COMMENTARY

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Immune play: defending the liver

Hepatic Oncology

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The notable emergence of immunotherapy as an effective and durable anti-tumor treatment strategy is driving intense clinical research into new agents. The recent approval of the first anti-programmed death (PD)-1 antibody, pembrolizumab, by the US FDA in August 2014 for metastatic melanoma, has highlighted the increasingly dominant role that cancer immunotherapy agents play in the oncology therapeutic arsenal. Unpredictably, tumors previously not considered immunogenic have noted encouraging therapeutic responses following treatment with these agents, with a side effect profile which is manageable and less toxic than conventional chemotherapy.

The unique anatomy and function of the liver leads it to be a singular specialty organ in immune surveillance and immune tolerance. The blood supply of the liver, both arterial and venous, is notable for filtering nutrients and gut pathogen-derived molecules, making this organ central to eliminating pathogens while maintaining tolerance to non-pathogenic antigens as well as metabolizing xenobiotics. Its immune function is also remarkable, given the

well documented early transplant experiments: organ transplantation required significant immunosuppressive therapy however allogeneic liver transplants were well tolerated overall. Though this phenomenon could be explained by a lack of reactive antigens in the liver, this is less likely given that subsequent organ transplantations were typically tolerated [1]. These findings would suggest that the microenvironment within the liver sustains a tolerant immune response, which has significant implications for liver infection and cancer.

The core tenet of cancer immunotherapy has been the realization that specific T cells can recognize human tumor-derived and tumor-specific antigens [2,3]. Immunogenic modulation of hepatocellular carcinoma (HCC) has been proposed, given the noted CD8⁺ specific T cells in response to antigens such as AFP, cancer testes antigen (NY-ESO-1) and glypican-3 (GPC-3), [4] and the correlation between the ratio of HCC-infiltrating CD8⁺ cells and progression-free survival [5]. Despite this, these immune responses have not been shown to have an impact on the growth of HCC.

KEYWORDS

• abscopal effect
• checkpoint inhibitor
• hepatocellular carcinoma
• immunotherapy • tumor cell death

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The extent of T-lymphocyte amplification in response to a specific tumor antigen is balanced by co-stimulatory and inhibitory immune checkpoint signals normally pivotal for self tolerance, but increasingly recognized as a mechanism of tumoral immune evasion if subverted by malignant cells [6]. For the liver, it has been proposed that the antigenic presentation by different types of liver cells induces incomplete activation of CD8⁺ T cells, with additional immunosuppressive signaling coming from Kupffer cells, myeloid-derived suppressor cells and liver dendritic cells expressing multiple inhibitory and co-inhibitory ligands and cytokines [7,8]. Preclinical data would suggest that liver-derived dendritic cells are capable of antigen presentation but require additional signaling to be truly immunostimulatory as they are competent in expressing indoleamine 2,3-dioxygenase (IDO) and programmed death ligand-1 (PD-L1) in response to IFN- γ [7–10]. Arguably, this type of suppressive environment is valuable in the setting of hepatitis infections where an intense intrahepatic cytotoxic immune response could prove fatal due to extensive liver destruction and a controlled inflammatory response can allow the liver to recover albeit with incomplete pathogen elimination.

Various immune-based treatment approaches have been explored, though in a limited fashion, in HCC. Oncolytic immunotherapies are conceptually designed to induce viral replication-dependent tumor cell lysis as well as inducing anti-tumor-specific immune responses. Intrahepatic injection of vaccinia JX-594 was performed in a randomized Phase II dose-finding trial, with a significant improvement in overall survival associated with the higher dose of vaccinia [11]. Further investigation into this approach continues.

Upregulation of immune checkpoints, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), and the programmed death-1 (PD-1)/PD-L1 axis, inhibits cytotoxic T cell and NK activation [4]. Following T-cell activation through the T-cell receptor, CTLA4 locates to the plasma membrane and interrupts the binding of CD28 to CD80 and 86, blocking T-cell function. There is limited preclinical and animal model data for either CTLA4 or PD-1/PD-L1 targeting inhibitors in HCC. Though CTLA4 blockade with ipilimumab, particularly in melanoma, has been very successful, there are no published trials of this agent in HCC.

Another CTLA4 blocking agent, tremelimumab, has been specifically studied in HCC patients in a recently reported Phase I study of 21 hepatitis C positive patients with good liver function (Child–Pugh A or B) and advanced disease not suitable for local therapy [12]. Tremelimumab was administered every 90 days at a dose of 15 mg/kg until progression or toxicity was recorded. Grade 3 serum transaminitis was noted in 45% of patients but this rise was transient and did not occur beyond the first dose of therapy. Partial responses were recorded in 17.5% of patients, with another 45% reporting stable disease for more than 6 months. Median overall survival was 8.2 months (95% CI: 4.64–21.34) with median time to progression of 6.48 months (95% CI: 3.95–9.14). Separately, a number of monoclonal antibodies targeting the PD-1/PD-L1 axis are also in development. The binding interaction between PD-1 and PD-L1 conveys a negative stimulatory cascade within the T cell that reduces proliferation and ultimately induces T-cell anergy or apoptosis [13]. Specifically for HCC, there are no published trials to date with these agents but there is currently an ongoing Phase I trial of nivolumab (an anti-PD-1 monoclonal blocking antibody) that will enroll three patient cohorts based on the viral etiology of their disease: hepatitis C, hepatitis B or no viral infection (NCT01658878).

There is growing evidence that checkpoint inhibition is only effective in the case where there is an underlying, endogenous anti-tumor response [14]. Conventional cytotoxic therapies such as chemotherapy and radiotherapy have been proposed to cause immunogenic cell death: post treatment, the dying tumor cell is taken up by antigen presenting cells, effectively stimulating antigen-specific T-cell responses by acting as a self ‘vaccine’ [15]. Immunogenic cell death has also been postulated to be the underlying mechanism of the abscopal effect, where radiation to a certain metastatic site causes regression of distal lesions. This phenomenon has been described in HCC following radiation to both intra-hepatic and extra-hepatic lesions [16–18], but retains relevance also due to the use of local ablative therapies, including radiofrequency ablation and transarterial chemoembolization which are widely used for the management of disease that is not amenable to curative intent interventions such as surgery [19]. These therapies, in causing tumor destruction by various mechanisms, permit the release of tumor

antigens and the theoretical ‘pick up’ of these by antigen-presenting cells that can trigger an immune response, which could prevent local tumor recurrences as well as distant metastasis.

Other than sorafenib, cytotoxic therapies have not been shown to have an impact in overall survival in HCC to date. A central question however, is how relevant are immune-based strategies in HCC? The significant successes of ipilimumab in melanoma and sipeuleucel-T in prostate cancer heralded the arrival of a new class of treatment agent and fueled the interest to explore these in other solid tumors. Immunotherapeutic treatment strategies in HCC remain experimental at this time; however, the immunogenicity of this disease is certainly a consideration given the demonstrated spontaneous T-cell responses to liver-specific antigens [4] as well as the frequency of spontaneous regressions [20]. A third consideration in justifying the exploration of immune-based therapies for this disease is the fact that many HCC patients have advanced cirrhosis, adding tremendously to the challenge of treating patients with cytotoxic or targeted therapy. Given also that HCC typically develops in the setting of chronic inflammation, as patients typically develop cirrhosis prior to cancer, it argues for a significant role of the

microenvironment and the immune responses to the pathogenic injury.

The rationale for combining immune-based approaches is a strategy our team continues to explore for HCC. A current trial looking at the efficacy of ablation together with tremelimumab (NCT01853618) will evaluate the safety and feasibility of this combination in HCC. Given the unique circumstances in which HCC develops, it is a challenge to design safe immune trials that take into account the impaired liver function patients with HCC typically present with and the mechanistic considerations underlying potential combinations using targeted agents such as sorafenib. This is an ongoing field of investigation, which we hope will lead to effective outcomes for our patients.

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References

- Calne RY, Sells RA, Pena JR *et al.* Induction of immunological tolerance by porcine liver allografts. *Nature* 223(5205), 472–476 (1969).
- Boon T, Cerottini JC, Van Den Eynde B, Van Der Bruggen P, Van Pel A. Tumor antigens recognized by T lymphocytes. *Ann. Rev. Immunol.* 12, 337–365 (1994).
- Vanky F, Klein E. Specificity of auto-tumor cytotoxicity exerted by fresh, activated and propagated human T lymphocytes. *Int. J. Cancer* 29(5), 547–553 (1982).
- Hato T, Goyal L, Greten TF, Duda DG, Zhu AX. Immune checkpoint blockade in hepatocellular carcinoma: current progress and future directions. *Hepatology* 60(5), 1776–1782 (2014).
- Flecken T, Schmidt N, Hild S *et al.* Immunodominance and functional alterations of tumor-associated antigen-specific CD8+ T-cell responses in hepatocellular carcinoma. *Hepatology* 59(4), 1415–1426 (2014).
- Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12(4), 252–264 (2012).
- Crispe IN. Immune tolerance in liver disease. *Hepatology* doi:10.1002/hep.27254 (2014) (Epub ahead of print).
- Kingham TP, Chaudhry UI, Plitas G, Katz SC, Raab J, Dematteo RP. Murine liver plasmacytoid dendritic cells become potent immunostimulatory cells after Flt-3 ligand expansion. *Hepatology* 45(2), 445–454 (2007).
- Sun X, Gong ZJ, Wang ZW *et al.* IDO-competent-DCs induced by IFN-gamma attenuate acute rejection in rat liver transplantation. *J. Clin. Immunol.* 32(4), 837–847 (2012).
- Jurgens B, Hainz U, Fuchs D, Felzmann T, Heitger A. Interferon-gamma-triggered indoleamine 2,3-dioxygenase competence in human monocyte-derived dendritic cells induces regulatory activity in allogeneic T cells. *Blood* 114(15), 3235–3243 (2009).
- Heo J, Reid T, Ruo L *et al.* Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nat. Med.* 19(3), 329–336 (2013).
- Sangro B, Gomez-Martin C, De La Mata M *et al.* A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J. Hepatol.* 59(1), 81–88 (2013).
- Topalian SL, Weiner GJ, Pardoll DM. Cancer immunotherapy comes of age. *J. Clin. Oncol.* 29(36), 4828–4836 (2011).
- Gajewski TF, Woo SR, Zha Y *et al.* Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. *Curr. Opin. Immunol.* 25(2), 268–276 (2013).
- Greten TF, Duffy AG, Korangy F. Hepatocellular carcinoma from an immunologic perspective. *Clin. Cancer Res.* 19(24), 6678–6685 (2013).
- Okuma K, Yamashita H, Niibe Y, Hayakawa K, Nakagawa K. Abscopal effect of radiation on lung metastases of hepatocellular carcinoma: a case report. *J. Med. Case Rep.* 5, 111 (2011).

- 17 Nakanishi M, Chuma M, Hige S, Asaka M. Abscopal effect on hepatocellular carcinoma. *Am. J. Gastroenterol.* 103(5), 1320–1321 (2008).
- 18 Ohba K, Omagari K, Nakamura T *et al.* Abscopal regression of hepatocellular carcinoma after radiotherapy for bone metastasis. *Gut* 43(4), 575–577 (1998).
- 19 Greten TF, Korangy F. Radiofrequency ablation for the treatment of HCC--maybe much more than simple tumor destruction? *J. Hepatol.* 53(4), 775–776 (2010).
- 20 Epstein RJ, Leung TW. Reversing hepatocellular carcinoma progression by using networked biological therapies. *Clin. Cancer Res.* 13(1), 11–17 (2007).