

Emerging role of Immune Checkpoint Inhibitors in Hepatocellular Carcinoma

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ABSTRACT

Hepatocellular carcinoma is the most common primary liver cancer and the fourth leading cause of cancer death worldwide. A total of 70-80% of patients are diagnosed at an advanced stage with a dismal prognosis. Sorafenib has been the standard of care for almost a decade until 2018 when FDA approved an alternative first-line agent namely lenvatinib. Whereas FOLFOX4 results an alternative first-line treatment for the chinese clinical oncology guidelines. In addition to cabozantinib, regorafenib, and ramucirumab, two therapeutics against the PD-L1/PD1 axis have been recently approved for subsequent-line therapy, as nivolumab and pembrolizumab. However, similar to other solid tumors, the response rate of single agent targeting PD-L1/PD1 axis is low. Therefore a lot of combinatory approaches are under investigation, including the combination of different immune checkpoint inhibitors, the addition of immune checkpoint inhibitors after resection or during locoregional therapy, immune checkpoint inhibitors in addition to kinase inhibitors, anti-angiogenic therapeutics, and others. This review focuses on the use of ICIs for the hepatocellular carcinoma with an attent evaluation of new ICIs based combinatory approaches.

KEYWORDS: Hepatocellular carcinoma, immune checkpoint inhibitors, HCC, pembrolizumab, nivolumab, immune microenvironment, targeted therapies

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the fourth leading cause of cancer death worldwide as stated in reports as of 2018. Chronic hepatitis C (HCV) or chronic hepatitis B (HBV) infections, alcohol abuse, and non-alcoholic steatohepatitis are the main risk factor [1, 2]. An association between type 2 diabetes mellitus and HCC has also been reported [3]. Treatment for the early stage include hepatectomy, liver transplant, hepatic transarterial chemoembolization (TACE), and radiofrequency ablation (RFA).

Nevertheless, 70%-80% of patients cannot benefit from such opportunities because they are diagnosed at an advanced stage and can receive only palliative care. Sorafenib has been the standard choice for a decade in advanced HCC, even if as well as other TKIs as well [4] is characterized by possible primary resistance or acquired resistance [5]. Recently, lenvatinib showed similar results in terms of survival in a non-inferiority randomized trial study considering the same subset of patients [6]. Whereas, cabozantinib, regorafenib and an anti-VEGFR2 molecules namely ramucirumab exhibited promising results in the second-line setting [7-9]. In the era of immunotherapy, immunecheckpoints inhibitors (ICIs) have also been tested for HCC patients [10], in particular nivolumab and pembrolizumab result approved for second-line therapy. However similar to other gastrointestinal malignancies [11] HCC response rate of ICIs as monotherapy is low, therefore new combinatory approaches comprising TKIs, the addition of different ICIs, anti-angiogenic therapeutics, locoregional therapy, kinase inhibitors, chemotherapy, and other drugs, are currently under intensive investigations

FROM LIVER IMMUNE SYSTEM TO HCC IMMUNE DISORDERS

The liver is an organ with a specific blood supply that influences its immune microenvironment, in particular 75% of the blood enters the liver through the portal vein that drains into smaller diameter structures called sinusoids. Therefore, a great amount of antigens are in contact trough the liver sinusoidal endothelial cells (LSECs) with the liver immune microenvironment, consisting in hepatic stellate cells (h-SCs) [12], Kupffer cells [13], fibroblasts [14], dendritic cells (DCs) [15], and lymphocytes [16]. The tumour microenvironment actively participate to drug-resistance acquisition in solid tumours [17-19] Both lymphocytes and DCs present multiple subtypes with different protumorigenic functions [20,21] that are included from the response to pathogenic non self-antigens to the tolerance to self-antigens. About the immune-tolerance, hepatic microenvironment show high expression of hepatocyte growth factor and colony-stimulating factor 1, which promote a tolerigenic phenotype that is required to overcome autoimmune mechanisms due to antigenic hyperstimulation coming from the bowel [22]. In addition, HCV and HBV infections lead to frequent chronic inflammatory liver insult resulting in a deregulation of T cell activities with an increase of the expression of immune checkpoint inhibitors [23]. At the same time, HCC patients exhibit a more immunosuppression of the liver microenvironment [24] regulatoty T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells are increased in HCC and correlate with tumor progression and poor prognosis. Given the tight

correlation between the endothelial and epithelial function in immune response modulation against different cancer types [25-27], extensive investigation in HCC patients pinpoint LSECs as immune tolerance inducer of CD8-positive T cells to tumor associated antigens, inducing Tregs, and also increase PD-L1 expression which correlate with recurrence after surgery and poor prognosis in advanced HCC [28].

CHECKPOINT INHIBITORS

In September 2017, based on the results of a phase I/II nonrandomized multi-institution study (CheckMate 040), the FDA approved a full human immunoglobulin G4 monoclonal antibody directed to PD1 namely Nivolumab for HCC patients who progressed on or after sorafenib. The trial included 48 patients in a dose-escalation phase and 214 patients in a dose-expansion phase. Among the dose-escalation phase, nivolumab resulted in a disease control rate of 55% and an objective response rate of 10%, with a median overall survival of the entire cohort of 7.6 months that increased to 9.8 months in sorafenib-naïve patients [29]. CheckMate 459, a multicentre, randomized, phase III trial evaluated nivolumab compared with sorafenib as a first-line treatment in 1009 HCC patients, results are currently in process (NCT02576509) [30], however a press release reported that the study did not achieve statistical significance for its primary endpoint of OS. More recently, FDA granted accelerated approval for pembrolizumab, another antibody targeting PD-1, for patients with HCC previously treated with sorafenib. In the nonrandomized, open-label, phase II KEYNOTE-224 trial including 104 patients who progressed on or were intolerant to sorafenib, pembrolizumab demonstrated an objective response of 17% with one complete response and 17 partial response, while 46 patients experienced a stable disease. The median PFS was 4.9 months and the median OS was 12.9 months [31]. However, likely to the phase III CheckMate 459, the phase 3 Keynote-240 trial comparing pembrolizumab to a placebo in second-line HCC did not meet its coprimary endpoints of OS and PFS [32]. About the monoclonal antibody targeting CTLA4, tremelimumab, was evaluated in a phase II multicenter clinical trial including 20 patients with advanced HCC from hepatitis C viral etiology. The infusion of tremelimumab at the dose of 15 mg/Kg IV every 90 days resulted in 18% of partial response and a 60% of stable disease [33]. Currently tremelimumab is being evaluated either alone or in combination with other ICIs.

Unfortunately, the response rate of single ICI remains low, differently from the circulating CD8⁺ T-cells that increased after ICIs treatment, no activity enhancement has been observed for intrahepatic CD8⁺ T-cells. The combination of anti-CTLA4 and antibody targeting the PD1/PD-L1 axis are also under investigation, based on preclinical studies demonstrating that the 2 pathways are not overlapping, indeed seem that the combination has a synergistic effect able to reverse the refractoriness of intrahepatic CD8⁺ T-cells [34]. The combination of the anti-CTLA4 antibody ipilimumab and nivolumab is currently evaluated in patients undergoing hepatic resection as a neoadjuvant treatment (NCT03682276, NCT03510871) [35,36]. Instead, in the adjuvant setting, for patients who have undergone a curative resection, toripalimab anti-PD-1 antibody has been evaluated in the JUPITER-04 trial with the primary end-point consisting in the recurrence free-survival [37].

COMBINATORIAL APPROACHES WITH CHECKPOINT INHIBITORS

Despite the fact that the impact of ICIs on malignancies treatment is unprecedented, unlike melanoma and non-small cell lung cancer, the response rate in HCC remains low. In regards to this, as well as for other malignancies, researchers are evaluating combined approaches to increase the efficacy of ICIs [38]. The combination of ICIs with anti-VEGF therapy is a major approach under investigation for HCC patients using the immunomodulatory effects of anti-VEGF drug as a means of decreasing CD4⁺ regulatory T-Lymphocytes and MDSCs as well as the activation and differentiation of dendritic cells [39,40]. The combination of atezolizumab and bevacizumab resulted in 61% partial response among 21 HCC patients with a relatively positive tolerability characterized by 35% of the subjects experiencing grade III/IV adverse events [41]. The combination of atezolizumab plus bevacizumab is also being evaluated in a phase III trial, open-label, multicenter, randomized study, with sorafenib in the control arm, in pts with locally advanced or metastatic and/or unresectable HCC (NCT03434379) [42]. Another randomized phase III EMERALD-2 (NCT03847428) [43] is evaluating the role of another ICIs targeting PD-L1, namely durvalumab, in addition to bevacizumab in the adjuvant setting.

As is known, after sorafenib others TKIs have been approved for HCC patients [6,9]. Interestingly, evidence suggests that these small molecule inhibitors could improve tumor immunogenicity through the increase of antigen expression and the activation of cytotoxic activity of CD8 cells. Several trials are exploring the combination of ICIs with TKIs approved for HCC patients, such as regorafenib, cabozantinib, and lenvatinib. Preliminary results of a phase 1b trial of lenvatinib with pembrolizumab demonstrated that among the 13 patients treated, 6 (46%) achieved a partial response and 6 (46%) a stable disease [44]. A phase 3 evaluating this combination for the first-line treatment of patients with advanced HCC is ongoing [45]. Two cohorts were added to the phase 1/2 nivolumab clinical trial to evaluate the tolerability and the effectiveness of nivolumab in combination with sorafenib and with cabozantinib.

Another combined approach under investigation is the addition of ICIs to local therapies, in particular the post-transarterial chemoembolization (TACE), which is associated with antigen release and the exposure of damage-associated molecular patterns. A phase 1 trial concerning HCC patients treated with TACE, radiofrequency ablation, or cryoblation in combination with tremelimumab, showed 23.5% (4 of 17 patients) partial response [46]. A tumor biopsy at 6 weeks showed an increase of CD8⁺ T-cells infiltration in patients who had a clinical benefit. Other trials combining ICIs with locoregional therapy are ongoing: pembrolizumab with TACE (phase I/II, NCT03397654) [47], nivolumab with TACE (phase I, NCT03143270) [48], pembrolizumab with yttrium-90 radioembolization (phase II, NCT03099564) [49], and nivolumab with yttrium-90 radioembolization (phase II, NCT03812562) [50], among others.

Even if HCC is known as a malignancy highly refractory to chemotherapy, based on the results of the EACH study, FOLFOX4 has been recently recommended as a clinical practice guideline by the China Food and Drug Administration [51]. Interestingly, similar to other chemotherapeutic agents, oxaliplatin can induce an antitumor immune response, activating dendritic cells, promoting the antitumor CD4⁺T cells phenotype, and by the down-regulation of MDSC and regulatory T cells. About this, a monoclonal antibody directed against PD-1 namely SHR-1210 combined with FOLFOX4 is under investigation in Chinese patients with advanced HCC (NCT03092895) [52].

Finally, besides the co-inhibitory receptors CTLA-4 and PD-1 other co-inhibitory molecules have been described, including T cell immunoglobulin and immune-receptor tyrosine-based inhibitory

motif domain (TIGIT), Lymphocyte activation gene-3 (LAG3), and T cell immunoglobulin containing the mucin domain 3 (TIM-3) [25]. The latter has been shown to be involved in HCC progression, with high infiltration of TIM-3 positive cells correlating with poor prognosis. The combination of anti-PD-L1/anti-PD1 therapy with therapeutics targeting TIM-3 (NCT03099109) [53] and LAG-3 (NCT03005782 and NCT01968109) [54,55] is under investigation.

CONCLUSIONS AND FUTURE DIRECTIONS

As discussed in this manuscript, ICIs are under intensive investigation for HCC patients, as well as other malignancies, ICIs are already approved for lines subsequent the first, as nivolumab and pembrolizumab. However, in accord with other solid tumors, the response rate is low, therefore several strategies to improve ICIs efficacy are under investigation, as the combinations of different ICIs, the addition of anti-angiogenic therapeutics or of kinase inhibitors, and others. Interestingly, a unique and promising combinatory approach that is evaluating in HCC patients is the addition of ICIs to locoregional therapy, future studies are needed for the validation of these treatments.

CONFLICT OF INTERESTS

The Authors declare the absence of conflict of interests.

ACKNOWLEDGMENTS

This research project was supported in part by Apulian Regional Project “Medicina di Precisione”.

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