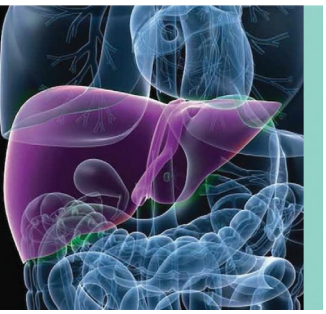

Immunotherapy for Hepatocellular Carcinoma: Is Latin America Ready for Primetime?

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Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related death worldwide, and its mortality is increasing steadily.[1] HCC incidence has grown in the last twenty years driven at least in part by the epidemic of nonalcoholic fatty liver disease (NAFLD).[2] Unfortunately, overall survival rate for patients with HCC at 5 years is near 18%, which reflects how complex and resistant to systemic therapies this devastating disease is.[2] Although new systemic drugs have been approved for advanced HCC in the first and second line, prolonging overall survival beyond a year is still difficult.[1]

Under physiological conditions, the liver has an important immune-regulatory role and has to constantly fight against the influx of diverse pathogenic and nonpathogenic antigenic stimuli derived from the gut. Experimental and clinical evidence support a steady-state role of the liver in promoting antigen tolerance.[3] However, during chronic hepatic inflammation and cirrhosis, the normal immune equilibrium of the liver is disrupted.[4] Chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, toxic liver damage induced by alcohol abuse, fat accumulation (in nonalcoholic steatohepatitis [NASH]), or liver damage caused by metabolic diseases

Abbreviations: APC, antigen-presenting cell; CTLA-4, cytotoxic T lymphocyte antigen 4; ER, endoplasmic reticulum; FDA, U.S. Food and Drug Administration; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; LRT, locoregional therapy; mAb, monoclonal antibody; MHC, major histocompatibility complex; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PD-1, programmed cell death receptor 1; PD-L1, PD-1 ligand; RECIST, response, evaluation criteria in solid tumors; TACE, transarterial chemoembolization; TCR, T cell receptor; TGF-β, transforming growth factor β.

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up-regulates proinflammatory signals and breaks the tolerance state.[5] As a result of necroinflammation, nonparenchymal hepatic cells are activated, and hepatocytes proliferation is altered due to DNA damage, endoplasmic reticulum (ER) stress, epigenetic modifications, chromosomal aberrations, and senescence alteration, which altogether generate chronic cell death, regeneration stimulation, and fibrosis. The linkage between the repeated cycles of necroinflammation and aberrant regeneration that takes place during chronic liver disease and tumor development has been clearly demonstrated.[5] At this stage, the immune system fails to eliminate transformed cells; therefore, the generation of therapeutic immunity against cancer in the liver is challenging.

Sia et al.[6] have recently developed an immune-based classification of HCC that might help to design immunotherapy strategies according to the tumor microenvironment immune status. This molecular classification allows the identification of an immune HCC class that could potentially respond to immunotherapy, and also defines an immune intermediate HCC class and immune excluded HCC class that could likely be resistant to standard immunotherapy and, therefore, will need innovative strategies to allow the eradication of the tumor by the immune system effectors[6] (Fig. 1).

The immune system is tightly regulated by a highly complex equilibrium of signals transmitted by stimulatory and inhibitory receptors.[3] Immunostimulatory monoclonal antibodies (mAbs) can be defined as agents with the ability to enhance ongoing immune responses.[7] In recent years, the use of immunostimulatory mAbs has revolutionized the treatment of cancer in such a way that a number of resistant metastatic cancers (e.g., melanoma, renal cell carcinoma, or lung cancer) now have the possibility of being successfully controlled with long-lasting clinical responses. The checkpoint inhibitors against programmed cell death receptor 1 (PD-1), PD-1 ligand (PD-L1), and/or cytotoxic T lymphocyte antigen 4 (CTLA-4) are at the forefront of this revolution (Fig. 2).

Why is it possible to think immunostimulatory mAbs would have a role in the present therapeutic

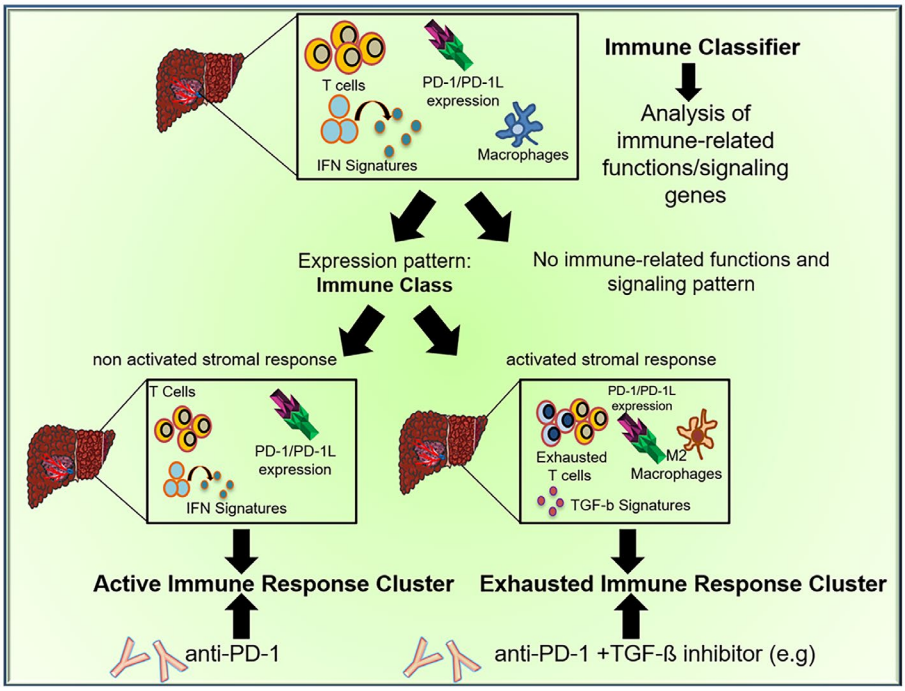

FIG 1 Immune-specific class of HCC: the molecular profile of immune-infiltrating tumor cells could determine whether immunotherapy might be effective in patients with advanced HCC. Patients with a significant presence of immune cells, signatures of IFN, and PD-1/PD-L1 signaling fit into the immune class. Patients within the immune class could have an inactivated or activated stroma. Patients with nonactivated stroma show a significant enhancement of T cells, IFN signature, and overexpression of adaptive immune response genes (activated immune response cluster), whereas patients with an activated stroma show a T cell exhausted signature with overexpression of TGF-ß signaling and the up-regulation of immunosuppressive factors (exhausted immune response cluster) without significant variation on immune cells infiltration and PD-1/PD-1L expression between clusters. These immune signatures could predict the response to immunotherapy with pembrolizumab/nivolumab or help to identify a subtype of HCC that could benefit from combinations.

armamentarium for HCC? Several reasons are in favor of this hypothesis: (1) in general, HCC can be considered an inflammatory and immunogenic tumor; (2) specific cytotoxic T cell responses have been reported in patients with HCC; (3) the density of immune cells, such as $CD8^+$ T cells, within the tumor microenvironment is correlated with a better prognosis in patients with HCC[3]; and (4) even microenvironments with exhausted immune cells could respond to immunotherapy.[6] After the arrival of checkpoint inhibitors to the clinic, the general thought was that the opportunity to promote immunity against HCC had arrived.

Tremelimumab (anti-CTLA-4) was first studied in 20 patients with advanced HCC and chronic hepatitis C, and showed a promising response rate and partial response according to Response, Evaluation Criteria in Solid Tumors (RECIST).[8] Later on, a phase I/II study using nivolumab, a fully human mAb targeting PD-1, showed that 40% of treated patients had either stable disease or showed partial response.[9] This promissory result allowed, in 2017, the approval by the U.S. Food and Drug Administration (FDA) of nivolumab in the second line for sorafenib-experienced HCC. Unfortunately, the phase III clinical trial (CheckMate 459) that compared nivolumab versus sorafenib as first-line

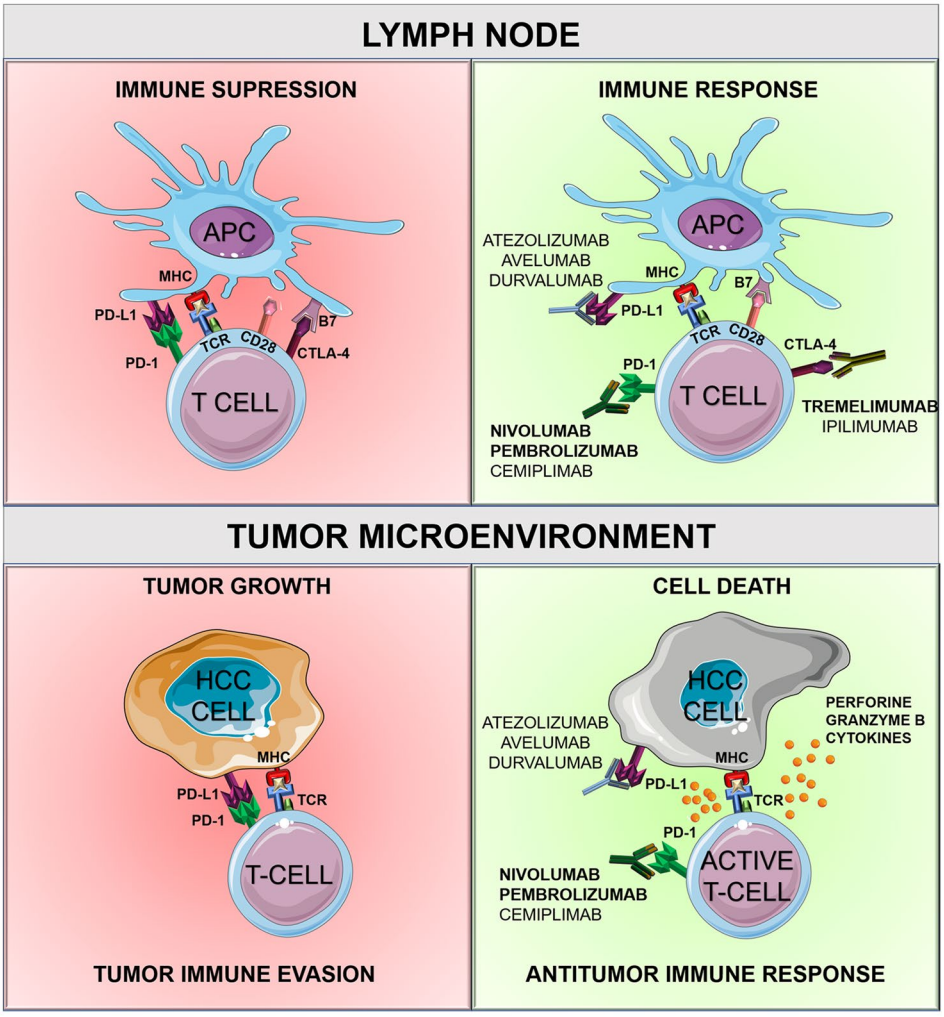

FIG 2 Immunotherapy with checkpoints inhibitors: tumor antigen is captured by an APC and delivered to naive T cells in the lymph node. T lymphocytes require two signals for activation and acquisition of effector capabilities. The first signal corresponds to the interaction of the TCR with its antigen coupled to an MHC molecule. The second signal involves costimulation proteins such as CD28 that recognize B7 in APC. PD-1 and CTLA-4 are negative regulators of the activity of T lymphocytes. PD-L1 expressed on the periphery tumor site also inhibits activated T cells. Immunostimulatory mAbs include the immune checkpoint inhibitors, which prevent T cell inhibition and promote their activation. Ipilimumab, anti-CTLA-4, was the first immune checkpoint inhibitor approved. Then tremelimumab, which also targets CTLA-4, and other antibodies targeting PD-1 (nivolumab, pembrolizumab, cemiplimab) or PD-L1 (durvalumab, atezolizumab, and avelumab) were clinically studied.

therapy did not meet its primary endpoint of improved overall survival.[10] Similarly, pembrolizumab (anti-PD-1) was studied in a phase II open-label trial (KEYNOTE-224); a total of 104 patients were enrolled, of whom 17% showed partial response and 62% disease control.[11] As a result of this trial, in 2018, the FDA granted accelerated approval to pembrolizumab for patients with HCC who have been previously treated with sorafenib. However, recently, a phase III trial of pembrolizumab (KEYNOTE-240) did not meet its coprimary endpoints of overall survival compared with placebo in patients with advanced HCC previously treated with sorafenib.[12]

The effectiveness of the immunotherapeutic approaches based on immunostimulatory mAbs is deeply hampered by the tumor's hostile repertoire that suppresses the effector immune response. Despite the first trials being very promising, the subsequent confirmations with several immune checkpoint inhibitor antibodies were not successful as expected as a monotherapy. The role of immunostimulatory mAbs either in combination with one another or in combination with other modalities such as targeted and/or locoregional therapy (LRT) might reveal potential future synergism for HCC treatment. For example, a combination of tremelimumab with LRT offered 26% partial responses.[13] In addition, different phase I/II trials of nivolumab or pembrolizumab + transarterial chemoembolization (TACE) therapy were initiated to evaluate the safety and efficacy of these combinations, and adjuvant or neoadjuvant immunotherapy accompanied with surgical resection is also being tested in clinical trials (CheckMate 9DX; ClinicalTrials.org: NCT03383458) (Fig. 3). Recently, in the IMbrave150 phase III trial (ClinicalTrials.gov NCT03434379) bevacizumab and atezolizumab combined therapy was compared to standard of care sorafenib, and the endpoints overall survival and progression free survival improved significantly compared with sorafenib (https://www.roche.com/media/releases/med-cor-2019-10-21.htm).

Future challenges in HCC include: (1) understanding the pathophysiology of atypical response patterns to immunotherapy (e.g., pseudoprogression caused by immune cell infiltration); (2) identifying a reliable biomarker to predict responses to immunotherapy; and (3) evaluating the potential benefit of combination strategies.

All in all, despite high expectations on immunostimulatory mAbs in HCC, there are still no major changes in the therapeutic armamentarium. It is clear that there is room for improvement based mainly on combinations among immunostimulatory mAbs, targeted therapy, and LRT.

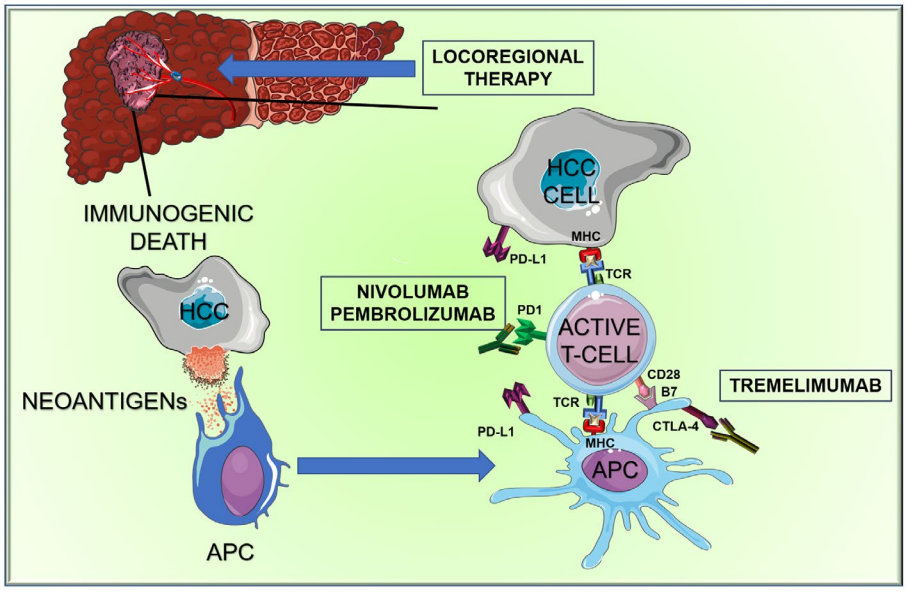

FIG 3 Combination strategies for HCC immunotherapy: LRT (transcatheter arterial chemoembolization or radiofrequency ablation) used in HCC could amplify the immune stimulation induced by immune checkpoint inhibitors. Chemotherapy drugs used in TACE (doxorubicin, cisplatin) might also induce the expression of neoantigens on dying tumor cells and make them detectable by the APCs, which have the ability to initiate an antitumor immune response. The induction of immunity against cancer cells by this specific form of cell death is called immunogenic cell death and might be an attractive approach to enhance the efficacy of immunostimulatory mAbs in patients with advanced HCC.

The public health system is responsible for the treatment of most people in Latin America. Compared with other regions, access to health care and delays in regulatory approval remain the main barriers that challenge the modern treatment of HCC. As a consequence, immune checkpoint inhibitors are not yet widely available. Finally, an important field to foster in Latin America is the development of research network through which we can support medical education and generation of regional information needed to apply and develop novel therapies for patients with advanced HCC.

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