Immunotherapy for nonalcoholic fatty liver disease-related hepatocellular carcinoma: Lights and shadows

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Abstract

About one-fourth of adults globally suffer from nonalcoholic fatty liver disease (NAFLD), which is becoming a leading cause of chronic liver disease worldwide. Its prevalence has rapidly increased in recent years, and is projected to increase even more. NAFLD is a leading cause of hepatocellular carcinoma (HCC), the sixth-most prevalent cancer worldwide and the fourth most common cause of cancer-related death. Although the molecular basis of HCC onset in NAFLD is not completely known, inflammation is a key player. The tumor microenvironment (TME) is heterogeneous in patients with HCC, and is characterized by complex interactions between immune system cells, tumor cells and other stromal and resident liver cells. The etiology of liver disease plays a role in controlling the TME and modulating the immune response. Markers of immune suppression in the TME are associated with a poor prognosis in several solid tumors. Immunotherapy with immune checkpoint inhibitors (ICIs) has become the main option for treating cancers, including HCC. However, meta-analyses have shown that patients with NAFLD-related HCC are less likely to benefit from therapy based on ICIs alone. Conversely, the addition of an angiogenesis inhibitor showed better results regarding the objective response rate and progression-free survival. Adjunctive diagnostic and therapeutic strategies, such as the application of novel biomarkers and the modulation of gut microbiota, should be considered in the future to guide personalized medicine and improve the response to ICIs in patients with NAFLD-related HCC.

Key Words: Hepatocellular carcinoma; Immunotherapy; Liver cancer; Nonalcoholic fatty

liver disease; Metabolic dysfunction-associated fatty liver disease; Obesity

Core tip: Complex interactions involving the immune system, angiogenesis and inflammation are associated with the pathogenesis of hepatocellular carcinoma (HCC). Recent reviews suggested lower efficacy of immunotherapy in patients with nonviral HCC. This calls into question the need to stratify patients to maximize the effectiveness of immunotherapeutic agents. In this study, we provided the latest report on the tumor microenvironment structure and its implications in response to immunotherapy in nonalcoholic fatty liver disease-related HCC and also discussed the efficacy of first-line systemic treatment in this patient population.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) includes a wide spectrum of hepatic abnormalities ranging from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH)[1]. About 24% of adults globally suffer from NAFLD[2,3]. This percentage has considerably increased in recent years, almost doubling between 2005 and 2010[3]. NAFLD starts developing with the accumulation of lipids in hepatocytes, while progression to steatohepatitis occurs in 20%–30% of the cases[4,5]. Cirrhosis occurs in 10%–20% of the cases due to the deposition of fibrous tissue (fibrosis) and alterations in the regeneration of hepatocytes[4,5]. Although it is difficult to precisely determine the prevalence of NAFLD-related cirrhosis, NAFLD is one of the leading causes of cirrhosis worldwide[6,7], and it is currently the second most common indication for liver transplantation[2]. As NAFLD is associated with metabolic disorders in almost all patients, a change in the terminology from NAFLD to metabolic dysfunction-associated fatty liver disease (MAFLD) was proposed[8]. This new classification might lead to a further increase in the prevalence of this disease, but there is still a lack of complete agreement among experts about redefining NAFLD as MAFLD.

Besides increasing in prevalence, NAFLD is becoming one of the leading causes of hepatocellular carcinoma (HCC), being responsible for 1%–38% of HCCs globally[9-12]. The high variation in estimating the prevalence of NAFLD-related HCC is due to the heterogeneous definition of NAFLD used in different studies (histological *vs* radiological *vs* clinical)[9].

Chronic viral hepatitis, caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, accounts for 80% of HCC cases globally[13]. However, some studies suggest that NAFLD is the main cause of HCC in some areas of Europe, while in the USA, the number of NAFLD-related HCC cases is steadily increasing[10]. Considering the growing prevalence of NAFLD and the progressive reduction in viral-hepatitis-related HCC (due to vaccination and effective antiviral treatments)[14-16], NAFLD/MAFLD might become the main cause of HCC in the next 10 years[10,14]. Although most cases of NAFLD-related HCC occur in a cirrhotic liver, retrospective studies have shown that NAFLD-driven HCC can occur even in the absence of cirrhosis in 20%–50% of the cases, especially when NASH is present[10,13,17,18].

Many factors contribute to making NAFLD a leading cause of HCC, even in the absence of cirrhosis. Specifically, type 2 diabetes mellitus and obesity increase the risk of HCC in NAFLD patients with or without cirrhosis[19-23]. Moreover, NAFLD-related HCC has higher mortality than HCC associated with viral hepatitis[11]. This is probably because HCC in patients with NAFLD is generally diagnosed in more advanced stages and mostly outside surveillance programs[17].

Recently, immunotherapy with immune checkpoint inhibitors (ICIs) was found to be a good therapeutic option for advanced HCC, either as an alternative to tyrosine kinase inhibitors (TKIs) or along with them[24]. Patients need to be categorized according to their likelihood of response, considering that ICIs were found to be less effective in certain patient subpopulations, particularly those with NAFLD. In this review, we describe the mechanism behind the progression of NAFLD to HCC and discuss the efficacy of ICIs in patients with NAFLD to determine the factors that might elucidate the best therapeutic choice for this patient population.

HCCIMMUNOBIOLOGY AND LIVER MICROENVIRONMENT

Many factors contribute to the progression of NAFLD to HCC, including individual (*i.e.*, genetics, epigenetics and gut microbiota) and environmental (*i.e.*, diet) factors[25]. Although the molecular basis of this process is not completely known[26], most authors agree that chronic inflammation and immune system disorders play a crucial role[25,27-30]. These interactions occur in the tumor microenvironment (TME) (Figure 1).

The immune system: ally or enemy in the TME?

Based on the complex interactions with other cell types and cytokines, each cell in the TME has a different function. To provide an overview of the TME, a classification of HCCs based on immunological features was proposed for determining prognostic phenotypes and predicting therapeutic responses[29-31]. Two main types of HCC can be identified: the inflamed class (high immune infiltration, increased PD-1/PD-L1 signaling, and markers of CD8^{+}T cell cytotoxic activity) and the noninflamed class (low abundance of tumor-infiltrating lymphocytes, low expression of immune checkpoints, and markers of CD8^{+}T cell cytotoxic activity)[32]. The inflamed class can be divided into two subclasses[29,31]: (1) The active immune class, characterized by a high abundance of CD8^{+}T cells, M1-phenotype macrophages, and overexpression of T cell effector genes, such as *CXCL9*, *CXCL10* and *IFNγ*, and granzymes, shows a favorable prognosis compared to the other classes; and (2) The exhausted immune class, characterized by an increase in M2 tumor-associated macrophages and T-cell exhaustion markers[29]. Transforming growth factor (TGF)-β signaling plays a key role in inducing T-cell exhaustion[33,34] and is associated with an increase in the expression of programmed cell death protein (PD)-1, TIM-3, LAG3 and TIGIT[35]. Another molecule involved in CD8^{+}T-cell exhaustion is the thymocyte selection-associated high mobility group box (TOX) transcription factor, whose expression is induced by the vascular endothelial growth factor (VEGF) signaling pathway[35]. Noninflamed subclasses include: (1) The intermediate class, in which immune infiltration is lower than that in the inflamed class; and (2) The immune excluded class, characterized by immunosuppressive gene upregulation in tissues surrounding the tumor, which leads to immunological desertification[32,36]. The immune excluded class has the worst prognosis and is unlikely to respond to immunotherapy[36,37].

Another classification considers immune cell tumor infiltration, with three subtypes of HCC phenotype: (1) The immune-high subtype, which has a high rate of T-cell, B-cell and plasma cell infiltration; (2) The immune-mid subtype, which has a moderate rate of immune cell infiltration; and (3) The immune-low subtype, which has a low rate of immune cell infiltration[30]. As in the active immune class, the high-immune subtype is associated with an increase in T helper 1 cells and CD8^{+} cell cytokines [30,32]. The high-immune subtype has a better prognosis than the other subtypes[30], particularly in poorly differentiated HCCs[30]. Furthermore, alterations in the T-cell count occur mostly during the shift from moderately to poorly differentiated HCC, resulting in immunological subtype differentiation in this phase[30].

Different roles of T cells in the TME

T cells play an important role in the progression of liver diseases to HCC. In an inflammatory setting, such as NASH, regulatory T (Treg) cells decrease while T-helper 17 cells increase[38]. IL-17 released by T-helper 17 exacerbates liver inflammation and promotes hepatocarcinogenesis[39]. After the development of HCC, the number of Foxp3^{+}GARP^{+} CTLA-4^{+}Treg cells increases in the TME, inhibiting the cytotoxic action of CD8^{+}T cells against tumor cells[40,41]. The infiltration of Treg cells in the TME is associated with the immune excluded class[29].

CD8^{+}T cells are directly cytotoxic to tumor cells. Two distinct phenotypes of CD8^{+}T cells in HCC were described: one with low cytotoxic activity, associated with an upregulation of the *KLRB* gene and a poor prognosis[42]; the other, with a high cytotoxic capacity, associated with the overexpression of the *XCL1* gene and a better prognosis[43]. The participation of T cells in the TME is strongly determined by the etiology of liver disease. In NASH-related HCC, there is an excess of CD8^{+}T cells expressing PD-1 [44,45]. These cells develop major-histocompatibility-complex-I-independent cytotoxicity against hepatocytes and lose tumor surveillance functions. This might be due to the metabolic dysregulation of immune system cells, which occurs in NASH[44,45]. In HBV-related HCC, the number of Treg cells increases with the overexpression of PD-1[46]. Furthermore, T cells are susceptible to Bcl-2-like protein 11-mediated apoptosis, which contributes to the tolerogenic milieu of HBV infection[47]. The number of CD8^{+}T resident memory cells, which probably have cytotoxic activity[46,48], is also enhanced in HBV-related HCC, and they are associated with a favorable prognosis[46]. However, these cells overexpress PD-1, suggesting an immune-exhausted microenvironment[46]. Chronic HCV infection also induces an exhausted phenotype in CD8^{+}T cells, causing a decrease in the production of interferon (IFN)γ, reduction in the expression of CD127, and overexpression of PD-1 and TIM[49]. Although the number of PD-1^{+}CD8^{+}T cells increases in both virus-related and NASH-related HCC, the response to anti-PD-1 ICIs is different in these two scenarios, suggesting that PD-1^{+}CD8^{+}T cells play a distinct function[44]. This is dependent on the type of cells with which PD-1^{+}CD8^{+}T cells interact. In the high-immune HCC subtype, PD ligand (PD-L)1 is mainly expressed by macrophages, which suggests that the PD-1/PD-L1

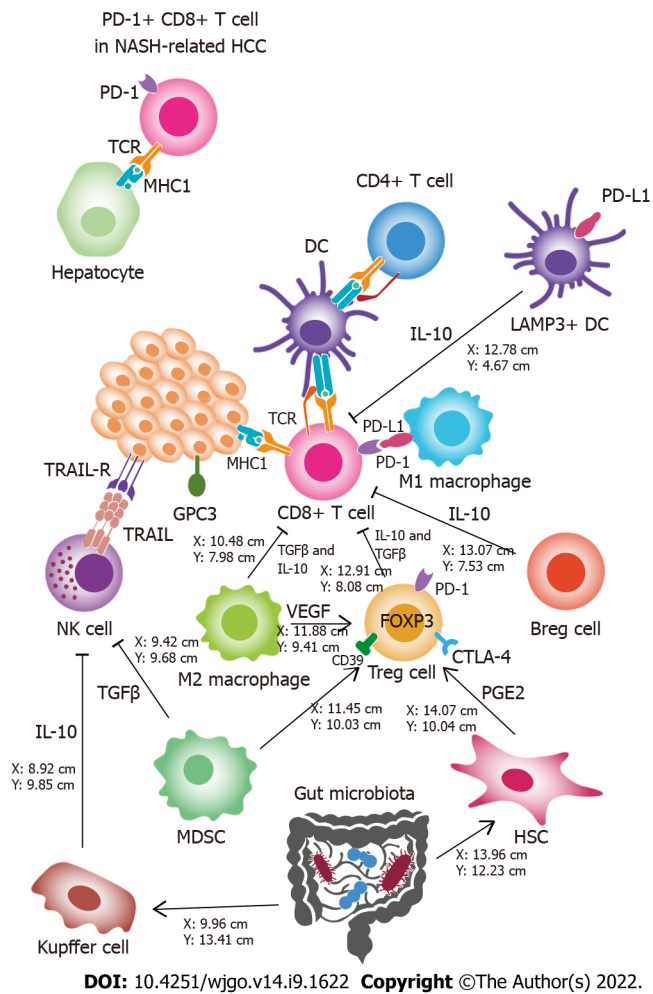**DOI:** 10.4251/wjgo.v14.i9.1622 **Copyright** ©The Author(s) 2022.

Figure 1 Hepatocellular carcinoma immunological microenvironment. Different elements that contribute to the antitumor activity or limit antitumor immunity are illustrated schematically. The main effectors against tumor cells are CD8+ T cells and natural killer (NK) cells. Dendritic cells (DCs), CD4+ cells, and M1 macrophages enhance CD8+ T cell cytotoxicity. Regulatory T cells, regulatory B cells, LAMP3+ DCs, and M2 macrophages inhibit CD8+ T cells and induce an immunosuppressive environment. Tumor cells attract M2 macrophages by expressing glypican-3 (GPC3). Myeloid-derived suppressor cells and Kupffer cells produce immunosuppressive cytokines in the tumor microenvironment and inhibit NK cells. The gut microbiota might play an indirect role in immunosuppression through persistent inflammation or other mechanisms leading to immune cell exhaustion. PD-1+ CD8+ T cells in NASH-related hepatocellular carcinoma show cytotoxic activity against hepatocytes, instead of exhibiting antitumor function. Breg: Regulatory B cells; DC: Dendritic cell; HSC: Hepatic stellate cell; MDSC: Myeloid-derived suppressor cell; MHC I: Major histocompatibility complex class I; NASH: Nonalcoholic steatohepatitis; NK: Natural killer; TCR: T cell receptor; TME: Tumor microenvironment; Treg: Regulatory T cells; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; TRAIL-R: Tumor necrosis factor-related apoptosis-inducing ligand receptor; VEGF: Vascular endothelial growth factor.

interaction plays a role in T cell–macrophage crosstalk rather than in T-cell inhibition by the tumor[30]. Furthermore, macrophages from high-immune subtypes overexpress CD169[30], which is an M1 phenotype marker associated with macrophage-dependent T-cell activation and favorable prognosis in several cancers[50]. Other T cells, such as - T cells, might also be involved in antitumor surveillance, considering that their depletion in tumor tissues is associated with a higher incidence of postoperative recurrence[51].

B cells play an ambivalent role in the TME

B cells play a dual role in HCC, depending on their interaction with other components of the TME. B-cell infiltration occurs in high-immune subtypes and is associated with a better prognosis[30]. On the contrary, B-cell infiltration, when associated with elevated interleukin (IL)-17 production, is a poor prognostic marker[52]. Moreover, regulatory B cells inhibit the activation and cytotoxic activity of CD8+ T cells in NASH-related HCC by expressing PD-L1 and producing IL-10[53].

Role of innate immunity in HCC TME

Along with CD8+ T cells, natural killer (NK) cells act as the main cytotoxic effectors in HCC, contributing to innate immune system tumor surveillance[54]. However, due to the abundance of Treg cells in the TME, the number of intratumoral NK cells decreases and impairs their cytotoxic activity, as well as the production of IFNγ[55,56]. NK T cells play a role in the development of HCC in patients with NASH by

triggering the transformation of hepatic stellate cells (HSCs) into fibroblasts[28] and promoting liver inflammation and injury *via* nuclear factor-B signaling and the production of cytokines[57]. NK T cells show antitumor activity and cooperate with CD4+ T cells to remove senescent hepatocytes from the liver following a chemically induced liver injury[58]. This antitumor property is associated with the chemokine CXC ligand (CXCL)16/CXCR6 hepatic chemokine pathway[58]. The interaction between bile acids and the gut microbiotaregulates CXCL16 expression in the liver sinusoidal endothelial cells[59], suggesting that microbiota the plays a role in the development of HCC.

Other innate immune cells, such as M2-phenotype tumor-associated macrophages and myeloid-derived suppressor cells, might suppress CD8+ T cells by releasing TGF-β and IL-10[39,60,61]. Tumor-associated macrophage abundance in the HCC tissue is associated with a poor prognosis[62,63]. Dendritic cells play an important role in PD-1/PD-L1 crosstalk. In the TME and lymph nodes, PD-L1+ LAMP3+ dendritic cells inhibit circulating CD8+ T cells activated by tumor antigens[64]. This immunoregulatory function primarily occurs through the secretion of IL-10 and the recruitment of Treg cells[65, 66].

Tumor cells modulate the immune response

Tumor cells can effectively control the HCC TME. The overexpression of the MYC proto-oncogene in tumor cells is associated with upregulation of PD-L1 on the cell surface[67]. Furthermore, alterations in the WNT–β-catenin signaling pathway decrease the secretion of chemokine CC ligand 5, which affects the recruitment of dendritic cells[68]. The immune-excluded HCC class shows a higher rate of *WNT/CTNNB1* gene mutation[36]. Tumor cells in the HCC TME directly recruit immunosuppressive neutrophils by upregulating the chemokine CXCL5[69].

The gut–liver axis influences the TME in HCC

The inflammatory condition associated with the progression of HCC is not limited to the liver. Intestinal inflammation acts as a cofactor in the pathogenesis of HCC[70,71]. This was confirmed by finding a higher fecal calprotectin concentration in patients with cirrhosis and HCC compared to that in healthy subjects or in patients affected by cirrhosis without HCC[70]. The gut microbiota might play an important role in modulating intestinal inflammation with noticeable effects on hepatocarcinogenesis [70-72]. The dominant phyla associated with HCC include *Bacteroidetes*, *Firmicutes* and *Proteobacteria*[73]. Changes in the gut microbiota occur commonly in patients with NAFLD-related cirrhosis, where the microbial diversity decreases compared to that in healthy people[74]. Other proinflammatory bacteria, such as *Bacteroides* and *Ruminococcaceae*, are overabundant in individuals with NAFLD-related cirrhosis and HCC[70]. In contrast, depletion of bacteria such as *Akkermansia* and *Bifidobacterium*, which have an anti-inflammatory effect, might also occur[70]. The integrity of the gut epithelial and vascular barrier plays an important role in preventing bacteria from entering the portal circulation[75]. Alterations in the gut–liver axis that occur in cirrhosis (such as changes in the gut microbiota composition and impaired bile acid production) might result in the disruption of the gut barrier, thus, increasing intestinal permeability[76]. Hence, intestinal bacteria, along with their antigens and products, such as lipopolysaccharides, can easily reach the liver *via* the portal system[77]. By binding Toll-like receptors, bacterial antigens activate Kupffer cells and HSCs[78]. This interaction enhances liver inflammation and plays a crucial role in hepatocarcinogenesis[71]. For example, Toll-like receptor 4 activation in Kupffer cells can increase IL-10 production, which suppresses the cytotoxic functions of NK cells[79]. Gram-positive bacteria might also play an important role in NAFLD-related HCC. Their metabolic products, such as deoxycholic acid and lipoteichoic acid, cause senescence of HSCs[80]. Senescent HSCs have a distinct phenotype characterized by higher production of cytokines, chemokines, and matrix-remodeling proteins[81]. In a preclinical mouse model, prostaglandin E2 generated by senescent HSCs was shown to interfere with the TME through the prostaglandin E receptor 4 signaling pathway[82]. Prostaglandin E receptor 4 is a G-protein-coupled receptor that is mostly expressed by immune cells[83]. Its activation may have an immunosuppressive effect by enhancing the infiltration of Tregs and PD1+ CD8+ T cells in the TME[82].

IMMUNOTHERAPY IN NAFLD-RELATED HCC: EVIDENCE AND CONCERNS

Systemic therapies represent the standard of care for unresectable HCC, either in an advanced or intermediate stage, that is unsuitable for further treatment[84]. Over the last few years, ICIs have shown positive therapeutic results, leading researchers to shift their focus from tyrosine kinase inhibitors (TKIs) to ICIs. Several agents have been approved, either alone or in combination, as first-line or second-line treatments for HCC, with some variations in the treatment regimen found in different countries[85] (Table 1).

Effectiveness of immunotherapy in NAFLD-related HCC

Subgroup analyses of survival outcomes based on trials evaluating the efficacy of ICIs as first-line treatment revealed a discrepancy between HCC associated with HBV or HCV infection (viral HCC)

Table 1 The main immune checkpoint inhibitors approved for treatment of advanced hepatocellular carcinoma

Drug	Mechanism of action	Efficacy	Safety	Approval
First-line				
Atezolizumab (1200 mg, IV) plus bevacizumab (15 mg/kg, IV) every 3 wk	ICI, anti-PD-L1 antibody (atezolizumab) plus antiangiogenic, anti-VEGF-A antibody (bevacizumab)	Improved OS, PFS, ORR *vs* sorafenib (IMbrave-150 phase III trial[93])	irAEs[1], hypertension, fatigue, proteinuria, pruritus, gastrointestinal bleeding	Approved by FDA and EMA for patients with advanced HCC
Tremelimumab (300 mg, IV) plus durvalumab (1500 mg, IV) once, followed by durvalumab (1500 mg, IV) every 4 wk	ICI, anti-CTLA-4 antibody (tremelimumab) plus ICI, anti-PD-L1 antibody (durvalumab)	Improved OS *vs* sorafenib and favorable benefit-risk ratio (HIMALAYA phase III trial[89])	Pruritus, irAEs[1]	Under evaluation for approval. Granted orphan drug designation by FDA for HCC treatment (2020)
Sintilimab (200 mg, IV) plus IBI305 (bevacizumb biosimilar; 15 mg/kg, IV) every 3 wk	ICI, anti-PD-1 antibody (sintilimab) plus antiangiogenic, anti-VEGF-A antibody (IBI305)	Better OS and PFS in HBV-related advanced HCC *vs* sorafenib (ORIENT-32 phase II/III trial[113])	Proteinuria, irAEs[1], thrombocytopenia, leukopenia, hypertension, fatigue	Approved by NMPA in China for patients with advanced HCC (2021)
Second-line				
pembrolizumab (200 mg, IV) every 3 wk plus best supportive care	ICI, anti-PD-1 monoclonal antibody	Better OS, PFS and ORR in patients post-sorafenib *vs* placebo (KEYNOTE-394 phase III trial[114] and KEYNOTE-224 phase II trial[115])	irAEs[1], fatigue, pruritus, anorexia	Approved by FDA for advanced HCC post-sorafenib (2018)
Nivolumab (1 mg/kg, IV) plus ipilimumab (3 mg/kg, IV) every 3 wk for 4 cycles, followed by nivolumab (240 mg, IV) every 2 wk	ICI, anti-PD-1 monoclonal antibody (nivolumab) plus ICI, anti-CTLA-4 antibody (ipilimumab)	Promising OS and durable response post-sorafenib (cohort 4 of CheckMate-040 phase I/II trial[90]). CheckMate 9DW phase III trial ongoing[116]	Pruritus, irAEs[1]	Approval by FDA for advanced HCC post-sorafenib (2020)

[1]Immune-related adverse events include hepatitis, colitis, pneumonia, endocrinopathy, skin rash, neurological disorders.

IV: Intravenous administration; ICI: Immune checkpoint inhibitor; VEGF-A: Vascular endothelial growth factor-A; OS: Overall survival; PFS: Progression free survival; ORR: Objective response rate; irAEs: Immune-related adverse events; FDA: Food and Drug Administration; EMA: European Medicines Agency; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; NMPA: National Medical Products Administration.

compared to liver disease of other etiology (nonviral HCC), including NASH-related HCC (Table 2). To our knowledge, none of the clinical trials that evaluated the efficacy of immunotherapy for the treatment of HCC differentiated the nonviral HCC subgroup of patients, thus including cases of HCC associated with NASH, alcohol use disorder, autoimmune hepatitis, primary biliary cholangitis, or sclerosing cholangitis.

In the CheckMate-459 trial, nivolumab treatment was found to be associated with slightly lower median overall survival (OS) than sorafenib in the nonviral HCC group (16 mo *vs* 17.4 mo; HR: 0.91; 95%CI: 0.72–1.16), while the best results were obtained in the viral HCC group (HCV-HCC patients: 17.5 *vs* 12.7 mo; HBV-HCC patients: 16.1 *vs* 10.4 mo)[86]. In the KEYNOTE-240 trial, pembrolizumab showed higher OS in HCC of any etiology compared to placebo, but better results were reported in patients with HBV-HCC (HR: 0.57; 95%CI: 0.35–0.94) than in those with nonviral HCC (HR: 0.88; 95%CI: 0.64–1.20)[87]. In the study 22 phase 1/2 trial that evaluated the effectiveness of the combination of tremelimumab plus durvalumab, HBV-HCC and nonviral HCC patients showed comparable OS results (14.4 and 13.8 mo, respectively), which differed from the results of the HCV-HCC patients (22.3 mo)[88]. In the subsequent HIMALAYA phase 3 study[89], tremelimumab plus durvalumab showed longer OS than sorafenib in HBV-HCC patients (HR: 0.64; 95%CI: 0.48–0.86) and in nonviral HCC patients (HR: 0.74; 95%CI: 0.57–0.95), which was opposite to that found in the HCV-HCC patients (HR: 1.06; 95%CI: 0.76–1.49).

Regarding second-line regimens, in cohort 4 of the CheckMate-040 trial, which investigated the therapeutic efficacy of three different dosing regimens of nivolumab plus ipilimumab, the OS benefit was similar in the nonviral HCC (14.7 mo) and HBV-HCC (15.2 mo) groups, while the OS in the HCV-HCC group was significantly higher (21.9 mo)[90].

Thus, the results of several studies supported the hypothesis that the underlying etiology might influence tumor response to immunotherapy. As shown by Foerster *et al*[91], this is particularly relevant in the case of NASH-related HCC, which is frequently identified at an advanced stage when systemic therapy becomes necessary. Regarding this, the authors highlighted some clinical issues. First, there are no effective strategies to prevent the development of HCC in NASH. Second, many cases of NASH-related HCCs arise in the absence of cirrhosis, but it is not known which subgroup of NASH patients might have a higher oncogenic risk and benefit from a surveillance program. Third, the low efficacy of ICIs in NASH-related HCC was concluded from post hoc analyses of phase III studies, which prevented definitive inferences from being drawn.

Table 2 Overall survival of patients with hepatocellular carcinoma receiving first-line immunotherapy alone or in combination, based on the etiology of the liver disease

Treatment	HCC etiology	HR (95%CI)	Trial	Phase
Atezolizumab plus bevacizumab *vs* sorafenib in first-line	Nonviral HCC	1.05 (0.68-1.63)	IMbrave150[93]	III
	HBV-HCC	0.58 (0.40-0.83)		
	HCV-HCC	0.43 (0.25-0.73)		
Nivolumab *vs* sorafenib in first-line	Nonviral HCC	0.91 (0.72-1.16)	CheckMate-459[86]	III
	HBV-HCC	0.79 (0.59-1.07)		
	HCV-HCC	0.72 (CI 0.51-1.02)		
Atezolizumab plus cabozantinib *vs* sorafenib in first-line	Nonviral HCC	1.18 (0.78–1.79)	COSMIC-312[99]	III
	HBV-HCC	0.53 (0.33-0.87)		
	HCV-HCC	1.10 (0.72-1.68)		
Tremelimumab 300 mg × 1 dose + Durvalumab 1500 mg *vs* sorafenib in first-line	Nonviral HCC	0.74 (0.57-0.95)	HIMALAYA[89]	III
	HBV-HCC	0.64 (0.48-0.86)		
	HCV-HCC	1.06 (0.76-1.49)		

HCC: Hepatocellular carcinoma; OS: Overall survival; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Tumor immune surveillance in NAFLD-related HCC and its association with efficacy of immunotherapy

Pfister *et al*[44] investigated the role of adaptive immunity in both NASH and NASH-related HCC and its effects on the efficacy of ICIs. In mice with steatohepatitis, an increase in hepatic resident-like CD8$^+$ PD1$^+$ T cells with characteristics such as exhaustion and effector functions were observed. Moreover, NASH severity was correlated with PD-L1 expression in hepatocytes and nonparenchymal cells. These results suggested that steatohepatitis-related HCC might significantly benefit from treatment with ICIs. However, when mice were administered anti-PD-1 immunotherapy, they did not show any tumor regression; instead, liver fibrosis increased. Conversely, mice affected by HCC of other origin had a positive response to anti-PD-1 immunotherapy. Furthermore, in NASH mice without HCC, the pre-emptive depletion of CD8$^+$ PD1$^+$ T cells significantly decreased liver damage and, consequently, the incidence of HCC. Pre-emptive treatment with ICIs increased PD1$^+$ CD8$^+$ T-cell infiltration in the liver and the incidence of HCC. When anti-tumor necrosis factor (TNF) or anti-CD8 antibodies were administered together with anti-PD-1 antibodies, liver damage and HCC incidence decreased compared to the reduction in liver damage after anti-PD-1 treatment alone. In humans, PD1$^+$ T cells were absent in healthy livers, but they were abundant in NASH livers and expressed the same gene profile as PD1$^+$ T cells found in mice. In summary, CD8$^+$ PD1$^+$ T cells failed to provide adequate antitumor immune surveillance in NASH-related HCC after immunotherapy. Instead, they triggered the transition to liver cancer through a TNF-dependent mechanism that was further enhanced by anti-PD-1 treatment. Although the study included data from patients with NASH, further investigations need to be conducted to elucidate the true effect of ICIs on NASH-related HCC in the clinical setting.

Benefits of antiangiogenic drugs in NAFLD-related HCC

Retrospective studies analyzed the response of tumors to lenvatinib and sorafenib, two TKIs with anti-VEGF activity, associated with the etiology of HCC. While some studies[92-94] did not find significant differences, Shimose *et al*[95] reported that NAFLD/NASH etiology was associated with greater survival of the patients treated with sorafenib. This was also confirmed by the REACH-2 trial, which showed that second-line treatment with ramucirumab (an inhibitor of VEGF receptor 2) achieved higher OS in nonviral than in viral HCC patients compared to placebo (HR: 0.633; 95%CI: 0.379–1.057 *vs* HR: 0.762; 95%CI: 0.435–1.334 *vs* HR: 0.838; 95%CI: 0.522–1.347, respectively)[96]. However, another study did not find any difference in the OS of patients with NAFLD/NASH who received sequential therapy after sorafenib treatment compared to those with viral or alcohol-related etiology of liver disease[95]. The favorable effect of angiogenesis inhibition on nonviral HCC was confirmed by administering combination therapies. In phase 3 IMbrave150 trial, the combination of atezolizumab plus bevacizumab showed considerable improvement in the OS compared to the OS after treatment with sorafenib in the HCV-HCC group (24.6 *vs* 12.6 mo; HR: 0.43; 95%CI: 0.25–0.73) and in the HBV-HCC group (19.0 *vs* 12.4 mo; HR: 0.58; 95%CI: 0.40–0.83). However, no improvement in the OS was observed in the nonviral HCC group (17.0 *vs* 18.1 mo; HR: 1.05; 95%CI: 0.68–1.63)[97]. There was also a significant improvement

in the objective response rate (ORR) (26.5% *vs* 9.4%; HR: 3.47; 95%CI: 1.24–9.65) and the progression-free survival (PFS) (7.1 *vs* 5.6 mo; HR: 0.80; 95%CI: 0.55–1.17) compared to the Sorafenib in the nonviral HCC group. Moreover, upon comparing the effectiveness of atezolizumab plus bevacizumab treatment with that of atezolizumab treatment, it became clear that the improvement in the PFS was related to the addition of the anti-VEGF drug (6.3 *vs* 3.4 mo; HR: 0.49; 80%CI: 0.26–0.92)[98]. Cabozantinib is another TKI featuring an anti-VEGF effect. The phase 3 COSMIC-312 trial[99] investigated its efficacy in combination with the anti-PD-L1 atezolizumab *versus* sorafenib in the first-line treatment of advanced HCC. The PFS and preliminary interim OS results were assessed, while follow-up for the final OS analysis is ongoing. Overall, cabozantinib plus atezolizumab did not show any improvement in the OS compared to sorafenib at the interim analysis (15.4 *vs* 15.5 mo; HR: 0.90; 96%CI: 0.69–1.18), while the PFS was significantly higher in the subgroup treated with the combination therapy (6.8 *vs* 4.2 mo; HR: 0.63; 99%CI: 0.44–0.91). Specifically, compared to sorafenib, atezolizumab plus cabozantinib showed the best results in the HBV-HCC patients (PFS: 6.7 *vs* 2.7 mo; HR: 0.46, 95%CI: 0.29–0.73; OS: 18.2 *vs* 14.9 mo; HR: 0.53, 95%CI: 0.33–0.87), whereas, modest improvements were observed in the HCV-HCC patients (PFS: 7.9 *vs* 5.6 mo; HR: 0.64, 95%CI: 0.38–1.09; OS: 13.6 *vs* 14.0 mo; HR: 1.1, 95%CI: 0.72–1.68) and no benefit was found in the nonviral HCC subgroup (PFS: 5.8 *vs* 7.0 mo; HR: 0.92, 95%CI: 0.60–1.41; OS: 15.2 mo *vs* not reached; HR: 1.18, 95%CI: 0.78–1.79). Two meta-analyses of the CheckMate-459, KEYNOTE-240, and IMbrave150 phase 3 trials[44,100] confirmed that anti-PD-(L)1 therapy resulted in lower OS in nonviral HCC compared to that in viral HCC. Haber *et al*[100] also conducted a meta-analysis of five phase 3 trials to assess TKIs or anti-VEGF in the second-line setting (REACH, REACH-2, METIV-HCC, CELESTIAL, and JET-HCC) and showed that survival outcomes were not influenced by the HCC etiology (viral HCC-pooled HR: 0.81; 95%CI: 0.71–0.92; nonviral HCC-pooled HR: 0.82; 95%CI: 0.67–1.01). These results highlighted the synergistic effect of anti-VEGF and anti-PD-(L)1 agents for the treatment of HCC associated with liver disease of any etiology, especially in the nonviral setting.

Potential applications of biomarkers in immunotherapy for HCC

So far, PD-L1 expression assessed by immunohistochemistry on tumor tissue is the only approved biomarker to identify patients with higher probability to respond to ICIs[101]. However, as discussed above, PD-1/PD-L1 signaling is involved in complex and partially unclear molecular pathways that could limit the role of PD-L1 tissue expression as a reliable predictive factor. Some PD-L1-negative patients benefit from immunotherapy, whereas some PD-L1-positive patients do not. Hence, biomarkers identified through liquid biopsy, such as circulating tumor DNA[102], miRNAs[103], tumor cells[104], and extracellular vesicles[105], have been considered. However, no biological marker has demonstrated a strong predictive value in patients with HCC[106]. Scheiner *et al*[107] developed and proposed the C-reactive protein and -fetoprotein in immunotherapy (CRAFITY) score as an easily applicable clinical tool to predict response to ICIs in patients with HCC. The score ranges from 0 (C-reactive protein < 1 mg/dL and -fetoprotein < 100 ng/mL) to 2 (C-reactive protein ≥ 1 mg/dL and -fetoprotein ≥ 100 ng/mL). The authors found that higher scores indicated shorter OS and a worse radiological response. The gut microbiota might play a significant role in predicting the response to ICIs and modulating the immune response, thus, affecting the effectiveness of immunotherapy. Microbiological changes and intestinal permeability that occur in cirrhosis increase the interaction between hepatic cells and proinflammatory intestinal bacteria, enhancing inflammation in the liver[70]. Anti-inflammatory bacteria, such as *Akkermansia* and *Bifidobacterium*, are usually scarce in NASH-related HCC patients and might lead to a persisting inflammatory response with the suppression of immune system surveillance in the long term. An increase in the abundance of *Akkermansia* along with a reduction in Enterobacteriaceae occurs in patients who respond to ICI therapy[108,109]. Furthermore, the composition of the gut microbiota changes over time during immunotherapy, which might be associated with a modification in the expression of immunomodulating pathways or vice versa may be a result of the modulating effect of the immune system on the gut microenvironment. Whether these modifications can predict responses that are essential for making further treatment decisions[106] needs further confirmation. Based on these findings, the oral administration of *Akkermansia muciniphila* was suggested to enhance the effect of ICIs[110]. Similar findings were observed in patients affected by epithelial cancer[110], colorectal cancer[111], and lung cancer[112].

CONCLUSION

In patients with HCC related to NASH, antitumor immune surveillance is impaired. The weaker efficacy of ICIs in NASH-related HCC contradicts the obesity paradox, in which mild obesity predicts a better response in patients with melanoma and other cancers treated with immunotherapy. Tremelimumab plus durvalumab was the only combination of two ICIs tested against sorafenib in the first-line setting and the only one that showed a relatively higher OS in the nonviral and viral HCC subgroups. Some studies showed similar efficacy in the treatment of viral *versus* nonviral HCC for the combination of nivolumab plus ipilimumab. Thus, regimens based on the combination of two ICIs rather than ICI monotherapy are promising for HCC treatment, regardless of the etiology of the liver

disease. Similar results were reported for TKIs with anti-VEGF activity, including anti-VEGF and anti-VEGF receptor agents, and some studies found better results in NAFLD/NASH patients. Therefore, the addition of antiangiogenic agents might increase the efficacy of immunotherapy and improve responses in patients who have an impaired immune system, such as patients with nonviral HCC. This was confirmed by the effectiveness of atezolizumab plus bevacizumab in improving survival regardless of the liver disease etiology, along with a higher PFS in nonviral HCC. However, this preliminary evidence generated by *post hoc* analyses or meta-analyses needs validation. Indeed, no clinical trial could differentially address the outcome of NASH-driven HCC compared to HCC of other nonviral etiology, such as alcohol-related HCC. Future studies should distinguish patient populations based on the underlying liver disease for a specific analysis of clinical outcomes and a better understanding of the mechanisms that trigger tumor immune escape in these patients.

Overall, these findings suggest that changes are required in the current algorithm of advanced HCC treatment toward a strategy that involves the administration of highly specific and optimized therapies based on the etiology of liver disease. The stratification of the patients is hampered by intragroup molecular heterogeneity. Thus, a model based on histological or circulating biomarkers might be critical for predicting responses to immunotherapy and defining a personalized strategy. However, biomarkers that can predict the outcome of immunotherapy have not been identified yet; the CRAFITY score provided encouraging results but required prospective validation. Despite compelling evidence regarding the role of the gut–liver axis in NAFLD-associated HCC, putting the theoretical knowledge into practice, either to categorize patients or enhance the response to treatment, is still a work in progress.

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