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REVIEW

Novel immunotherapy combinations in clinical trials for hepatocellular carcinoma: will they shape the future treatment landscape?

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ABSTRACT

Introduction: Underlying liver disease and the intrinsic chemoresistance have historically hampered the development of efficacious treatments in HCC. However, in the last few years, immunotherapy-based combinations have emerged as efficacious therapeutic strategy in this setting. This paper critically summarizes the recent therapeutic progress in the systemic treatment of HCC.

Area covered: This paper examines the preclinical rationale of the following combinations in HCC: dual checkpoint inhibitors, immune checkpoint inhibitors plus anti-angiogenic agents, and immune checkpoint inhibitors plus tyrosine kinase inhibitors. Results of recent clinical studies are presented, along with a brief overview of ongoing and future trials.

Expert opinion: The approval of atezolizumab plus bevacizumab and the positive results of the HIMALAYA trial have broadened the therapeutic scenario for advanced HCC, opening, at the same time, new challenges. First of all, predictive biomarkers to allocate patients to the best treatment are eagerly required; second, specific studies are urgently needed to define the use of new combinations in patients usually excluded from clinical trials, e.g. those with deranged liver function and HIV or transplant recipients. Finally, with new combinations being translated into earlier stages, profound changes are soon expected in the adjuvant and neoadjuvant setting.

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1. Introduction

Hepatocellular carcinoma (HCC) is a highly aggressive cancer type and currently ranks sixth by incidence rate and fourth by mortality rate of all cancers worldwide [1]. Both environmental and genetic risk factors contribute to the development of HCC, which constitutes a heterogeneous spectrum of disease manifestations with different etiologies, genomic drivers, and immune microenvironments. Despite increasing awareness of etiologic and prognostic risk factors, most patients present with unresectable advanced HCC at the time of diagnosis, and less than 30% are eligible for curative treatment options [2]. Early-stage tumors (Barcelona Clinic Liver Cancer-BCLC 0/A) can be cured through successful surgical procedures such as liver transplant or local resection or with radiofrequency ablation (RFA). Locoregional therapies, including transarterial chemoembolization (TACE) and transarterial radioembolization (TARE), compose major alternative approaches; however, their feasibility largely depends on tumor location, burden, and liver function status [3]. For intermediate and advanced-stage HCC (BCLC-B and BCLC-C), the intent of treatment is not curative, and the majority of patients will eventually die from HCC.

In 2008, sorafenib, a multi-targeted tyrosine kinase inhibitor (TKI), has been the first systemic therapy approved to treat advanced or intermediate-stage HCC nonamenable for locoregional therapy based on the results of the phase III SHARP trial [4]. Later on, three additional TKIs, lenvatinib, regorafenib, and cabozantinib, have been approved and became available for use in first-line (lenvatinib) and second-line (regorafenib and cabozantinib) treatments [5–7]. In addition, ramucirumab, a vascular endothelial growth factor (VEGF) receptor-2 monoclonal antibody, has shown significant survival benefit post-sorafenib in patients with increased AFP (>400 ng/ml) [8]. Recent developments in the field of immune-based therapies have ushered a new era in the management of unresectable HCCs. While immune checkpoint inhibitor (ICI) monotherapy achieves good responses in up to 15–20% of patients, inhibitors of the programmed cell death-1 (PD-1) pathway did not demonstrate superiority to TKIs in phase III trials [9,10]. In 2020, the IMBrave150 study provided evidence of survival benefit for the combination of atezolizumab, an anti-PD-L1 monoclonal antibody, plus bevacizumab over sorafenib [11]. In 2021, the combination of tremelimumab, an anti-CTLA-4 antibody to durvalumab, and anti-PD-L1 antibody has also demonstrated clinically

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Article highlights

- HCC has an immune-exhausted microenvironment characterized by a prevalence of regulatory and immune-suppressive cells, which ease tumoral immune-escape and hamper the response to single-agent anti-PD-1/PD-L1 therapy.
- The combination of anti PD-1/PD-L1 with anti CTLA-4 with tyrosine kinase inhibitors or anti-angiogenic agents has been reported to overcome the intrinsic immune-resistance of HCC.
- The IMbrave 150 phase III trial has established the superiority of atezolizumab plus bevacizumab over sorafenib in terms of overall survival, progression-free survival, and objective response rate. The combination is now the new standard of care for advanced HCC.
- The HIMALAYA phase III trial has recently showed that the combination of single dose tremelimumab plus durvalumab prolongs median overall survival compared to sorafenib. At the same time, durvalumab monotherapy has been reported to be non-inferior to sorafenib.
- The association of ipilimumab and nivolumab, after positive results in the phase I/II CheckMate-040 study, is currently being tested against sorafenib or lenvatinib as first line for advanced HCC in the phase III CheckMate 9DW trial.
- The association of lenvatinib and pembrolizumab yielded promising results in the dedicated phase Ib study and is currently under investigation in the phase III LEAP-002 trial. The accrual has been completed, and the results are currently pending.
- Predictive biomarkers of response to each strategy are lacking but eagerly required to guide treatment decisions.
- There is still a conspicuous proportion of patients who are not eligible for new combinations. Specific studies should be conducted to define the best treatment for Child-Pugh B patients, HIV positive, and transplant recipients.
- The use of immune-based combinations in earlier stages will be defined in the next few years.

and statistically meaningful survival benefit over sorafenib [12]. These two regimens have entered the latest Barcelona Clinic Liver Cancer (BCLC) treatment algorithm update in 2022 as recommended therapies for advanced stage HCC [13].

Encouraged by the positive trial results for unresectable HCCs, a large number of randomized controlled trials (RCT) are currently testing ICIs in combination with other systemic or locoregional therapies across different stages of the disease. Use of ICIs in HCC is supported by a strong preclinical and biologic rationale, given the high prevalence of immune-exhausted T-cell responses within the tumor/stroma immune interface [14]. HCC cells are in fact known to evade host immunity through a multiplicity of mechanisms including overexpression of negative regulators of T-cell immunity in the tumor microenvironment. A key mechanism of action of ICIs is to revert cancer-related immunosuppression and to restore anti-cancer immunity [15] by targeting co-inhibitory receptors expressed on T cells or their ligands on antigen-presenting cells. A number of key actionable drivers of anti-cancer immunity are being identified for their clinically meaningful immune-reconstitution potential and include, among others, the cytotoxic T-lymphocyte-associated protein 4 (CTLA4; CD152) – CD80/86 (B7-1/B7-2), programmed death-1 (PD1; CD279) – programmed death ligand-1 (PD-L1; B7-H1, CD274), lymphocyte-activation gene 3 (LAG3) – MHC-I-II, T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) – galectin-9 (GAL9) [16]. Among them, CTLA-4 and PD-1 are the best characterized pathways, having led to transformative changes in the clinical management of growing number of malignancies including HCC.

With the liver being a profoundly immunesuppressive organ and taking into account the strongly inflamed cirrhotic background that contributes to the pathogenesis of over 80% of the cases of HCC [17], inhibition of a single pathway including for instance CTLA-4 or PD-1 has led to low-level responses and suggests the need to integrate an ICI backbone with concomitant inhibition of multiple pathways.

In this review, we will describe immunologic rationale and corresponding clinical data in support of ICI combinations in HCC focusing in particular on dual checkpoint combinations, combinations between ICIs, and anti-VEGF therapies as well as ICI-TKI combinations.

1.1. Dual checkpoint inhibitors: preclinical rationale

The liver is constantly exposed to exogenous antigens originating from the inward portal circulation. An immune-tolerant microenvironment is therefore required to avoid spontaneous triggering of liver-related and systemic inflammatory responses [18]. Key mechanisms of tolerance within the liver relate to the high prevalence of immune-regulatory cells, to immunosuppressive cytokines such as IL-10, TGF-beta, and prostaglandin E2 (PGE2), and by low expression of Major Histocompatibility Complex (MHC) class II and co-stimulatory ligands such as CD80 and CD86 [19]. Furthermore, most of the cases of HCC arise on the background of chronic liver inflammation, either driven by viral infections (HBV and HCV), metabolic factors (NASH/NAFLD), or exogenous toxins (alcohol related disease), which further promote immune suppression through various mechanisms, including T-cell exhaustion, MHC loss, T-reg differentiation, and metabolic alteration of the microenvironment (e.g. arginine depletion and disruption of cholesterol homeostasis) [17]. The overexpression of regulatory checkpoint molecules such as PD-1, CTLA-4, TIM-3, LAG-3, and OX-40, is one of the key mechanisms leading to immune exhaustion, a process characterized by suppression of effector T-cell activity in the context of both chronic inflammation and cancer [17]. The immune dysregulation associated with chronic inflammation contributes to promote both HCC development and progression, and these factors, taken together, suggest a strong rationale for the use of immunotherapy in HCC. Clinically available immunotherapies for HCC include ICIs targeting the PD-1/PD-L1 axis and the CTLA-4 pathway. Clinically, responses to CTLA-4 monotherapy are low and short lived [20]. The interaction between PD-1 and its ligands—PD-L1 and PD-L2—suppresses CD8+ T-cell function, promoting the phosphorylation of activating kinases [21]; therefore, anti PD-1/PD-L1 antibodies act mainly restoring the effector capacity of CD8+ T cells. Physiologically, CTLA-4 is expressed on activated T cells, and it exerts its action within the immune synapse, preventing the interaction between the co-stimulatory molecule CD28 and its ligand B7 on antigen-presenting cells [22]. Thus, anti-CTLA4 antibodies facilitate the interaction between CD28 and B7, increasing the number of

naïve CD4+ and CD8+ T cells. Combining anti PD-1/PD-L1 with anti-CTLA4 antibodies has been proven to be more effective single-agent ICI in several malignancies, as a likely consequence of synergy stemming from co-inhibition of both pathways. The increased risk of toxicity from cumulative exposure to CTLA-4 and PD-1 pathway inhibitor suggests the clinical challenge of uncoupling toxicity from efficacy and further highlights the urgent need to develop predictive factors of response to ICI therapy [23].

1.2. Dual checkpoint inhibitors: clinical trials

Data from oncological indications other than HCC have eloquently demonstrated that the blockade of two checkpoint pathways is feasible and characterized by enhanced ICI monotherapy. In HCC, PD-1/PD-L1 inhibitors are associated with response rates of <20% [9,10], whereas the association of anti-PD-1/anti PD-L1 with anti-CTLA-4 has recently been demonstrated to be a successful strategy in both first- and second-line settings [24]. The CheckMate-040 phase I/II trial has been the first study to report on dual checkpoint inhibition in HCC. Overall, 148 patients with advanced HCC previously treated with sorafenib were randomized to receive the combination of the anti-PD-1 nivolumab with the anti-CTLA-4 ipilimumab at three different doses [25]. Participants enrolled in arm A received ipilimumab 3 mg/kg (4 doses) and nivolumab 1 mg/pro kg every 3 weeks and achieved the best results, reporting an objective response rate (ORR) of 32% (vs 27% and 29% in arms B and C, respectively) and a median overall survival (mOS) of 22.8 months (vs 12.5 and 12.7 months in arms B and C, respectively). An important finding from the study is the association between improved outcome at the expense of higher toxicity: with 94% of arm A participants reporting any grade of treatment-related adverse event (TRAE), compared to 71% in arm B and 79% in arm C, and 18% of patients in arm A interrupted the treatment due to TRAEs; the incidence of immune-related AEs was higher in arm A. Overall, about 10% of subjects with viral hepatitis reported virologic breakthrough even if without any repercussions on liver function, and this confirm the feasibility of dual checkpoint inhibition also in virally induced HCC. Efficacy results were independent from PD-L1 expression or etiology of chronic liver disease; however, the majority of the population had a viral cause of HCC. Following the CheckMate-040, the combination of ipilimumab and nivolumab at the same dose of arm A was approved by Food and Drug Administration (FDA) for the treatment of advanced HCC in Child-Pugh A patients after sorafenib. The same dosing regimen is being tested in first-line in the phase III CheckMate 9DW trial (NCT04039607), which is randomizing patients to receive ipilimumab plus nivolumab versus sorafenib or lenvatinib.

The combination of durvalumab (anti PD-L1) plus tremelimumab (anti CTLA-4) has showed positive results in both phase II [26] and III studies [12]. The phase II study of this combination demonstrated that a single high dose of tremelimumab (300 mg) followed by durvalumab (1500 mg every 4 weeks) was tolerable and associated with the highest ORR (24%) and median OS (18.5 months) compared to the other arms testing tremelimumab monotherapy, durvalumab monotherapy, and durvalumab plus low dose tremelimumab (75 mg every 4 weeks). Durvalumab monotherapy and the combination of durvalumab plus tremelimumab at the two different doses were brought forward in the subsequent phase III HIMALAYA trial (NCT03298451). Overall, the study randomized 1324 Child-Pugh A patients, with advanced HCC without main portal vein tumor thrombosis to receive sorafenib, durvalumab plus tremelimumab (750 mg single dose), durvalumab plus tremelimumab (75 mg every 4 weeks), or durvalumab alone. Recruitment to low-dose tremelimumab was prematurely closed as no differences with durvalumab alone emerged at the interim analysis. The primary endpoint was OS, evaluated comparatively across the high dose tremelimumab plus durvalumab cohort versus sorafenib. Secondary endpoints include PFS, ORR, DOR, and OS of durvalumab monotherapy versus sorafenib, the latter being tested for non-inferiority with a 1.08 margin. The study met its primary endpoint and reported a median OS of 16.4 months for the experimental arm compared to 13.8 months for sorafenib (HR: 0.79; CI: 0.65–0.93; p = 0.0035). Durvalumab monotherapy was proven to be non-inferior to sorafenib (HR: 0.96, CI: 0.73–1.03), reporting a median OS of 16.6 months. The combination yielded an ORR of 20.1%, compared to 5.1% in patients receiving sorafenib. The safety profile of the combination mirrored that reported in the phase II study, showing an incidence of grade 3 or higher AEs in the combination of 25.8%, compared to 12.9% for durvalumab monotherapy and 36.9% for sorafenib [12].

The positive results of the HIMALAYA trial and the good tolerability resulted in recommendation by the BCLC 2022 guidelines [27], pending regulatory approval.

Overall, no new safety concerns were raised, and the risk of bleeding was not increased in patients treated with the experimental regimen. However, some considerations about the safety of dual checkpoint inhibition in HCC patients should be made. In particular, even if the risk of immune-related adverse events (irAE) is not increased in the presence of cirrhosis, specific expertise is required for the diagnosis and management of irAE in patients with underlying liver disease. In fact, symptoms of irAE could overlap to the extra-hepatic manifestation of cirrhosis, and from the one side, delaying the diagnosis of an immune-related adverse event could lead to life-threatening consequences, and from the other, starting immunosuppressive treatments without a real need in the presence of chronic liver disease might worsen hepatic function [28].

Dual checkpoint inhibition is being translated into earlier stages in both the adjuvant and neoadjuvant settings. The rationale for the neoadjuvant use is to boost the immune system prior to resection to both achieve response rate to ease surgery, to reduce the incidence of early recurrence, and to ideally improve OS. Several combinations are being explored in early phase studies; currently no phase III trial testing dual checkpoint inhibition is being recruited in the adjuvant or neoadjuvant setting. The association of low-dose ipilimumab and nivolumab is currently being explored in a Ib study in operable HCC [29]. The same combination, administered with a different dosing schedules, is also the objective of an ongoing phase II study (NCT03510871). Same combinations

have been proposed in the adjuvant setting after locoregional therapies or surgery (NCT04340193). The association of immunotherapy with locoregional therapies relies on the hypothesis that checkpoint inhibitors can further stimulate immune system against tumoral antigens released after locoregional treatments [30]. Durvalumab and tremelimumab are further being investigated in early phase studies, in association with TACE (NCT03638141) or TARE (NCT04605731).

The combination of immune checkpoint inhibitors is emerging as a practice changing strategy in advanced HCC, and results from ongoing clinical trials beyond advanced HCC are eagerly awaited to define its role across the various stages of the disease. Blockade of other immune-checkpoint pathways including TIM-3, LAG-3, TIGIT, and OX-40, alone or in combination, even if not specifically tested in HCC yet, is the objective of basket early-phase trials and could potentially enter the therapeutic armamentarium in the future, should early experience justify expansion to HCC (NCT04215978) (NCT04370704).

1.3. Open issues

With several phase III trials being conducted in the first-line setting, treatment choice and sequencing remain main challenges for clinicians treating HCC patients. All the most recent phase III trials have been conducted to test new treatments against sorafenib or lenvatinib, which were the standard-of-care at the time of trial design but are no longer the only first-line option. Therefore, no direct comparisons between new treatment strategies are available, and they are unlikely to be prospectively conducted in the near future. The choice among different treatment options depends on patients' characteristics including baseline comorbidities, predicted risk of toxicities, patients' and physicians' preference, and local reimbursement considerations. Further studies are urgently required to identify predictive and prognostic biomarkers to select the best therapy for each patient [31].

Another open issue stems from the broadening of ICI therapy to earlier stages of HCC. Dual immune-checkpoint inhibitors in the neoadjuvant setting are being investigated in early phase trials, and they are expected to provide potentially practice changing results. However, the use of this strategy in transplant candidates is still controversial and not recommended outside of clinical trials due to the intrinsic risk of organ rejection following immune system stimulation, which also limits the design of specific studies [32,33].

At the same time, the use of dual checkpoint inhibitors in patients with autoimmune disease or HIV has not been thoroughly investigated, even though real-world evidence from other oncological indications suggests the feasibility of this approach even in these subgroups [34,35]. Patients with Child-Pugh B liver dysfunction are another subgroup usually excluded from clinical trials but conspicuous in clinical practice [36]. The rationale for excluding these subjects derives from the presence of underlying liver dysfunction, which acts as a competitive risk factor for mortality and treatment-related hepatic decompensation. Real-world evidence suggests that Child-Pugh B patients treated with ICI monotherapy have a worse OS compared to Child-Pugh A patients, but they do not have a higher risk of toxicities [37]. Safety of dual checkpoint inhibitors in Child-Pugh B patients is currently lacking, and it is not likely to be investigated in phase III trials.

1.4. Checkpoint inhibitors plus anti-VEGF: preclinical rationale

The induction of angiogenesis is one of the hallmarks of cancer [38], and it plays a major role in HCC tumorigenesis. HCC is a richly vascularized tumor, as it relies on aberrant neoangiogenesis for tumor growth and distant metastasis. The pathogenesis of HCC is driven by several pro-angiogenic factors, the most relevant of which is the VEGF family (A-E) and their receptors (VEGFR1-3) [14]. Among the five isoforms of VEGF, VEGF-A, upon interaction with its receptor VEGFR-2, plays a predominant role in determining neoplastic angiogenesis [39].

In particular, the increased tumor VEGF expression is linked to higher vascular density, tumor invasiveness, and metastasis, eventually leading to poor prognosis [39]. The whole range of targeted therapies approved for HCC treatment are characterized at least in part by an antiangiogenic mechanism of action. However, in addition to its widely studied role in promoting angiogenesis, a whole body of research has unraveled the immunomodulatory role of VEGF and the possible interactions between the VEGF pathway and the anti-cancer immune response [40]. The intracellular signaling induced by VEGFR activation can impair the immune-mediated antitumor effect by acting on the innate and the adaptive immunity. For instance, high levels of VEGF are associated with an increase of immature dendritic cells, and in mouse models, VEGF has shown to inhibit the expression of the nuclear factor-κB (NF-κB), a key effector of immune response [41]. VEGF pathway exerts an immunosuppressive effect by acting on a number of different cell types, including the immunomodulatory myeloid-derived suppressor cells (MDSCs) and the tumor-associated macrophages (TAMs), whose enrichment within the tumor microenvironment (TME) correlates with poorer prognosis. Furthermore, the majority of pro-angiogenic factors negatively interacts with the T-cell compartment of the TME by inhibiting replication and the cytotoxic action of T cells and inducing the expression of PD-1 on intratumoral CD-8+ T cells. The VEGF pathway further induces an immunosuppressive switch by promoting the expression of Fas ligand (Fas-L) on endothelial cells, thus leading to selective apoptosis in effector CD8+ T cells but not in immunosuppressive regulatory T cells (T-regs) [42].

Overactivation of the VEGF pathway mainly promotes aberrant neoangiogenesis. The dysfunctional vessels show altered permeability, mechanically impairing the physiological immune cells extravasation and increasing local hypoxia, which is a known factor of immune escape [43].

However, the use of antiangiogenic agents can revert VEGF-induced immunosuppression.

The use of anti-angiogenic agents has been shown to have a pleiotropic range of immune-stimulating effects by counterbalancing VEGF-mediated immunosuppression. Both in mouse models and in humans, evidence shows that VEGF-targeting agents can boost dendritic cell maturation and their

proliferation in lymphoid organs, while reducing the concentration of MDSCs in peripheral blood [42]. Furthermore, antiangiogenic therapies modulate the accumulation of immunosuppressive T-regs in various mouse models by blocking the conversion of conventional CD4+ Foxp3- T cells into regulatory CD4+ Foxp3+ T-cells and by reducing the proliferation of preexisting T-regs [44].

Based on this evidence, the strategy of combining antiangiogenic agents with immune checkpoint inhibitors has unsurprisingly been shown to exert a synergistic immune-mediated antitumor effect, as shown by increased responsiveness to combination therapy versus PD-1 axis inhibition alone. Preclinical models have demonstrated that the inhibition of PD-1 can enhance the restoring of a physiological angiogenesis induced by anti-VEGFR2 agents, while the use of an antiangiogenic agent can overcome tumor resistance to immune checkpoint inhibitors [45]. Also, the concomitant inhibition of the VEGF and PD-1/PD-L1 pathways can reprogram the tumor microenvironment by shifting the balance between M1 (antitumoral) and M2 (immunosuppressive) TAMs and by reducing the presence of T-regs.

1.5. Checkpoint inhibitors plus anti VEGF: clinical trials

Based on a strong preclinical rationale, the combination of immune checkpoint inhibitors and anti-VEGF monoclonal antibodies has rapidly entered clinical testing in patients with advanced HCC. After the promising safety and efficacy signals in the phase Ib GO30140 trial, with an ORR of 36% [46], the combination of atezolizumab, an anti-PD-L1 inhibitor, and bevacizumab was investigated in the randomized phase III IMbrave150 trial, randomizing a total of 501 patients with advanced HCC naïve to any prior systemic treatment to receive either the combination or sorafenib in a 2:1 ratio [11]. After an updated median follow-up of 15.6 months [47], atezolizumab and bevacizumab therapy was shown to extend the median OS to 19.2 versus 13.4 months in the sorafenib arm (hazard ratio [HR] 0.66; 95%CI, 0.52–0.85; p = 0.0009), together with a significant improvement of the median PFS to 6.9 months versus 4.3 months (HR 0.65; 95%CI 0.53–0.81; p = 0.0001) [11]. The updated overall response rate (ORR) evaluated by independent review per response evaluation criteria in solid tumors (RECIST) criteria v1.1 was 29.8% (95% CI, 24.8–35.0) with atezolizumab plus bevacizumab versus 11.3% (95% CI, 6.9–17.3) with sorafenib, with 7.7% patients treated with the combination achieving a complete response (CR). The most common G ≥ 3 treatment-related AEs (TRAEs) of the combination were hypertension (10.3%), aspartate aminotransferase (AST) increase (4.3%), and proteinuria (2.7%), mirroring the two known different spectra of immune-related and antiangiogenic-related toxicity. Atezolizumab plus bevacizumab outperformed sorafenib also in terms of the quality of life (QoL) [48]. The analyses of the patient-reported outcomes (PROs) showed that the combination achieved a clinically meaningful delay time-to-deterioration of QoL, with a significant reduction of the risk of deterioration for a number of cancer-related symptoms. In light of these results, atezolizumab plus bevacizumab has become the new standard of care for first-line treatment of patients with unresectable or metastatic HCC, leaving TKIs (sorafenib and lenvatinib) to patients unfit for the combination or with contraindications to either drug [27,49].

The same combination is currently being investigated alone or in combination with TACE in intermediate stage HCC (NCT04712643) (NCT04803994) and as adjuvant treatment after surgical resection or ablation (NCT04102098) (32352320).

A similar treatment option was tested in the ORIENT-32 [50], a phase II/III study investigating the combination of sintilimab, an anti-PD-1 mAb, and a bevacizumab biosimilar in a mainly HBV-predominant Chinese population of patients with unresectable or metastatic HCC. A total of 595 patients were randomized in a 2:1 ratio to either the combination or sorafenib. After a median follow-up of 10.0 months, the experimental treatment met both the co-primary endpoints, with a median PFS of 4.6 months [95% CI 4.1–5.7] for the experimental arm versus 2.8 months [95% CI 2.7–3.2] for sorafenib (HR 0.56, 95% CI 0.46–0.70; p < 0.0001); at the time of data cutoff, less than 50% of patients in the experimental arm had died, and so the median OS could not be calculated, but the combination showed a 43% reduction in the risk of death (median not reached [NR] versus 10.4 months [8.5–NR] for sorafenib; HR 0.57, 95% CI 0.43–0.75; p < 0.0001). The independently assessed ORR per RECIST criteria v1.1 was 21% (95% CI, 17–25), compared to 4% (95%CI, 2–8) for sorafenib. The safety profile was not dissimilar to the IMbrave150 study findings, with the most frequent grade 3 or above treatment-emergent AEs being hypertension (14%), decreased platelets count (7%), and proteinuria (5%).

The combination of durvalumab (anti-PD-L1) plus bevacizumab is being investigated in the adjuvant settings after TACE and RFA/surgery in the EMERALD-1 (NCT03778957) and 2 trials. EMERALD-1 is a phase III trial, randomizing patients with intermediate stage HCC to receive either TACE, or TACE followed by durvalumab plus placebo, or TACE followed by durvalumab plus bevacizumab. The trial has completed accrual, and the results are currently pending (NCT03778957). EMERALD-2 study is another phase III trial currently recruiting patients to be randomized to receive placebo, durvalumab plus placebo, or durvalumab plus bevacizumab after curative resection or ablation (NCT03847428).

1.6. Checkpoint inhibitors plus anti-VEGF: open issues

The main limitation to the use of anti-angiogenic agents and bevacizumab, in particular, is related to the risk of gastrointestinal bleeding in HCC patients [51]. Rate of bleeding events was reported at 7% in patients recruited to the IMbrave150 trial; however, rates of severe gastrointestinal bleeding was less than 2% [11]. Assessment of esophageal varices through esophago-gastro-duodenoscopy was mandatory 6 months before entering the trial, and high-risk varices were required to be treated according to local guidelines. This recommendation has been translated into clinical practice: in case of high-risk varices requiring endoscopic treatment, bevacizumab

should not be administered for 2–6 weeks after definitive treatment [17]. Delayed access to endoscopy in the routine practice and the requirement to hold bevacizumab until adequate treatment of varices has been achieved are important factors to consider as they may impact the clinical outcomes.

Only patients with preserved liver function (Child-Pugh A) were included in IMbrave150 and ORIENT-32 studies, and as a consequence, the safety and efficacy of the combination in Child-Pugh B patients are unknown. Recently, real-life data showing the feasibility of this approach in Child-Pugh B patients are emerging [52], and the association of atezolizumab plus bevacizumab is being prospectively investigated in a phase II trial specifically conducted in Child-Pugh B7 patients (NCT04829383).

A subgroup analysis of the IMbrave 150 showed that the benefit of the combination is not significant in patients with non-viral liver disease [11], and recent translational data suggest that nonalcoholic steatohepatitis (NASH) hampers response to anti PD-1 therapy in mice models [53]. Currently, there are no definitive data to recommend a different treatment in patients with non-viral underlying liver disease; in fact, patients enrolled in the IMbrave 150 were not stratified according to HCC etiology, and preclinical data coming from mice models might not exactly reflect the complex heterogeneity of non-viral hepatitis in humans. However, this evidence highlights the need to stratify patients according to HCC etiology in clinical trials.

1.7. Checkpoint inhibitors plus tyrosine kinase inhibitors: preclinical rationale

TKIs are promiscuous inhibitors of a number of signal transduction pathways including VEGF, epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDGF), and fibroblast growth factor (FGF), albeit with different affinity. Sorafenib, regorafenib, lenvatinib, and cabozantinib have all been shown to have activity against HCC in the clinical setting, with the common mechanism of action being anti-angiogenesis. Moreover, the TKIs all are immuno-modulatory, enhancing the effect of immunotherapy. Preclinical work in HCC illustrates that the TKI, sorafenib, induces hypoxia and over-expression of PD-L1 within the tumor, resulting in accumulation of T-reg and M2-macrophages [54,55]. Moreover, in an elegant study by Shigeta and colleagues, dual blockade with anti-PD-1/VEGFR-2 therapy significantly inhibited HCC growth and improved survival in vivo. The authors illustrated that dual therapy resulted in an increase in cytotoxic T-cell infiltration and activation, an increase in M2 tumor-associated macrophages, and a reduction in T-regs [56]. Normalization of vessel architecture with dual therapy was also observed lending preclinical support for the use of combination ICI and anti-angiogenic therapy in the clinical setting. The authors illustrated similar normalization of tumor vasculature with regorafenib and increased CD8 T cell infiltration with combined PD-1 therapy [56]. Moreover, they reported an increased concentration of regorafenib within the tumor. When considering combination of lenvatinib and PD-L1 inhibition, a number of preclinical studies illustrate activation of immune pathways, reduction of regulatory T-cells infiltration within the tumors, and inhibition of TGFβ signaling and enhanced interferon-γ signaling, resulting in tumor regression [57–60]. These preclinical studies clearly highlight the biological rationale behind the use of combination immune checkpoint inhibition (ICI)-TKI therapy for HCC. There has been considerable interest in the combination of TKIs and immunomodulatory agents, with over 45 ongoing trials investigating this combination therapy (Table 1).

1.8. Checkpoint inhibitors plus tyrosine kinase inhibitors: clinical trials

There are a number of combination studies of TKIs and immunotherapy in the first-line setting, the majority of which are with lenvatinib, a TKI that has a strong affinity for VEGF receptors 1–3 and FGF receptors 1–4. Finn et al. recently evaluated the combination of lenvatinib and pembrolizumab in a phase Ib trial in 104 patients [61]. The median duration of follow-up was 10.6 months with an objective response of 46% per mRECIST and 36% per RECIST reported. The median progression-free survival by IIR was 9.3 months per mRECIST and 8.6 months per RECIST v1.1, and the median overall survival was 22 months. Importantly, no dose-limiting toxicities were observed. The results of the confirmatory phase III study are awaited for readout in late 2022 (NCT03713593). A further, phase III study of toripalimab combined with lenvatinib versus lenvatinib with placebo in 519 participants with advanced HCC (NCT04523493) is ongoing. Several other clinical trials are ongoing, investigating lenvatinib combined with different type of anti-PD-1/PD-L1 (NCT04740307, NCT03418922, NCT03841201, NCT04443309, NCT04542837, NCT04401800, NCT04444167, NCT04728321, NCT04368078, NCT04523493, NCT04194775, and NCT04425226).

Cabozantinib predominantly inhibits RET, MET, and VEGFR and has been already approved as a monotherapy for patients progressing under sorafenib [7]. It has been tested in association with ICI in different settings. The combination of cabozantinib and atezolizumab has been investigated as first-line treatment in the multi-arm phase III COSMIC-312 trial, testing the combination versus sorafenib, and cabozantinib monotherapy versus sorafenib, OS, and PFS was selected as co-primary endpoints. Recently, results of the first interim analysis have been announced, showing a significant benefit in terms of PFS (6.8 vs 4.2 months; HR: 0.63; 99% CI 0.44–0.91) but not an OS advantage (15.4 vs 15.5 months; HR: 0.90;96% CI 0.69–1.18) for the combination arm and an ORR of 11% for atezolizumab plus cabozantinib. Final results are awaited to determine if the trial will be formally positive, meeting both the co-primary endpoints, and to determine if PFS is a reliable surrogate of OS in HCC. Differently from previous studies, COSMIC-312 was stratified according to HCC etiology, and the highest benefit for the combination, for both OS and PFS, was observed in HBV patients [62]. Nivolumab is a human IgG4 monoclonal antibody that blocks interaction with PD-L1 and PD-L2. Yarchoan et al. investigated the combination of cabozantinib and nivolumab in the neoadjuvant setting in 15 patients with locally advanced or borderline resectable HCC in an open-label single-arm phase 1 trial (NCT03299946).

Table 1. Summary of the main trials that are currently recruiting patients with HCC to test immunotherapy combinations.

Name	Status	Phase	Setting	Arms	Primary endpoint (s)
NCT03211416	Recruiting	Ib/II	Advanced HCC, first line	Pembrolizumab + sorafenib	ORR
NCT03439891	Recruiting	II	Advanced HCC, first line	Nivolumab + sorafenib	ORR, safety
NCT02988440	Completed	Ib	Advanced HCC, first line	PDR001 + sorafenib	Safety
NCT04069949	Recruiting	I/II	Advanced HCC, first line	Toripalimab + sorafenib	PFS, safety
NCT0416323	Recruiting	III	Advanced HCC, first line	PD-1 + sorafenib versus sorafenib	DFS
NCT04770896	Recruiting	III	Advanced HCC, second line after atezolizumab plus bevacizumab	Atezolizumab + lenvatinib/sorafenib versus lenvatinib/sorafenib	OS
NCT04740307	Recruiting	II	Advanced HCC, first line	Pembrolizumab/quavonlimab + lenvatinib	ORR, safety
NCT03713593	Recruiting	III	Advanced HCC, first line	Pembrolizumab + lenvatinib versus lenvatinib	OS, PFS
NCT03841201	Recruiting	II	Advanced HCC, first line	Nivolumab + lenvatinib	ORR, Safety
NCT04443309	Recruiting	I/II	Advanced HCC, first line	Camrelizumab + lenvatinib	ORR
NCT04401800	Recruiting	II	Advanced HCC, first line	Tislelizumab + lenvatinib	ORR
NCT04728321	Recruiting	II	Advanced HCC, first line	AK104 + lenvatinib	ORR
NCT04523493	Recruiting	III	Advanced HCC, first line	Toripalimab + lenvatinib versus lenvatinib	OS, PFS
NCT04194775	Recruiting	III	Advanced HCC, first line	CS1003 + lenvatinib versus lenvatinib	OS, PFS
NCT04696055	Recruiting	II	Advanced HCC, second line after ICI-based therapy	Pembrolizumab + regorafenib	ORR
NCT04170556	Recruiting	I/IIa	Advanced HCC, second line after sorafenib or atezolizumab plus bevacizumab	Nivolumab + regorafenib	Safety
NCT04310709	Recruiting	II	Advanced HCC, first line	Nivolumab + regorafenib	ORR
NCT04718909	Recruiting	II	Advanced HCC, second line after sorafenib or lenvatinib therapy	Sintilimab + regorafenib versus regorafenib	PFS
NCT04183088	Recruiting	II	Advanced HCC, first line	Tislelizumab + regorafenib	ORR, safety, PFS
NCT04442581	Recruiting	II	Advanced HCC, first line	Pembrolizumab + cabozantinib	ORR
NCT04503902	Recruiting	I/II	Advanced HCC, first line	Toripalimab + donafenib	ORR, Safety
NCT03970616	Recruiting	Ib/II	Advanced HCC, first line	Durvalumab + tivozanib	Safety
NCT04601610	Recruiting	Ib/II	Advanced HCC, first line	KN046 + nigantinib	ORR, Safety
NCT04639180	Recruiting	III	Adjuvant after curative resection or ablation	Camrelizumab + apatinib	RFS
NCT04418401	Recruiting	I	Adjuvant after curative resection	Donafenib plus anti PD-1	RFS
NCT04615143	Recruiting	II	Neoadjuvant, resectable HCC	Tislelizumab or tislelizumab + lenvatinib	DFS
NCT04615143	Recruiting	I	Neoadjuvant/adjuvant, resectable HCC	Nivolumab or nivolumab +relatlimab	Safety
NCT03847428	Recruiting	III	Adjuvant after curative resection or ablation	Durvalumab or durvalumab + bevacizumab versus placebo	RFS
NCT03682276	Recruiting	I/II	Neoadjuvant, resectable HCC	Ipilimumab + nivolumab	Safety
NCT04472767	Recruiting	II	Adjuvant after trans-arterial chemo-embolization in intermediate stage HCC	Ipilimumab + nivolumab + cabozantinib	PFS; Rate of complete response
NCT04803994	Recruiting	III	Intermediate stage HCC	Atezolizumab + bevacizumab Versus trans-arterial chemo-embolization	Time to failure of treatment strategy
NCT04857684	Recruiting	I	Neoadjuvant, resectable HCC	Atezolizumab + bevacizumab + Stereotactic Beam Radiation Therapy	Safety
NCT04541173	Recruiting	II	Intermediate stage HCC	Y-90 radio-embolization versus Atezolizumab + bevacizumab + Y-90 radio-embolization	PFS
NCT05097911	Recruiting	I	Advanced HCC, first line	MTL-CEBPA + atezolizumab + bevacizumab	ORR

Fourteen patients completed neoadjuvant therapy and 12 patients underwent successful R0 surgical resection. Five out of 12 resected had a major or complete pathological response [63]. This trial is ongoing and expected to be completed in March 2022. There are several clinical trials testing cabozantinib with other immune checkpoint inhibitors (NCT03170960, NCT03755791, NCT03539822, NCT04442581, and NCT04514484) (Table 1).

Avelumab is an antibody that binds to PD-L1, inhibiting interaction with PD-1 and PD-L1. Axitinib is a second-generation tyrosine kinase inhibitor that inhibits VEGFR 1–3, thus blocking angiogenesis, tumor growth, and metastases. In the VEGF Liver 100 trial, Kudo et al. tested avelumab and axitinib in 22 patients with advanced/metastatic HCC in a phase 1b study for a minimum follow up time of 18 months [64]. The overall median survival was 14.5 months. Progression-free survival was 5.51 months, and 13.6% achieved an objective response per RECIST 1.1 and 31.8% per mRECIST for HCC. The median duration of response was 7.29 months (95% CI 3.71–12.94). Additionally, no grade 4 TRAEs or treatment-related deaths occurred. Similarly, the combination of regorafenib with pembrolizumab (NCT03347292) is being investigated in the first-line setting. Sorafenib combination therapy with other immune checkpoint inhibitors is also in progress (NCT03439891, NCT02988440, NCT04069949, NCT0416323, NCT04770896, NCT03211416, and NCT04163237).

Combination of ICIs and TKIs has not been largely investigated beyond first line. The association of the anti-PD-1 camrelizumab and the selective oral VEGF-2 TKI apatinib has been tested in the phase II RESCUE trial, in both first- and second-line settings [65]. Overall, 120 out of 190 participants were treated in the second line. The combination reported a tolerable safety profile and signs of activity independently from previous treatment . However, none of the patients enrolled in the RESCUE trial had received prior ICI-based treatment [65]. The IMbrave251 (NCT04770896) is currently investigating the combination

of atezolizumab with either lenvatinib or sorafenib versus lenvatinib or sorafenib monotherapy following progression to atezolizumab and bevacizumab.

1.9. Open issues

The main issue with the use of combination therapy stems from the increased risk of additive adverse effects. Finn et al. reported TRAEs in 95% of patients treated with combinatorial lenvatinib and pembrolizumab [61]. Overall, 67% of patients had grade ≥3 TRAEs (63% for grade 3, 1% for grade 4, and 3% for grade 5). Three treatment-related deaths were caused by acute respiratory failure, intestinal perforation, and abnormal liver function. TRAEs led to treatment interruption, discontinuation, or dose reduction in 62%, 14%, and 52% of patients, respectively. Similarly, Kudo et al. reported TRAEs in 95.5% of patients, with five (22.6%) patients discontinuing due to adverse events [64]. The most common adverse events reported were hypertension, decreased appetite, and hand-foot syndrome, and ten patients (45.5%) developed immune-related adverse effects such as thyroid disorders, adrenal insufficiency, and rash. The combination of cabozantinib plus atezolizumab resulted in 93% of TRAEs of any grade and 55% of TRAEs of grade 3 or higher, with diarrhea and palmar-plantar erythrodysesthesia occurring in 42% of the participants. The tolerability of ICI-TKI therapy remains to be elucidated compared with TKI monotherapy, and the results of phase III studies are awaited, in particular any quality-of-life outcomes.

Despite the promise of immunotherapy combinations, the response to treatment remains moderate. Indeed, Finn et al. reported an ORR of 30% in atezolizumab/bevacizumab combination therapy, and there is still a proportion of patients that fail to achieve any clinical benefit [11]. Future studies should aim to identify any predictive biomarkers or baseline characteristics that are associated with therapeutic response. Currently, investigation in other biomarkers such as tumor mutational burden, gut microbiome, and other genetic profiles are ongoing; however, the absence of reliable biomarkers of response to TKIs further hampers the discovery of biomarkers for TKIs-based combinations [31].

With the introduction of TKI-ICI combination in the first-line setting, there is now a paucity of evidence for treatments in the second-line setting. Extrapolating from renal cell carcinoma, another tumor driven by angiogenesis, sequential TKI use following ICI therapy is associated with incremental OS benefit, leading to international guidelines to recommend the use of any multi-targeted TKI that has not been used in the first-line setting in combination with ICI, an approach that is gaining traction in HCC [10,66,67]. Another therapeutic approach is the evaluation of novel therapies that target ICI resistance mechanisms or alternate signaling pathways in HCC.

2. Conclusion

In the past decades, ICIs have ushered a paradigm shift in the therapy of cancer, having emerged from novel and experimental therapeutic options to drugs that have now integrated fully into the standard of care. ICI combination therapies have already changed the therapeutic landscape in advanced-stage HCC. With accumulating evidence of benefit, further development of ICI combination therapies promises improvement in the outlook of the various stages of HCC. While the benefit of combination therapy is substantial in terms of OS benefit, several issues still need to be addressed. First, combination therapies are invariably associated with superadded toxicity compared to ICI monotherapy, leading to the need for careful patient selection. Risk of bleeding reduces the suitability to VEGF combinations, whereas risk of immune pathology is a greater concern in checkpoint doublet combinations.

Second, we lack predictive biomarkers to determine the candidates who can benefit from each combination therapy. PD-L1 expression does not aid stratification due to biologic [68] and analytical heterogeneity [69]. The challenge of treating patients with impaired liver function, impaired performance status, and population who were originally excluded from clinical trials (transplant recipients, HIV, etc.) remains a difficult task. Further research efforts are required to optimize the development and delivery of ICI combinations. The balance between efficacy and toxicity of ICIs before might be reached with the integration of molecular identification of immunebiologic traits to predict for treatment response and toxicity.

3. Expert opinion

After initial skepticism due to safety concerns and difficulties in demonstrating OS advantage over sorafenib, ICIs are now an essential component of the treatment algorithm for advanced HCC [27], and almost all the ongoing trials, in both advanced and earlier setting, involve the use of ICIs. In this rapidly changing landscape, there are several open issues, and among them the most relevant are the absence of predictive and prognostic biomarkers to stratify patients; the absence of clear data about the role of ICI monotherapy in HCC; the lack of prospective data defining the best treatment sequencing after progression to ICI-based therapy; the treatment of subgroups generally underrepresented in clinical trials, as elderly, Child-Pugh B patients, HIV positive patients, and patients affected autoimmune diseases; the role of ICI in transplant candidates; and the use of systemic therapy in adjuvant and neoadjuvant settings and in the intermediate stage.

The development of predictive and prognostic markers in HCC has been mainly hampered by the lack of histological samples, which are not always required for diagnostic purposes [70]. Recently, a classification based on the expression of immune-related genes has been proposed [71]. According to this classification, the 'Immune class,' characterized by high immune infiltration and by a genomic signature similar to that of other tumors responding to ICIs, accounts for about 25% of HCC cases. However, it is not clear if this percentage corresponds to those patients better responding to immunotherapy. Prospective studies to validate the predictive role of this classification are required. Similarly, mutations in *CTNNB1*, the gene encoding for beta-catenin, which is mutated in about 30% of HCC, have been associated with resistance to ICIs in both preclinical and

clinical settings [72]; up to date, prospective validation of these findings in patients treated with ICIs, alone or in combination, is lacking. The absence of reliable predictive biomarkers has consequences for both clinicians and researchers: in fact, on the one hand, it limits treatment selection only to clinical factors, and on the other, it prevents the design of clinical trials stratified according to biological criteria. As a consequence, most of the ongoing studies are testing new combinations independently from preclinical data, with the risk to expose patients to potentially toxic drugs without certainty of benefit. The absence of patients' stratification has, at least in part, also contributed to the formal failure of trials testing ICI monotherapy [9,10]: currently, ICI monotherapy, despite showing signs of antitumoral activity and having a better safety profile compared to TKIs [9], is not approved as a first-line treatment for HCC. However, the recent results of the HIMALAYA trial [12], reporting non-inferiority of durvalumab monotherapy compared to sorafenib, have prompted 2022 BCLC guidelines to consider durvalumab monotherapy as a front-line treatment for patients with contraindication to combination strategies, which, however, do not reflect the population treated with durvalumab in the HIMALAYA study [27]. Further studies are required to deliver high-quality data for special populations as Child-Pugh B patients, elderly, patients with HIV or autoimmune diseases, or transplant candidates, who represent a conspicuous percentage of subjects in daily practice but have been too often neglected by clinical trials, and whose management mainly depends on real-world data [37,73,74] and clinicians' experience.

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•• **Phase III trail showing the superiority of atezolizumab plus bevacizumab over sorafenib**

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