of PD-L1 in MDSCs; however, by applying PD-L1 blockade, MDSC suppressive activity is abrogated in this system. Beyond hypoxia, regional therapy in patients with HCC has been shown to alter cytokine profiles; the neutrophil-to-lymphocyte ratio; and, importantly, CD4+/CD8+ T-cell subset populations and level of T-cell activation. The kinetics of these changes last from hours (as is the case with IL-6/IFN-γ/tumor necrosis factor-α sequestration after embolization) to months (as in the case of expansion and activation of T-cell subsets). In one study, expansion and activation of tumor-specific CD4+ T cells against 3 AFP epitopes occurred after embolization. Importantly, heightened levels of AFP-specific CD4+ T cells after treatment were associated with a tumor necrosis rate of >50% and improved clinical outcome. Tremelimumab appears to be feasible with transarterial hepatic artery chemoembolization and other locoregional therapies, but more data are required to assess the safety of this approach (ClinicalTrials.gov Identifier NCT01853618). Emerging data from this small series have demonstrated a high response rate (40%) for extrahepatic disease, perhaps supporting the hypothesis of a synergistic antitumor response. Given these data, it will be of critical importance to assess how different methods of local tumor destruction and their timing can augment or be augmented by checkpoint blockade.

**Biomarkers and Imaging Assessment**

A critical variate of responsiveness to checkpoint blockade appears to be the mutational landscape of specific cancer histology. Recent work has indicated that a higher tumor mutation burden enriches for responsiveness to CTLA-4 blockade in patients with melanoma, and to PD-1 inhibition in those with non-small cell lung and mismatch repair-deficient colorectal cancers. The operant hypothesis is that high mutational burden leads to a greater number of neoantigens for presentation to effector T cells. HCC, a cancer with a moderate mutational burden (mean somatic mutation rate of approximately 1.3 mutations per mega base) and rare examples of hypermutation may therefore be less sensitive to checkpoint blockade than other tumor types. Alternative tumor-specific factors that might modulate responsiveness to immunotherapy include HCC etiologic factors, the presence/absence of TILs, TIL effector composition, and concentrations of checkpoint molecules in the tumor microenvironment (ie, CTLA-4, PD-1, or PD-L1). One germane example is tumoral PD-L1 expression, which is variably expressed on HCC and may be a prerequisite for responsiveness to PD-1 and PD-L1 checkpoint blockade. Inconsistent expression is due to experimental technique, tumor heterogeneity (ie, both in sampling and etiology), and disease stage (PD-L1 expression increases with more advanced disease). Furthermore, PD-L1 expression is protean, increasing with modalities that induce hypoxia (ie, embolization or sorafenib). Thus, tumoral PD-L1 will need to be assessed as an integrated biomarker rather than an integral biomarker that is required for clinical trial enrollment with anti-PD-1/PD-L1 therapy. Host factors also may play a role in determining responsiveness to checkpoint blockade. The neutrophil-to-lymphocyte ratio, circulating cytokine levels (ie, IL-10 and TGF-β), and baseline and on-treatment immune effector composition (Tregs and MDSCs) appear to correlate with antitumor activity in other disease systems and require assessment in HCC as well.
Radiographic surrogates of HCC outcomes have been difficult to establish because tumors rarely shrink on the background of the cirrhotic liver. Several criteria have been assessed in HCC, including RECIST, modified RECIST (ie, incorporates intratumoral enhancement as a response criteria), the ratio of tumor necrosis to tumor volume, volumetric measurement, and the application of functional and hypoxia-based magnetic resonance imaging. Pseudoprogression, as a result of an immunologic infiltrate, occurs after checkpoint blockade in several solid tumors, and in these patients improvement in outcomes is also apparent. Thus, radiographic response assessment in patients with HCC will have to be tailored further to account for the delayed response kinetics of immunotherapy.

Conclusions
Immunotherapy is rightfully gaining momentum as an important therapeutic strategy in HCC. The greatest promise appears to surround checkpoint blockade inhibitors, given their clear activity in other disease systems, safety, feasibility, and the recent modest signal in HCC. Several clinical trials, based on compelling preclinical data, investigating immune checkpoint inhibitors alone or in various combinations currently are in progress or being planned. Hopefully, these new lines of investigation will lead to new therapies that will improve the care of patients with this devastating disease.

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Conflict of Interest Disclosures
Ghassan K. Abou-Alfa has acted as a paid consultant for Medimmune, Bristol-Myers Squibb, Merck, SillaJen, and AstraZeneca for work performed outside of the current study.

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Cancer February 1, 2016 375
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