Cancer Immunoprevention: Challenges and Potential Opportunities for Use of Immune Checkpoint Inhibitors

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ABSTRACT

Cancer immunoprevention is achieved through promoting antitumor immune surveillance to block tumor formation and progression. Following the success of prophylactic vaccines against human papillomavirus (HPV) in preventing HPV-associated cancer, immunopreventive cancer vaccines targeting tumor antigens have been increasingly evaluated against cancers of noninfectious origin. While advances in cancer immunotherapy with immune checkpoint inhibitors (ICI) have clearly shown that the host immune system can mount effective antitumor immunity against tumor antigens when immune checkpoints are optimally blocked, the use of ICIs in the prevention setting has not been widely explored because of concerns of ICI-associated adverse events. In this issue of *Cancer Prevention Research*, Chung and colleagues demonstrate that the human cirrhotic liver harbors neoantigens, which accumulate further as the disease progresses to hepatocellular carcinoma (HCC), suggesting that cirrhotic liver may be susceptible to ICI therapy. Utilizing an established mouse model of carcinogen-induced liver fibrosis and HCC, they show that intermittent intervention by ICI, anti-mouse PD-1 (CD279) antibody, can prevent the progression of the precancerous stage of cirrhosis to HCC accompanied by increased T-cell infiltrates in the liver parenchyma. Importantly, there were no overt ICI-associated toxicities in the treated mice, indicating that safe dosing regimens could be established. This work is both significant and timely, opening the door to future studies, where the utility of ICI therapy can be further investigated not only in cirrhosis but other high-risk precancerous conditions. In this perspective, we discuss the implications of their findings, and the challenges and potential opportunities for use of ICIs for cancer immunoprevention.

See related article by Chung et al., p. 911

Chronic inflammation and tissue injuries can lead to persistent exposure to antigens, resulting in progressive loss of immune effector cell functions, the state often referred to as immune exhaustion. In a physiologic setting, "self-tolerance" is an important mechanism to prevent autoimmunity and is mediated by immune checkpoint pathways. Immune exhaustion has been observed not only in chronic infections (viral or otherwise), but also in cancer. It is widely accepted that tumor growth is facilitated by immune escape through upregulation of immune inhibitory checkpoint molecules and proinflammatory cytokines in tumor microenvironment (TME). Enhanced antitumor immunity was first demonstrated by inhibition of immune checkpoint molecules, cytotoxic T-lymphocyte antigen 4 (1) and programmed cell death 1 (PD-1; ref. 2). Unlike cancer vaccination strategies that are intended to elicit adaptive antitumor immunity, immune checkpoint inhibitors (ICI) unleash the host's antitumor immune responses by putting the brakes on the immune checkpoint system.

Antitumor immunity directed against neoantigens expressed only by transformed, abnormal, or tumor cells can minimize immune tolerance and the risk of autoimmunity. While ICIs have demonstrated considerable promise for the treatment of melanoma, non–small cell lung cancer, and other cancers, ICI therapies have also been associated with serious autoimmune adverse events, as unleashing the host immune system can result in immune recognition of not only tumor neoantigens, but also self-antigens. Ongoing concern regarding toxicities associated with ICI utilization for therapy has led to specific funding opportunities under the NCI Moonshot program [Advancing Cancer Immunotherapy by Mitigating Immune-Related Adverse Events (irAE), RFA-CA-19-044]. Increased understanding of these potentially serious autoimmune toxicities from ICI therapy can lead to better management and reduction of irAEs, and encourage its exploratory use in the prevention setting that targets high-risk but relatively healthy individuals. Apart from irAEs, potential challenges of ICI use include accumulating evidence that ICI immunotherapy exerts efficacy only in a select group of cancer types and in a subset of patients. Reasons for variability in ICIs' ability to stimulate or

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enhance antitumor immunity to fight the cancer have yet to be fully elucidated, but may include tumor heterogeneity, the extent of immune compromise, the activation level of ICI-targeted immune checkpoint molecules, and/or the stages of cancer when they are administered. Nevertheless, ICI intervention may be effectively deployed in the context of intercepting early precancerous lesions that tend to have a lower degree of precursor cell heterogeneity and more intact immune system, if safe regimens are established.

Worldwide, liver cancer is one of the most commonly diagnosed cancer and is the fourth leading cause of cancer-related deaths (3). Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer representing approximately 90% of all liver cancer cases. Surgery and transplantation are reserved for candidates with limited tumor burden, while systemic therapies for advanced HCC extend life only by a few months. Prevention is, therefore, an urgent unmet need and an important priority to reverse the current trend of increasing HCC incidence and mortality worldwide. Nonalcoholic steatohepatitis (NASH), viral hepatitis, heavy alcohol consumption, and autoimmune hepatitis are common etiologies of cirrhosis and HCC. Particularly, NASH and cirrhosis represent a premalignant state, and the decades-long latency before progression to HCC offers a window of opportunity for intervention.

Encouraging evidence that HCC responds to immunotherapy has been demonstrated in the recent IMBRAVE150 clinical trial, where combination of atezolizumab with bevacizumab led to better overall and progression-free survival in patients with unresectable HCC (4). Previous studies with single-agent ICI therapy failed to demonstrate a survival benefit in patients with HCC. While understanding the mechanistic basis of the combination regimen's efficacy, as well as patients with HCC who would likely respond to the combination treatment is of utmost importance (4), whether ICIs can intercept and stop progression of precancerous cirrhotic lesions to HCC is also an important next step to address. Chronic inflammation, early immunologic changes in precancerous TME, and accumulation of neoantigen burden most likely suggest upregulation of immune checkpoint pathways, which provides an opportunity for immunoprevention strategies (5, 6). In this study, Chung and colleagues (7) performed systematic studies by first establishing the evidence for the presence and accumulation of neoantigens during cirrhosis compared with noncirrhotic livers. Their data show that neoantigen burden progressively increases with worsening liver fibrosis and subsequent progression from cirrhosis to clinically detectable HCC. On the basis of the previous studies that demonstrated a correlation between tumor mutational burden and sensitivity to ICI therapy, the accumulation of neoantigens during cirrhosis strongly suggests that immune interception strategies using ICIs may block the tumor formation and progression to invasive stages. The authors then examined the efficacy of ICI therapy in a well-characterized model of carcinogen-induced liver fibrosis and inflammation-associated HCC in the immunocompetent C3h/HeJ mouse strain. Intraperitoneal injection of a potently mutagenic carcinogen, diethylnitrosamine, followed by repeated administration of a profibrogenic agent, carbon tetrachloride (CCL_4), in the mice has strong hepatic mutagenic effects with enhanced tumorigenesis and fibrosis that recapitulate the chronic liver damage and fibrosis associated with HCC in humans. The authors observed that once-a-week intraperitoneal injections of anti-PD-1 mAb given every other week during the premalignant stage prevented up to 46% of HCC with no noticeable systemic adverse effects as compared with the IgG control antibody–treated mice. As expected from previous studies with ICI therapies, the authors observed an increased infiltration of both $CD4^+$ T cells and $CD8^+$ T cells into the liver parenchyma correlating well with tumor prevention response, although ICI therapy did not show uniform efficacy. Finally, when the tumors from untreated and treated mice were examined by PCR for known point mutations in the *Hras, Egfr*, and *Braf* genes, there were no differences in the proportion of tumors with mutations in any of these oncogenes, suggesting that the preventive response to ICI therapy was not dependent on the presence of specific recurrent oncogenic mutations.

While the current study illustrates the pivotal first step in the road map toward the development of ICI therapy in the prevention of HCC from the cirrhotic liver, there are key questions yet to be answered. It is unknown whether mutational burden during precancerous stages, such as cirrhosis, could be predictive of sensitivity to ICI therapy. In addition to the point mutation analysis in the three oncogenes, *Hras, Egfr*, and *Braf*, hepatic mutational burden should have been evaluated before and after progression from cirrhosis to HCC and compared between ICI-treated versus control mice in the current study. Such analysis would have provided further insight into the utility of mutational burden as one of predictive markers for ICI sensitivity in the cirrhosis and might have validated the current diethylnitrosamine + CCL_4 mouse model as a valuable preclinical model for future studies, including potential co-clinical trials of ICI therapy for HCC immunoprevention. Furthermore, the authors did not provide the expression level of PD-1 and PD-L1 in the mouse liver tissues in the current study. Because the predictive value of immune checkpoint signaling pathway status for the efficacy of ICI therapy has yet to be established for HCC, it would have been a valuable piece of information. Finally, the authors analyzed the extent of $CD3^+/CD4^+$ T-cell and $CD3^+/CD8^+$ T-cell infiltrates in the ICI-treated versus control mouse liver tissues without further delineating their subsets. More detailed analysis on the immune consequences of ICI therapy in the liver microenvironment would have provided further mechanistic insight on ICI effects including the possible impact on immunosuppressive regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages, as well as other immune checkpoint molecules in the precancerous and TME. Nevertheless, there are important lessons that can be learned from the current study and it should inspire future studies on

ICI-mediated interception not only of cirrhosis, but also other high-risk precancerous conditions. As an increasing number of studies are now including immune profiling of premalignant lesions (8), more detailed ICI target profiles for precancer immune interception are expected to emerge in the near future.

Known determinants of susceptibility to ICI therapies include the expression level of PD-1/PD-L1 and hypermutation often associated with mismatch repair deficiency. Roles of other immune checkpoint molecules (e.g., LAG3), the immune cell contexture in the TME, and interplay of tumor drivers and immune checkpoint pathways are currently under investigation. When developing ICIs for immunoprevention of HCC or other cancers, the following challenges and questions will have to be addressed: (i) candidate high-risk cohorts that may benefit from ICI therapy for immunoprevention must be carefully selected on the basis of the expected risk–benefit (prevention efficacy) ratio, as well as availability of reliable clinical, biochemical, and immune biomarkers for monitoring during and after ICI therapy; (ii) ICI regimens should be specifically designed for immunoprevention purposes including optimization of timing of ICI therapy initiation (i.e., at what precancerous stages), dosing, scheduling, and treatment duration, as well as possible combination regimens with other prevention agents, as has been demonstrated with combination chemoprevention approaches (9); (iii) availability of optimal preclinical models for ICI immunoprevention therapies should be considered, as it affects translatability of preclinical study data, which should always be carefully scrutinized, not only from the point of prevention efficacy and ICI regimen optimization, but also from the point of ICI-related short-term and long-term immune-related toxicities. In addition, the use of mAbs targeting PD-L1 and other immune checkpoint receptor ligands, as well as chemical compounds known to have immune checkpoint inhibitory activities may be of interest to explore.

Chung and colleagues' latest article opens the door to reveal the challenges and important questions, while presenting the feasibility and a glimpse of success with intermittent use of ICI for HCC immunoprevention. This work is both significant and timely, and invites further studies to confirm these findings and examine the utility of ICI therapy not only for HCC prevention in patients with cirrhosis, but those with other high-risk precancerous conditions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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The opinions expressed by the authors are their own and this article should not be interpreted as representing the official viewpoint of the U.S. Department of Health and Human Services, the NIH, or the NCI.

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