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Immunotherapy and Hepatocellular Carcinoma

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Hepatocellular Carcinoma

Hepatocellular Carcinoma (HCC), the most common primary liver tumor, is the third leading cause of cancer death worldwide and the ninth leading cause of cancer death in the United States [1]. The incidence of HCC in the United States has increased from 2.7 to 3.2 per 100,000 persons over the period 2001-2006, according to the most recent data from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) surveillance system [2]. Moreover, HCC related to Hepatitis C Virus (HCV) infection is among the fastest-rising causes of cancer death in the US owing to the peak incidence of HCV infection in the aging cohort born between 1945 and 1965 [3].

Because HCC arises in a background of chronic inflammation often due to viral infection, tumor cells accumulate myriad mutations thus making the cancer resistant to traditional chemotherapy, and treatment with chemotherapeutics is further limited by impaired hepatic function.

HCC is frequently diagnosed in an advanced stage, as only 5% of patients diagnosed in the United States are eligible for surgical resection. For the majority of patients with advanced stage disease, treatment options are limited to sorafenib, a tyrosine kinase inhibitor of Raf kinase and vascular endothelial growth factor receptor, the only therapy which prolongs survival by about three months as compared to placebo, and with a relatively high incidence of adverse effects [4].

Even in patients who are eligible for surgical resection, recurrence of the tumor occurs in up to 70% of cases at 5 years [5]. For the fifteen percent of highly selected patients who are eligible for liver transplantation, recurrence is much less frequent, but many become ineligible prior to surgery owing to rapid disease progression. There is thus an urgent need for new therapeutics in the treatment of advanced HCC, and immunotherapy has emerged as one promising approach.

The Anti-Tumor Role for the Adaptive Immune System

The adaptive immune system is the host's response to specific foreign antigens that evade the non-specific, or innate immune response. In addition to recognizing infectious particles, such as viruses, bacteria and fungi, the immune system also recognizes many cancer cells as non-self. Antigens are displayed to T-cells by class I major histocompatibility (MHC) proteins found on most human cells. A subset of cells, including dendritic cells, macrophages and B-cells, display antigens by class II MHC proteins and also use co-stimulatory pathways to activate CD8+ T-cells, inducing clonal expansion of an army of antigen-specific cytotoxic CD8+ T-cells with an effector phenotype. On recognizing the target epitope, these effector cells, with the assistance of CD4+ helper T-cells and others, organize cytolytic activities including release of perforin, which forms pores in the membrane of the cell under attack, and granzyme, a serine protease which results in cellular apoptosis.

In order for the adaptive immune system to provide an anti-tumor response, effector CD8+ T-cells must recognize antigens associated with cancer cells as foreign. Diverse work has shown that CD8+ T-cells can recognize various HCC-associated tumor antigens presented by MHC molecules [6]. The expression of Alpha Fetoprotein (AFP), which normally stops after birth, is found in up to 80% of HCC. AFP-specific CD8+ T-cells have been shown to produce detectable, circulating interferon-gamma in the peripheral blood of HCC patients [7]. Glypican-3 (GPC-3) is another fetal oncoprotein found in over half of HCC, but not present on normal liver tissue. T-cell responses was elicited after in-vitro peptide stimulation in about half of HCC patients with an HLA-A2 or HLA-A24 allele [9]. CD8+ T-cells specific for NY-ESO-1, a cancer testis antigen expressed by HCC, have been found in HCC patients, but notably the cells have impaired cytolytic function [8,9]. Responses to several other cancer testis antigens also expressed de novo in HCC and first described in melanoma, such as SSX-2 and MAGE, have been reported with in-vitro stimulation in HCC cell lines [10,11].

An anti-tumor response by the host's adaptive immune system has been thoroughly described in several cancers in both pre-clinical and clinical models [12]. CD8+ T-cells can be activated by cancer epitopes which may then induce apoptosis in tumor cells and lead to tumor regression. In a randomized trial of 150 HCC patients after curative resection, patients receiving autologous T-lymphocytes activated *in-vitro* as compared to no adjuvant therapy experienced a 41% lower risk of overall recurrence (p=0.01) and higher rates of disease specific survival (p=0.04), although no change in overall survival [13]. This trial, published in 2000, provided early evidence for the potential clinical benefit of immunotherapy. In a study of 302 patients with HCC, also after curative resection, patients with the highest ratio of effector CD8+ T-cells to regulatory T-cells in tumor biopsies had longer overall survival (86 months versus 31 months; p<0.0001) and disease-free survival (105 months versus 23 months; p=0.003) as compared patients with the lowest ratio [14]. This work suggests the important anti-tumor role of effector CD8+ T-cells.

Mechanisms of Immune Tolerance in HCC

Despite various cancer epitopes recognized by CD8+ T-cells, a clinically significant immune response to tumor cells, in both HCC and other cancers, has been difficult to elicit by autologous infusion, vaccination and other strategies. Rapidly advancing research has uncovered diverse mechanisms of immune tolerance, which avoid excessive or persistent activation of the immune system that may cause harm to host tissues, and several of these mechanisms are exploited by cancer cells. Two of the most important such mechanisms involve

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the activity of regulatory T-cells, which play a key role in the balance between tolerance and rejection, and inhibitory receptor pathways, such as those associated with cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death receptor 1 (PD-1).

Regulatory T-cells play an immunosuppressive role in various clinical settings. In HCC patients, an increased number of regulatory T-cells within tumor regions predicts worse clinical outcomes and a lower number of tumor infiltrating cytotoxic CD8+ T-cells. In an illustrative cohort of 75 HCC patients not previously treated with anti-tumor therapy, increased numbers of regulatory T-cells were associated with shorter survival (Cox proportional hazard ratio 2.29, $p<0.001$), even when controlling for other prognostic factors [15]. The regulatory T-cells were further found to impair the function of the CD8+ cytotoxic T-cells by limiting their activation and proliferation as well as their release of the cytolytic molecules perforin and granzyme: an effect which could be reversed when the regulatory T cells were depleted.

Clinical Results with CTLA-4 Inhibitors

Two membranes associated inhibitory receptor pathways have been shown to play a role in hindering T-cell function in cancer [16]. CTLA-4 competes against CD28 for binding to the co-stimulatory receptors CD80 and CD86 on antigen presenting cells, thus impairing the initial stages of T-cell activation and proliferation. Ipilimumab, an anti-CTLA-4 monoclonal antibody, has been approved for use in advanced melanoma. In a phase III trial combining the drug with a glycoprotein 100 peptide vaccine in 676 patients with unresectable stage III or IV melanoma who had received prior therapy, the group receiving dual therapy had an overall survival of 10.1 months as compared 6.4 months with the vaccine alone ($p<0.0001$) [17]. Notably, grade 3 or 4 adverse events were seen in 15% of patients, and 14 deaths were attributed to the study drug. Ipilimumab was later shown to substantially improve overall survival in previously untreated melanoma patients when combined with dacarbazine [18].

A similar anti-CTLA-4 monoclonal antibody was recently studied as a single agent in a small non-controlled open-label phase II trial among patients with advanced HCC and chronic HCV infection [19]. Here, 21 patients were treated with intravenous tremelimumab at a dose of 15 mg/kg on day one of every 90 day cycle for up to 4 cycles. Over half of patients had received prior therapy, with one quarter having received sorafenib. Of 17 patients evaluated, three patients exhibited a partial response, none had a complete response, and ten patients showed stable disease. Notably, transient grade 3 or higher abnormalities in transaminases occurred in almost half of patients, although there was no evidence of liver failure. Among diverse anti-CTLA-4 trials, inflammatory or autoimmune side effects were evident in up to 30% of patients, and this has been attributed to the important role this pathway plays in the initial events of T-cell activation [20]. Experience with CTLA-4 has nonetheless suggested that immunotherapy could provide durable tumor control in HCC.

Pre-Clinical Evidence for the PD-1 Pathway in HCC and Hepatitis Related HCC

PD-1 is a T-cell coinhibitory receptor with two known ligands, PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273). PD-1 is a type I transmembrane protein with an intracellular tyrosine-based inhibitory motif. The receptor was first described in 1992 in association with T-cell apoptosis, and in 1999 a distinctive autoimmune phenotype was noted in PD-1 knockout mice manifest by autoimmune glomerulonephritis and a lupus-like proliferative arthritis, suggesting its role in inhibition of the adaptive immune response [21]. Ligand binding of PD-L1 to PD-1 subsequent to T-cell activation leads to numerous immunosuppressive effects, including inhibition of both the cell survival factor Bcl-xL and various transcription factors associated with effector T-cell function [22]. PD-L1 is found on resting T and B lymphocytes, dendritic cells and macrophages as well as tumor cells, whereas PD-L2, whose role remains less clear, has been found on dendritic cells and macrophages. Importantly, the interaction of PD-1 with its ligands occurs mostly peripherally, in the tumor microenvironment, in contrast to CTLA-4, whose interactions often occur in lymph nodes at the time of T-cell activation.

In the HCC tumor microenvironment, PD-L1 is primarily expressed by Kupffer cells, with low level expression on other antigen presenting cells and tumor cells [23]. Expression of PD-L1 by Kupffer cells appears to be driven, at least in part, by Interleukin (IL)-10 productions in HCC. IL-10 has diverse functions in the immune response but has been identified as an important cytokine for tempering the immune response [24].

The proportion of CD8+ T-cells that express PD-1 is much higher in both tumor regions and peripheral blood as compared to healthy controls and controls with cirrhosis [25]. The effector PD-1+CD8+ T-cells found in tumor regions are less proliferative as compared control CD8+ T-cells, as measured by the proliferative marker Ki67, and also express less perforin and granzyme B, and this impaired effector function can be reversed in vitro with blockade of the PD-1 pathway.

In addition to Kupffer cells, PD-L1 is also expressed in low levels on HCC cells, where its upregulation appears to be driven, at least in part, by interferon-gamma secretion from CD8+ T-cells. The PD-L1+ HCC cells are then associated with T-cell apoptosis which will in turn protect the HCC cells from attack by the immune system, an effect which can be reversed in-vitro with a monoclonal antibody against PD-L1. Interestingly, in immunohistochemical analysis, a pattern of PD-L1 expression in aggressive tumor regions with distribution of CD8+ T-cells around the tumor periphery – and relatively few infiltrated CD8+ T-cells - was often seen.

In a study looking at PD-1 expression in 54 patients with HCC and HBV coinfection who underwent surgical resection, higher versus lower expression of intrahepatic PD-1 on T cells was asssociated with lower disease free survival (13.6 months versus 28.7 months; $p<0.001$). A similar relationship between disease free survival and level of PD-1 expression on peripheral circulating T-cells was also found. Moreover, the frequency of circulating PD1+CD8+ T-cells was positively correlated with stage of disease. Similarly, higher expression of PD-L1 in the tumor microenvironment is also associated with worse prognosis [26].

The high rate of HCC recurrence after surgical resection is often associated with chronic viral infection and a persistent inflammatory state which promotes de novo tumorigenesis. Interestingly, the PD-1/PD-L1 pathway has recently emerged as an important contributor to viral persistence in chronic HBV and HCV infection. Several studies have found that most HBV-specific circulating CD8+ T-cells in chronic HBV infection are PD-1+, with this expression likely due to chronic antigenic stimulation, as T-cells specific for Cytomegalovirus (CMV) and influenza in these same patients are much less likely to express PD-1 [27].

The upregulation of PD-1 in a state of chronic antigenic stimulation

may thus represent a mechanism of immune tolerance. Blockade of PD-1/PD-L1 in-vitro increases the peripheral HBV-specific CD8+ T-cell population and is associated with higher interleukin (IL)-2 and interferon gamma production by these cells, which suggests more potent effector activity. Similar findings have been reported in HCV infection. In HCV patients, peripheral CD8+ T-cells maintained high levels of PD-1 expression and were functionally impaired, with restoration of their effector function after blocking the PD-1/PD-L1 pathway [28]. Using CMV-specific CD8+ T-cells as controls, peripheral HCV-specific CD8+ T-cells show impaired synthesis of interferon-gamma and Tumor Necrosis Factor (TNF) alpha, impaired de granulation, and lower stores of granzyme and perforin [29]. Moreover, the impaired PD-1+CD8+ T-cells are enriched in the liver as compared to the peripheral blood, suggesting that the site of impairment may be tied to the site of persistent viral replication, the liver [30].

Emerging Clinical Promise for PD-1 in Cancer

The therapeutic promise of the PD-1 pathway has already translated into clinical progress for some cancers, with evidence strongest for melanoma, non-small cell lung cancer, and renal cell carcinoma. Monoclonal antibodies against both PD-L1 and PD-1 have been developed.

BMS-936559 (Bristol Myers Squibb, Princeton, NJ), an anti-PD-L1 monoclonal antibody, was administered in a phase I trial to patients with diverse cancers, including melanoma, renal cell, ovarian, gastric, pancreatic, breast and non-small cell lung carcinomas [31]. Patients in this study had progression after at least one prior course of therapy for their advanced or metastatic disease and were treated every 14 days for up to 16 cycles. A maximum tolerated dose was not reached. Treatment related grade 3 or 4 adverse events were noted in 9% of patients, and infusion related reactions were noted in 10%, almost all of which were of low severity. An objective response was seen in 9 of 52 melanoma patients (including 3 complete responses), 5 of 49 non-small cell lung cancer (NSCLC) patients, 1 of 17 ovarian cancer patients and 2 of 17 renal cell carcinoma patients. Stable disease was seen in 14 of 52 melanoma patients, 6 of 49 NSCLC patients, 3 of 17 ovarian patients, and 7 of 17 renal cell patients.

Nivolumab (BMS-936558, Bristol Myers Squibb, and Princeton, NJ) is a human IgG4 blocking monoclonal antibody against PD-1. A phase I dose-escalation study assessed the safety and efficacy of the drug administered to heavily pretreated patients with advanced melanoma, non-small lung cancer, renal-cell cancer, prostate cancer and colorectal cancer. It was given as an infusion every 2 weeks for 12 cycles [35]. No maximum tolerated dose was reached. Across dosage ranges, objective responses were seen in 26/94 melanoma patients, 14/76 NSCLC patients with both squamous and non-squamous subtypes, and 9/33 RCC patients. Stable disease was observed in an additional 7%, 6% and 27% of patients, respectively. 61 pre-treatment tumor specimens were analyzed; notably, none of the 17 patients with PD-L1 negative tumors had an objective response, as compared 9 of the 25 patients who did express PD-L1. Drug-related serious adverse events occurred in 11% of patients, including characteristic autoimmune phenomena such as pneumonitis, colitis and thyroiditis. Drug-related pneumonitis was noted in nine patients (3%) with three patients dying due to that complication.

Promising results have been recently reported for an expansion cohort of 34 renal cell carcinoma patients, where an objective response rate of 30%, one-year overall survival rate of 72% and three-year overall survival rate of 52% were seen for nivolumab [32]. Additionally, in a 52 patient NSCLC expansion cohort, an objective response rate of 22% was seen [33]. In 135 advanced melanoma patients treated with an anti-PD-1 antibody, a confirmed objective response rate of 38% was observed, with the rate reaching 52% in the highest dose category, along with the majority of responses durable [34].

Future Direction of PD-1 Immunotherapy in HCC

Given mounting evidence for the PD-1/PD-L1 pathway in the balance between anti-tumor activity and immune tolerance in HCC, as well as encouraging activity across a spectrum of immunogenic tumors, most notably melanoma and renal cell cancers, a phase I study of nivolumab (Bristol Myers Squibb) is presently recruiting patients with advanced HCC with disease progression despite at least one line of therapy. The patient population will be stratified according to viral infection status with HBV or HCV. This eagerly anticipated trial may herald a long awaited new era in treatment options for HCC [35].

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