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REVIEW

Immune-Based Combination Therapies for Advanced Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is the fourth most frequent cause of cancer-related death worldwide. HCC frequently presents as advanced disease at diagnosis, and disease relapse following radical surgery is frequent. In recent years, immune checkpoint inhibitors (ICIs) have revolutionized the treatment of advanced HCC, particularly with the introduction of atezolizumab/bevacizumab as the new standard of care for first-line treatment. Recently, dual immune checkpoint blockade with durvalumab plus tremelimumab has also emerged as an effective first-line treatment for advanced HCC and most of the research is currently focused on developing combination treatments based mainly on ICIs. In this review, we will discuss the rationale and ongoing clinical trials of immune-based combination therapies for the treatment of advanced HCC, also focusing on new immunotherapy strategies such as chimeric antigen receptor T cells (CAR-T) and anti-cancer vaccines.

Keywords: hepatocellular carcinoma, VEGF, PD-1, tislelizumab, atezolizumab, durvalumab

Introduction

Primary liver cancers represent the seventh most frequently occurring cancer in the world and the fourth most frequent cause of cancer-related death worldwide.[1] Hepatocellular carcinoma (HCC) accounts for more than 75% of all primary liver cancers and its incidence is increasing both in the Americas and in most European countries.[2] Hepatitis B virus (HBV) and hepatitis C virus (HCV) remain the most relevant risk factors worldwide, but their impact is decreasing due to HBV vaccination and effective antiviral treatments for HCV. By contrast, particularly in USA and Europe, non-alcoholic fatty liver disease (NAFLD) is becoming one of the most relevant risk factors, representing the most rapidly growing cause of HCC among patients listed for liver transplantation in USA.[3]

In most cases HCC is diagnosed at advanced stage and up to 70% of resected patients develop disease recurrence for which systemic treatment is required.[4] For many years tyrosine kinase inhibitors represented the only systemic treatment available for advanced HCC. Improvement in terms of median overall survival (mOS) was modest with approximately 12 months with first-line sorafenib or lenvatinib, while cabozantinib, regorafenib and ramucirumab were used as second-line treatment with an improvement in terms of mOS of approximately two months compared to placebo.[5–8] Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of several cancer types and their role has also been investigated in advanced HCC. Despite promising results of first Phase I/II trials with ICIs monotherapy, the subsequent large Phase III trials failed to demonstrate an advantage in terms of OS compared to sorafenib or lenvatinib. In the phase III randomized CheckMate-459 trial, nivolumab (an anti PD-1 antibody) was compared to sorafenib as first-line treatment in advanced HCC. Despite a clinically relevant

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improvement in mOS (16.4 vs 14.7 months) and in overall response rate (16% vs 7%), OS did not reach statistical significance.[9] The results of the phase III RATIONALE-301 study have been recently presented; in this trial, 674 advanced HCC patients were randomized to receive tislelizumab (an anti-PD-1 antibody) or sorafenib as first-line treatment. The study, which was a non-inferiority trial, met its primary endpoints, with an mOS of 15.9 vs 14.1 months and a median progression free survival (mPFS) of 2.2 vs 3.6 months for tislelizumab and sorafenib respectively, while overall response rate (ORR) was markedly improved in tislelizumab arm (14.3% vs 5.4%) [ESMO Congress 2022, LBA36]. Additionally, durvalumab (an anti-PD-1 antibody) proved to be non-inferior compared to sorafenib in the phase III Himalaya trial which will be discussed later.[10] Finally, pembrolizumab (an anti-PD-1 antibody) was tested as second-line treatment in advanced HCC patients whose disease progressed on sorafenib in the phase III KEYNOTE-240 trial. A total of 413 patients were randomized to receive pembrolizumab or placebo, and the experimental arm showed a clinically meaningful improvement in mOS (13.8 vs 10.6 months; HR 0.781; 95% CI, 0.611 to 0.998; p = 0.0238) and ORR (18.3% vs 4.4%; nominal one-sided p = 0.00007), but the study did not meet its primary endpoints as per specified criteria.[11]

Given the modest results shown by ICIs monotherapy, growing attention has been paid to immunotherapy-based combinations with the aim to increase ICIs efficacy. The phase III IMbrave150 trial demonstrated the superiority of atezolizumab (an anti-PD-L1 antibody) plus bevacizumab compared to sorafenib as first-line treatment, thus becoming the new standard of care, and other immunotherapy-based combinations are currently under investigation.[12] In this review, we will discuss immunotherapy-based combinations under investigation for treatment of advanced HCC, focusing on the biological rationale and ongoing clinical trials.

Combination of ICIs Plus mTKI or Anti-VEGF Agents

The introduction of ICIs, such as anti-PD-1/PD-L1 and anti-CTL4, has revolutionized the therapeutic approach to solid tumors. Despite their increasingly widespread employment, the mechanisms which are responsible for the onset of primary and secondary resistance to ICIs have not been well identified and explored yet. Thus, it is also not surprising that the lack of specific and reliable predictors of tumor response to ICIs is still an unmet clinical need. In the attempt to unravel the determinants of tumor resistance, increasing interest has been directed toward tumor microenvironment (TME).[13]

Indeed, many studies suggest a thorough involvement of the TME - as well as paracrine and juxtacrine signaling - as pivotal player both in tumorigenesis and in the development of ICI resistance.[14,15] It is enough to say that either hypoxic environment or the vascular endothelial growth factor (VEGF) secreted by tumor-associated cells induce PD-L1 upregulation, hence resulting in tumor immuno-resistance.[16,17] Similarly, also the intrinsic mechanisms underpinning the tumor immune escape seem to be sustained by tyrosine kinase-dependent signaling pathways. In fact, several oncogenic pathways, such as PI3K/Akt-, MEK/ERK- and RAS-dependent signaling, are deemed to converge in PD-L1 upregulation.[18] Nevertheless, TKIs should also have, per se, an anti-proliferative role, directly acting on HCC cells, as evidenced by in vitro assays.[19] Altogether, these considerations represent a thorough biological rationale supporting the association of ICIs and TKIs as a valuable therapeutic strategy in HCC.

Over the years, several clinical trials have been designed, with some of these studies often showing contrasting results. In such a context, IMbrave-150 trial has revolutionized the therapeutic standard of care in advanced HCC, demonstrating that the combination of atezolizumab plus bevacizumab is the first-line treatment of choice. Conversely, in the same disease setting, other studies have failed to demonstrate an overall significant and general benefit of the association of ICI plus TKI over TKI alone.

The Phase III, global, open-label IMbrave-150 study enrolled up to 501 patients, who were randomized 2:1 to receive either atezolizumab plus bevacizumab or sorafenib treatment. Remarkably, both primary data and updated analysis have met the primary endpoints of the study, showing an improvement in mOS and mPFS - 19.1 months (HR 0.66) and 6.8 months, respectively, with atezolizumab plus bevacizumab superior over sorafenib (mOS: 13.4 months; mPFS: 4.3 months). The benefit duration, expressed as median duration of response, was longer in patients treated with combinatory therapy compared to sorafenib alone, settling at 18.1 months and 14.9 months, respectively. Moreover, atezolizumab plus bevacizumab treatment performed better also in terms of activity, showing 29.8% ORR and 7.7% CR, despite the unfavorable prognostic features of the study population (ie, macro-vascular or bile duct invasion). In addition, HCC patients treated with the co-administration of ICI and anti-VEGF experienced a better QoL, as witnessed by 11.2% of mTTD (median time to deterioration), compared to 3.6% with sorafenib.[12,20] Similarly, camrelizumab/apatinib

association (ie, an anti-PD-1 monoclonal antibody and a VEGFR-2 TKI) showed promising results, supporting its value as alternative first line treatment in advanced HCC. Based on the results of a successful Phase II pivotal study, the RESCUE trial, SHR-1210-III-310, a phase III trial, the results of which have been recently presented at ESMO 2022 Conference, evaluated the clinical benefit of camrelizumab plus apatinib over sorafenib. The study enrolled 543 patients who were randomized to receive either the combination treatment or TKI alone. The trial has shown the superiority of camrelizumab/apatinib in terms of mOS and mPFS, 22.1 months (HR 0.62) and 5.6 months, respectively. Nonetheless, the efficacy data are equally encouraging, exhibiting 25.4% of ORR[21] [ESMO 2022, LBA35]. Unlike IMbrave-150 trial, characterized by more heterogeneous population, in SHR-1210-III-310 trial 83% of the study population is Asiatic and 75% patients have been affected by HBV-related HCC. Thus, further investigation is needed to better clarify the actual impact and the relevance of these results in a more assorted population, such as non-Asiatic people and non-viral HCC.

Unlike ground-breaking results evidenced by IMbrave-150 and SHR-1210-III-310 trials, LEAP-002 and COSMIC-312 trials did not meet their primary endpoints. LEAP-002 was aimed at assessing the effect of lenvatinib/pembrolizumab regimen in first-line treatment of advanced HCC patients. 743 eligible patients were enrolled and randomized 1:1 to receive either combo active treatment or lenvatinib/placebo. The coprimary endpoint of PFS and OS was not met because the pre-specified statistical significance was not reached. One of the most putative factors responsible for such an outcome might be found in using placebo in control arm; indeed, this choice could lower the drop-out rate and/or investigator-assessed clinical progression events [ESMO 2022, LBA34]. Nevertheless, the combination of lenvatinib/pembrolizumab achieved mOS and mPFS similar to that detected in control arm, 21.2 vs 19 months and 8.2 vs 8.1 months, respectively. As regards antitumor activity, the doublet lenvatinib/pembrolizumab displayed an advantage in ORR compared to lenvatinib alone - 26.1% vs 17.5%, respectively. Although the study was negative, the mOS of 19.0 months with lenvatinib monotherapy supports its role as a valuable standard of care in first-line advanced HCC.

COSMIC-312, a phase III randomized trial, explored the efficacy and safety of cabozantinib/atezolizumab over sorafenib with regard to mOS and mPFS as primary end-points. The ad-interim analysis showed no benefit of the combo therapy over sorafenib in terms of mOS. Conversely, mPFS at final analysis was significantly improved by the co-administration of cabozantinib/atezolizumab (6.8 vs 4.2 months), and a specific sub-set of patients (ie, Asiatic and HBV-affected population) benefited from the drug association compared to control arm also in terms of mOS. Of note, ICI/TKI co-administration offers higher ORR (11% vs 4%), comparable CR and longer duration of response (10.6 vs 8.8 months).[22]

Finally, a special mention should be reserved for a population-specific recent clinical trial. Indeed, the phase II/II ORIENT-32 study aimed to assess sintilimab plus IBI305, an anti-PD-1 and bevacizumab biosimilar, respectively, versus sorafenib as a first-line treatment for advanced HBV-associated HCC. The study randomized 595 Chinese HCC patients to receive either combination treatment or sorafenib alone, in a ratio 2:1. The co-primary endpoints were OS and mPFS; the trial was positive, demonstrating a superiority of combinatorial treatment in both endpoints. In particular, sintilimab/IBI305 showed longer mPFS, (4.6 months vs 2.8 months) and mOS, at least at the first ad interim analysis (median not reached vs 10.4 months).[23] By comparing ORIENT-32 study and the other two positive phase III trials, IMbrave-150 and SHR-1210-III-310, it emerged that mPFS and mOS of control arm (ie, sorafenib treated patients) in Chinese study were shorter. This phenomenon might be attributable to the different population enrolled in the trials. In fact, in IMbrave-150, SHR-1210-III-310, and ORIENT-32 the ratio of Asians vs non-Asians (including Japanese people) progressively increases from 40% to around 83% and 100%, respectively. Moreover, the subset of HBV-positive patients, which significantly varied among the studies, ranging from 49% in IMbrave-150 to 94% in ORIENT-32, could have impact on both mPFS and mOS. Therefore, the major caveat of this study is the homogeneity of the enrolled population. Further investigation is needed to clarify the actual role of sintilimab/IBI305 co-administration in a wider and more heterogeneous population, with careful attention to non-viral HCC and Caucasian ethnicity.

Besides the previously mentioned studies, several clinical trials are currently ongoing. Table 1 displays a list of most recent studies and their details.

Dual Immune Checkpoint Blockade

It has been demonstrated that agents targeting PD-1/PD-L1 and antibodies against CTLA-4, even if they present some points of convergence in their respective downstream pathways, lead to distinct patterns of immune activation in vivo.[24]

Table 1 Clinical Trials Investigating the Association of Immune Checkpoint Inhibitors with Multikinase Inhibitors or Anti-VEGFR Agents

NCT Identifier	Treatment	Strategy	Setting	Study type	Sample Size	Results
NCT03434379	Atezolizumab + Bevacizumab vs Sorafenib	Anti-PD-L1 mAb + anti-VEGF-A mAb vs mTKI	aHCC	Phase III	558 (R 2:1)	mOS 19.2 vs 13.4 mo mPFS 6.8 vs 4.3 mo ORR 29.8 vs 11.3%
NCT03463876/ RESCUE trial	Camrelizumab + Apatinib	Anti-PD-1 mAb + mTKI	aHCC	Phase II	190 (70 first line + 120 second line)	ORR 34.3% (first line) and 22.5% (second line). Grade ≥ 3 AEs 77.4%
NCT03764293/ SHR-1210-III-310 tral	Camrelizumab + Apatinib vs Sorafenib	Anti-PD-1 mAb + mTKI vs mTKI	aHCC	Phase III	543 (R 1:1)	mOS 22.1 vs 15.2 mo mPFS 5.6 vs 3.7 mo ORR 25.4 vs 5.9%
NCT03713593/ LEAP-002 trial	Pembrolizumab + Lenvatinib vs Lenvatinib	Anti-PD-1 mAb + mTKI vs mTKI	aHCC	Phase III	794 (R 1:1)	mOS 21.2 vs 19 mo mPFS 8.2 vs 8.1 mo Results did not meet pre-specified statistical significance
NCT03755791/ COSMIC-312 trial	Atezolizumab + Cabozantinib vs Sorafenib vs Cabozantinib	Anti-PD-L1 mAb + mTKI vs mTKI vs mTKI	aHCC	Phase III	840 (R 2:1:1)*	mOS: no statistically significant differences (ad interim analysis) mPFS 6.8 vs 4.2 (A+C vs S)
NCT04523493	Toripalimab + Lenvatinib Vs Lenvatinib	Anti-PD-1 mAb + mTKI vs mTKI	aHCC	Phase III	519 (R 2:1)*	NA Recruitment ongoing
NCT04560894	SCT-110A + SCT510 vs Sorafenib	Anti-PD-1 mAb + anti-VEGF-A mAb (Bevacizumab biosimilar) vs mTKI	aHCC	Phase II/III	621*	NA Recruitment ongoing (China)
NCT05101629	Pembrolizumab + Lenvatinib	Anti-PD-1 mAb + mTKI	aHCC (second line after Atezolizumab + Bevacizumab)	Phase II	34	NA Recruitment ongoing (Germany)
NCT04696055	Pembrolizumab + Regorafenib	Anti-PD-1 mAb + mTKI	aHCC (second line after anti-PD-1 or anti-PD-L1	Phase II	95	NA
NCT03439891	Nivolumab + Sorafenib	Anti-PD-1 mAb + mTKI	aHCC	Phase II	24*	NA Recruitment ongoing (USA)
NCT05162352	Sintilimab + Donafenib	Anti-PD-1 mAb + mTKI	aHCC	Phase II	30*	NA Recruitment ongoing (China)

NCT05039736	Cabozantinib (6 wks) → Nivolumab	mTKI followed by aanti-PD-1 mAb	aHCC	Phase II	30*	NA Not yet recruiting (USA)
NCT02519348	Durvalumab + Bevacizumab	Anti-PD-L1 mAb + anti-VEGF-A mAb	aHCC	Phase II	47	ORR 21.3% Grade ≥ 3 AEs 70.2%
NCT04050462	Nivolumab Vs Nivolumab + Cabiralizumab Vs Nivolumab + BMS-986253	Anti-PD-1 mAb vs Anti-PD-1 mAb + anti-CSF-1R mAb vs Anti-PD-1 mAb + anti-IL-8 mAb	aHCC	Phase II	23 (75 estimated)	NA Recruitment ongoing (USA)
NCT04605796	Toripalimab + Bevacizumab	Anti-PD-1 mAb + anti-VEGF-A mAb	aHCC	Phase II	54 (China)	ORR 32.7% mPFS 9.9 mo mOS not reached Grade ≥ 3 AEs 25.9%
NCT04741165	HX008 + Bevacizumab or Lenvatinib	Anti-PD-1 mAb + anti-VEGF-A mAb or mTKI	aHCC	Phase II	72*	NA Recruitment ongoing (China)
NCT03973112	Serplulimab + HLX04	Anti-PD-1 mAb + anti-VEGF-A mAb (Bevacizumab biosimilar)	aHCC (second line)	Phase II	20	ORR 30% mPFS 2.2 mo mOS 11.6 mo
NCT04401800	Tislelizumab + Lenvatinib	Anti-PD-1 mAb + mTKI	aHCC	Phase II	64	NA Active but not recruiting (China)
NCT04183088	Tislelizumab + Regorafenib vs Regorafenib	Anti-PD-1 mAb + mTKI vs mTKI	aHCC	Phase II	125*	NA Recruitment ongoing (Taiwan)
NCT03439891	Nivolumab + Sorafenib	Anti-PD-1 mAb + mTKI	aHCC	Phase II	24*	NA Recruitment ongoing (USA)
NCT05441475	Atezolizumab + ABSK-011	Anti-PD-L1 mAb + FGFR4 inhibitor	aHCC	Phase II	62*	NA Recruitment ongoing (China)
NCT04443322/ DULECT2020-1 Trial	Durvalumab + Lenvatinib	Anti-PD-L1 mAb + mTKI	aHCC	Phase II	20*	NA Recruitment ongoing (China)
NCT03841201/ IMMUNIB trial	Nivolumab + Lenvatinib	Anti-PD-1 mAb + mTKI	aHCC	Phase II	50	NA Active but not recruiting (Germany)

(*Continued*)

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Table 1 (Continued).

NCT Identifier	Treatment	Strategy	Setting	Study type	Sample Size	Results
NCT04443309	Camrelizumab + Lenvatinib	Anti-PD-1 mAb + mTKI	aHCC	Phase I/II	53*	NA Recruitment ongoing (China)
NCT04503902	Toripalimab + Donafenib	Anti-PD-1 mAb + mTKI	aHCC	Phase I/II	46*	NA Recruitment ongoing (China)
NCT02795429	Spartalizumab + Capmatinib	Anti-PD-1 mAb + mTKI	aHCC	Phase I/II	89	NA
NCT03893695	Nivolumab + GT90001	Anti-PD-1 mAb + anti-ALK-1 mAb	aHCC	Phase I/II	20	ORR 43.75%
NCT03418922	Nivolumab + Lenvatinib	Anti-PD-1 mAb + mTKI	aHCC	Phase I	30	ORR 76.7%
NCT03970616/ DEDUCTIVE trial	Durvalumab + Tivozanib	Anti-PD-L1 mAb + VEGFR1-3 inhibitor	aHCC	Phase I/II	42*	NA Active but not recruiting (USA)
NCT03539822	Durvalumab + Cabozantinib ± Tremelimumab	Anti-PD-L1 mAb + mTKI ± anti-CTLA-4 mAb	aHCC and other GI malignancies	Phase I/II	117 (24 were HCC)*	NA Recruitment ongoing (USA)
NCT03289533	Avelumab + Axitinib	Anti-PD-L1 mAb + mTKI	aHCC	Phase I	22	ORR 13.6% Grade ≥ 3 AEs 72%
NCT03347292	Pembrolizumab + Regorafenib	Anti-PD-1 mAb + mTKI	aHCC	Phase I	57	ORR 31%
NCT03006926	Pembrolizumab + Lenvatinib	Anti-PD-1 mAb + mTKI	aHCC	Phase I	104	ORR 36% mPFS 8.6 mo mOS 22 mo

Note: *Estimated number.

Abbreviations: AEs, Adverse events; aHCC, Advanced hepatocellular carcinoma; GI, Gastrointestinal; mAb, Monoclonal antibody; mo, Month; mOS, Median overall survival; mPFS, Median progression-free survival; ORR, Overall response rate; mTKI, Multiple tirosin-kinase inhibitor; NA, Not available; wks, Weeks, PD-1, Programmed cell death protein 1; PD-L1, Programmed death-ligand 1; CTLA-4, Cytotoxic T-lymphocyte antigen 4; VEGF, Vascular endothelial growth factor; CSF-1R, Colony stimulating factor 1 receptor; FGFR, Fibroblast growth factor receptor.

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Given this, in recent years the association of two ICIs has received growing attention, with several clinical trials investigating this therapeutic approach and it has been demonstrated to be an effective strategy in the treatment of several malignancies such as melanoma, renal cell carcinoma and non-small cell lung cancer.[25–27] The open-label phase I/II CheckMate 040 study was one of the first studies to investigate this therapeutic strategy in HCC. In the fourth cohort of this trial, 148 advanced HCC patients, previously treated with sorafenib, were randomized to receive nivolumab (an anti-PD-1 antibody) plus ipilimumab (an anti-CTLA-4 antibody) at three different dosages, and obtaining an ORR of 32% with an mOS and a 3-year OS of 22.2 months and 42% respectively (arm A: nivolumab 1 mg/kg + ipilimumab 3 mg/kg every 3 weeks, 4 doses, followed by nivolumab 240 mg every 2 weeks).[28,29] Interestingly, an increased dosage of ipilimumab seems to be associated with an improved outcome, even if the study was not powered enough to detect differences between arms.[30] Safety profile was acceptable with 53% of patients in arm A who reported a grade 3–4 adverse event (AE).[28] The association of nivolumab plus ipilimumab is currently under investigation in the phase III CheckMate 9DW trial as first-line treatment for advanced HCC compared to monotherapy with sorafenib or lenvatinib.[31]

One of the most relevant novelties in the field of dual immune checkpoint blockade for the treatment of advanced HCC is represented by the recently published results of the phase III Himalaya trial. A total of 1171, previously untreated, advanced HCC patients were randomized to receive the so-called STRIDE regimen (one dose of tremelimumab 300 mg plus durvalumab 1500 mg, followed by durvalumab 1500 mg every 4 weeks), durvalumab 1500 mg every 4 weeks or sorafenib until disease progression. STRIDE regimen significantly improved mOS compared to sorafenib (HR 0.78; 96% CI 0.65–0.93; p=0.0035) with an mOS of 16.4 months, 16.6 months and 13.8 months for STRIDE regimen, durvalumab, and sorafenib respectively. ORR and 3-year OS were 20.1% and 30.7% for STRIDE regimen, 17.0% and 24.7% for durvalumab, while sorafenib-treated patients had 5.1% and 20.2%.[10] Grade 3–4 AEs were 25.8%, 12.9% and 36.9% for STRIDE regimen, durvalumab and sorafenib respectively.[10] Even if it is not possible to compare different studies, STRIDE regimen seems to be associated with a reduced risk of grade 3–4 AEs compared to nivolumab plus ipilimumab, where hepatitis was the second most frequent immune-mediated adverse event requiring immune-modulating medication.[28] This difference may be explained by the reduced dose of anti-CTLA-4 administered in the STRIDE regimen where there was only a single priming dose of tremelimumab.[32] Currently, a phase III study (NCT05557838) is investigating the efficacy of durvalumab plus tremelimumab also in Child Pugh B patients, who were excluded from Himalaya trial.[33] Interestingly, dual immune checkpoint blockade seems to exert comparable efficacy also in nonviral-related HCC. In the phase I/II study 22 the association of durvalumab plus tremelimumab showed comparable mOS between HBV-related HCC and nonviral HCC (14.4 and 13.8 months, respectively), while in HCV-related HCC mOS was further increased reaching 22.3 months.[34] This trend seems to be confirmed also in the subsequent Himalaya trial where the advantage of durvalumab plus tremelimumab over sorafenib was maintained also in nonviral HCC subgroup (HR: 0.74; 95% CI: 0.57–0.95).[10] Similar results were obtained in second-line setting in the CheckMate 040 study with an mOS of 14.7, 15.2 and 21.9 months in the nonviral, HBV and HCV subgroups respectively.[28] Even if the previously mentioned studies were not designed to detect differences in terms of efficacy based on HCC etiology, this observation could be quite relevant especially if we consider that nonviral-related HCC seems to derive less benefit from atezolizumab/bevacizumab or monotherapy with ICIs. In the IMbrave 150 trial atezolizumab plus bevacizumab showed no benefit over sorafenib in the nonviral HCC subgroup (mOS 17.0 vs 18.1; HR 1.05; 95% CI 0.68–1.63), while also single-agent nivolumab and pembrolizumab, in their respective phase III trials demonstrated a reduced OS benefit in nonviral HCC compared to sorafenib (nivolumab HR: 0.91; 95% CI: 0.72–1.16; pembrolizumab HR: 0.88; 95% CI: 0.64–1.20).[9,11] These results are supported by two recently published meta-analyses which showed that therapy with anti PD-1/PD-L1 alone, or in combination with bevacizumab, resulted in lower OS in nonviral HCC compared to that in viral HCC.[35,36] In addition, a recently published large retrospective study suggests that lenvatinib is associated with a survival benefit compared to atezolizumab/bevacizumab treatment in patients with NAFLD-related HCC.[37] These clinical data are supported by preclinical evidence demonstrating the presence of a subgroup of exhausted, unconventionally activated CD8+PD-1+ T cells in NASH-mice models. These cells had tissue-damaging functions and are increased by treatment with anti-PD-1 agents without leading to tumor regression. Interestingly, a similar subgroup of cells has been found also in human NASH-affected livers, thus hypothesizing a possible contribution to the unfavorable effects of anti-PD-1 treatment.[36] Stratification of HCC patients based on

etiology is warranted in future clinical trials in order to better understand the real advantage of dual immune checkpoint blockade over anti-PD-1/PD-L1 monotherapy and anti-PD-1/PD-L1 plus anti-VEGF agents in nonviral HCC.

IBI310, an anti-CTLA-4 monoclonal antibody, in association with sintilimab (an anti-PD- antibody) has demonstrated promising antitumor activity and a manageable safety profile in a Phase I study enrolling advanced HCC patients.[38] Currently sintilimab plus IBI310 is under evaluation as first-line treatment in a randomized phase III trial compared to sorafenib.[39]

Dual immune checkpoint blockade has demonstrated promising antitumor activity after progression to prior ICI monotherapy in melanoma, non-small-cell lung cancer and renal cell carcinoma.[40–42] Nivolumab or pembrolizumab plus ipilimumab has been tested as second line therapy, after progression to monotherapy with ICIs, also in HCC patients in a small retrospective study. Twenty-five patients were included and an ORR of 16% with an mOS of 10.9 months were reported. ORR did not differ between primary resistance group and acquired resistance group while mOS was 4.4 and 11.4 months respectively; all responders were Child Pugh A HCC patients.[43] If this suggestion is confirmed in larger prospective studies, dual immune checkpoint blockade could represent a promising candidate for second-line treatment after atezolizumab plus bevacizumab in advanced HCC patients. Currently, a phase II trial is evaluating the association of nivolumab plus ipilimumab in advanced HCC patients after progression to atezolizumab plus bevacizumab.[44]

In recent years, other new inhibitory immune checkpoint molecules turned out to play an important role in immune tolerance in HCC, such as Lymphocyte-activation gene 3 (LAG-3), T cell immunoglobulin mucin-3 (TIM-3) and T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT).[45–47] Both LAG-3, TIM-3 and TIGIT contribute to resistance to ICIs monotherapy as they are upregulated under PD-1/PD-L1 blockade, similar to what happens with CTLA-4.[48–50] This provides a strong rationale for combining anti-PD-1/PD-L1 antibodies with agents targeting these new inhibitory immune checkpoint molecules. An increased expression of TIM-3 on tumor-infiltrating lymphocytes and tumor-associated macrophages has been correlated with poor prognosis and a higher risk of recurrence in HCC patients, and several clinical trials are evaluating the role of anti-TIM-3 agents in advanced solid tumors (eg, NCT02817633, NCT03708328, NCT03680508).[51–55] Of note, NCT03680508 is a phase II trial investigating the association of cobolimab (an anti-TIM-3 antibody) with dostarlimab (an anti-PD-1 antibody) in advanced HCC patients as first-line treatment, and the results of this study are expected in 2024.[54] IL-27 plays a central role in inducing the expression of TIM-3 on immune cells, thus representing a potential therapeutic target.[56] SRF388 (an anti-IL-27 antibody) in association with pembrolizumab will be tested in a phase I study in advanced HCC and renal cell carcinoma.[57] The association of relatlimab (an anti-LAG-3 antibody) plus nivolumab has been recently approved by FDA for the treatment of advanced melanoma, given the results of the large phase III RELATIVITY-047 trial.[58,59] Currently, relatlimab is under evaluation in a phase II trial in association with nivolumab in immunotherapy naïve advanced HCC patients (NCT04567615).[60] Clinical trials investigating dual immune checkpoint blockade in advanced HCC are summarized in Table 2.

Other Immune-Based Strategies

Triplets

Given the modest results shown by large phase III studies of ICIs in association with TKIs, such as LEAP 002 and COSMIC-312 trials, the addition of a second ICI to combination therapies with anti-PD-1/PD-L1 plus TKIs or anti VEGF agents has received growing attention[22] [LBA34 ESMO 2022]. Triplets under investigation for treatment of advanced HCC could be divided into two groups: (i) anti-PD-1/PD-L1 plus anti-CTLA-4 with anti-angiogenics (TKIs or anti-VEGF); (ii) anti-PD-1/PD-L1 plus anti-VEGF with agents targeting alternative immune pathways.

Early clinical data of the association of dual immune checkpoint blockade with TKIs were provided by an arm of the Checkmate 040 trial in which 71 advanced HCC patients were randomized to receive nivolumab 240 mg every two weeks plus cabozantinib 40 mg daily or nivolumab 3 mg/kg every two weeks + ipilimumab 1mg/kg every six weeks + cabozantinib 40 mg daily. Although the small sample size did not allow drawing definitive conclusions, the triplet arm showed an improved ORR and mPFS with 26% vs 17% and 6.8 vs 5.5 months respectively.[61] The rationale for considering the association of cabozantinib with dual immune checkpoint blockade is provided by the evidence that cabozantinib may exert an immunomodulatory effect not only via the inhibition of VEGF, but also through the inhibition of other targets such as MET and TAM family of receptor kinases. In particular, the inhibition of TAM family of kinases determines an increase in circulating and tumor-infiltrating cytotoxic T lymphocytes, while MET inhibition impaired the

Table 2 Clinical Trials of Dual Immune Checkpoint Blockade for Advanced Hepatocellular Carcinoma

NCT Identifier	Treatment	Strategy	Setting	Study Type	Sample Size	Results
NCT01658878/ CheckMate 040 (4th court)	Nivolumab + ipilimumab[a]	Anti-PD-1 mAb + anti-CTLA-4 mAb	aHCC (second line)	Phase I/II	148 (R 1:1:1)	Arm A: ORR: 32% mOS 22.2mo Grade ≥ 3 AEs 53%[d]
NCT04039607/ CheckMate 9DW	Nivolumab + ipilimumab vs sorafenib or lenvatinib	Anti-PD-1 mAb + anti-CTLA-4 mAb vs mTKI	aHCC (first line)	Phase III	732 (R 1:1)	NA
NCT02519348/ Study 22	Tremelimumab + durvalumab[a] vs durvalumab vs tremelimumab	Anti-PD-L1 mAb + anti-CTLA-4 mAb vs Anti-PD-L1 mAb vs anti-CTLA-4 mAb	aHCC	Phase II	332 (R 1:1:1:1)	STRIDE-regimen: ORR 24.0% mOS: 18.7mo Grade ≥ 3 AEs 37.8%; durvalumab + tremelimumab: ORR 9.5% mOS 11.3mo Grade ≥ 3 AEs 24.4%; durvalumab: ORR 10.6% mOS 13.6mo Grade ≥ 3 AEs 20.8%; tremelimumab: ORR 7.2% mOS 15.1mo Grade ≥ 3 AEs 43.5%
NCT03298451/ Himalaya	Tremelimumab + durvalumab vs durvalumab vs sorafenib	Anti-PD-L1 mAb + anti-CTLA-4 mAb vs Anti-PD-L1 mAb vs mTKI	aHCC (first line)	Phase III	1504 (R 1:1:1)[b]	STRIDE-regimen: ORR 20.1% mOS 16.4mo Grade ≥ 3 AEs 25.8% (Durvalumab: ORR 17% mOS 16.6mo Grade ≥ 3 AEs 12.9% Sorafenib: ORR 5.1% mOS 13.8mo Grade ≥ 3 AEs 36.9%[c]
NCT04401813	Sintilimab + IBI310	Anti-PD-1 mAb + anti-CTLA-4 mAb	aHCC	Phase I	97	ORR 17.2% Grade ≥ 3 AEs 34.5%, mPFS 3.9mo[c]
NCT04720716	Sintilimab + IBI310 vs sorafenib	Anti-PD-1 mAb + anti-CTLA-4 mAb vs mTKI	aHCC (first line)	Phase III	490 (R 1:1)[b]	NA Recruitment ongoing
NCT04567615	Nivolumab vs nivolumab + relatlimab[a]	Anti-PD-1 mAb + anti-LAG-3 mAb vs Anti-PD-1 mAb	aHCC	Phase II	250 (R 1:1:1)[b]	NA Recruitment ongoing
NCT03680508	Cobolimab + dostarlimab	Anti-TIM-3 Antibody + anti-PD-1 mAb	aHCC (first line)	Phase II	42 [b]	NA Recruitment ongoing
NCT04374877	SRF388 + pembrolizumab (part C of the study)	Anti-IL-27 mAb + anti-PD -1 mAb	aHCC and aRCC	Phase I	220[b]	NA Recruitment ongoing

(*Continued*)

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Table 2 (Continued).

NCT Identifier	Treatment	Strategy	Setting	Study Type	Sample Size	Results
NCT04823403	Ipilimumab (intra-arterial administration) + nivolumab	Anti-PD-1 mAb + anti-CTLA-4 mAb	aHCC	Phase I	27[b]	NA Recruitment ongoing (France)
NCT05557838	Durvalumab + tremelimumab	Anti-PD-L1 mAb + anti-CTLA-4 mAb	aHCC (first line)	Phase III	300[b]	NA Recruitment ongoing (China)
NCT05451043	Durvalumab + tremelimumab + propanolol	Anti-PD-L1 mAb + anti-CTLA-4 mAb	aHCC, aPDAC and aBTC	Phase II	62[b]	NA Not yet recruiting (Canada)
NCT05199285	Nivolumab + ipilimumab	Anti-PD-1 mAb + anti-CTLA-4 mAb	aHCC (second line after Atezolizumab + Bevacizumab)	Phase II	40[b]	NA Not yet recruiting (USA)

Notes: [a]More than one arm with different dosages. [b]Estimated number. [c]Preliminary results. [d]Arm A: nivolumab 1mg/kg + ipilimumab 3g/kg every three weeks (4 doses) followed by nivolumab 240 mg every 2 weeks.
Abbreviations: AE, Adverse event; aHCC, advanced hepatocellular carcinoma; mAb, monoclonal antibody; mo, month; mOS, median overall survival; mPFS, median progression-free survival; mTKI, multiple tirosin-kinase inhibitor; NA, not available; ORR, overall response rate; wks, weeks, PD-1, Programmed cell death protein 1; PD-L1, Programmed death-ligand 1; aRCC, advanced clear cell renal cell carcinoma; aBTC, advanced biliary tract cancer; aPDAC, advanced pancreatic ductal adenocarcinoma.

recruitment of immunosuppressive neutrophils into tumor bed in response to immunotherapy.[62,63] Moreover, the addition of cabozantinib to dual immune checkpoint blockade seems able to overcome primary resistance to anti-PD-1 plus anti-CTLA-4 by targeting myeloid-derived suppressor cells in castration-resistant prostate cancer mice models.[64] Cabozantinib is under evaluation in association with nivolumab plus ipilimumab and transarterial chemoembolization (TACE) in a phase II study (NCT04472767), while the CAMILLA study (NCT03539822) is evaluating the role of cabozantinib in association with durvalumab plus tremelimumab in advanced HCC.[65,66]

It has been demonstrated in HCC mice models that lenvatinib reduced regulatory T lymphocytes differentiation and the number of tumor-associated macrophages (TAMs), while it increased the percentage of activated CD8+ T cells producing interferon-γ.[67,68] Interestingly, also tremelimumab treatment is associated with an increased median proliferating CD8+ T-cell counts, compared to durvalumab treatment, with a tremelimumab dose-dependent increase in T-cell clonal expansion which is associated with improved ORR and OS.[69,70] These data provide a rationale for combining lenvatinib with anti-PD-1 plus anti-CTLA-4 and by hypothesizing a possible synergistic effect. Lenvatinib plus durvalumab and tremelimumab is now under investigation in the phase III EMERALD-3 trial in association with TACE, while MK-1308A (a coformulation of pembrolizumab and the anti-CTLA-4 antibody quavonlimab) is under evaluation in association with lenvatinib as first-line treatment in advanced HCC.[71,72]

It has been demonstrated that VEGF promotes tumor growth not only via the stimulation of tumor vascularization, but also through an immunosuppressive effect.[17] In particular, preclinical data on mice models showed that VEGF induces the expression of immune checkpoint molecules, including PD-1, CTLA-4 and LAG-3, resulting in a reduced activity of anti-PD-1 treatment.[16] In addition, VEGF reduces the number of infiltrating CD8+ effector T lymphocytes by increasing the expression of FasL on tumor endothelial cells, making them capable of killing CD8+ T lymphocytes.[73] In melanoma patients the addition of bevacizumab to ipilimumab determined an increase in infiltrating CD8+ T cells compared to ipilimumab alone, suggesting a possible synergistic effect with anti-CTLA-4 antibodies.[74] Immunosuppressive effects of VEGF are summarized in Figure 1. Combination treatment with atezolizumab plus bevacizumab has already proven its synergistic effect in HCC, thus adding a second ICI could represent a promising therapeutic strategy. Clinical trials investigating the association of anti-VEGF agents plus anti-PD-1/PD-L1 antibody and agents targeting CTLA-4, LAG-3, TIGIT and IL-27 in advanced HCC are summarized in Table 3.

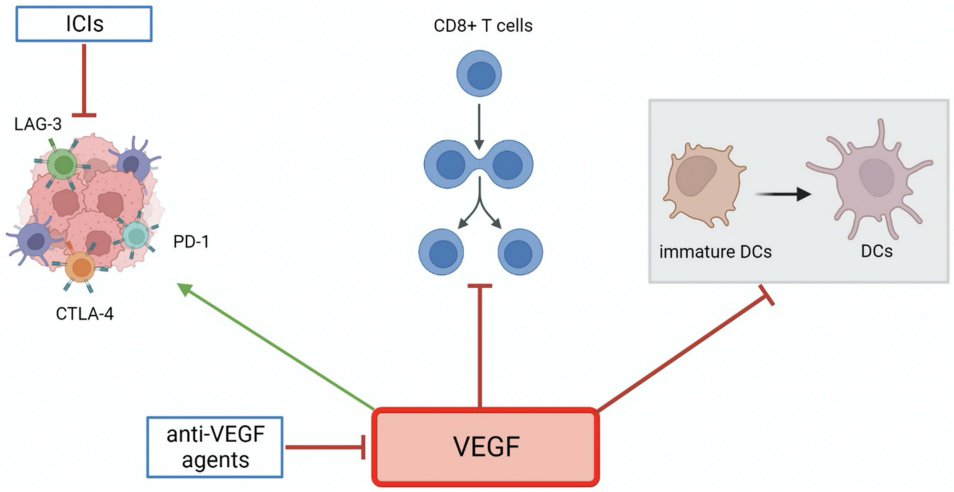

Figure 1 Immunosuppressive effects of VEGF. VEGF induces the expression of immune checkpoint molecules, such as PD-1, CTLA-4 and LAG-3 and inhibits DCs maturation through the inhibition of NF-κB pathway in immature DCs. In addition, VEGF reduces the number of infiltrating CD8+ effector T lymphocytes by increasing the expression of FasL on tumor endothelial cells, making them capable of killing CD8+ T cells.

Abbreviations: VEGF, Vascular endothelial growth factor; ICIs, Immune checkpoint inhibitors; DCs, Dendritic cells; PD-1, Programmed cell death protein 1; CTLA-4, Cytotoxic T-lymphocyte antigen 4; LAG-3, Lymphocyte-activation gene 3.

Table 3 Clinical Trials Investigating Triplet Systemic Strategy for Advanced Hepatocellular Carcinoma

NCT Identifier	Treatment	Strategy	Setting	Study Type	Sample Size	Results
NCT01658878/ CheckMate 040 (5th court)	Nivolumab + ipilimumab + cabozantinib vs nivolumab + cabozantinib	Anti-PD-1 mAb + anti-CTLA-4 mAb + mTKI vs Anti-PD-1 mAb + mTKI	aHCC (second line)	Phase I/II	71 (R 1:1)	Triplet: ORR: 26% mPFS 6.8mo Grade ≥ 3 AEs 71% Doublet: ORR 17% mPFS 5.5mo Grade ≥ 3 AEs 42%
NCT04948697	Ociperlimab + tislelizumab + BAT1706 vs tislelizumab + BAT1706	Anti-PD-1 mAb + anti- TIGIT mAb + anti-VEGF-A mAb vs Anti-PD-1 mAb + anti-VEGF-A mAb	aHCC (first line)	Phase II	90 (R 1:1)	NA
NCT05337137	Nivolumab + relatlimab + bevacizumab vs nivolumab + bevacizumab	Anti-PD-1 mAb + anti-LAG-3 mAb + anti-VEGF-A mAb vs Anti-PD-1 mAb + anti-VEGF-A mAb	aHCC	Phase I/II	162 (R 1:1)[b]	NA Recruitment ongoing
NCT03539822	Tremelimumab + durvalumab + cabozantinib vs durvalumab + cabozantinib	Anti-PD-1 mAb + anti-CTLA-4 mAb + mTKI vs Anti-PD-1 mAb + mTKI	aHCC and other GI malignancies	Phase I/II	117[b]	NA Recruitment ongoing
NCT05363722	Sintilimab + IBI310 + bevacizumab[a]	Anti-PD-1 mAb + anti-CTLA-4 mAb + anti-VEGF-A mAb	aHCC (first line)	Phase I	80 (R 1:1)[b]	NA Not yet recruiting
NCT04740307	Coformulated pembrolizumab/quavonlimab + lenvatinib	Coformulated anti-PD-1/anti CTLA-4 + mTKI	aHCC	Phase II	110	NA
NCT05359861	SRF388 + atezolizumab + bevacizumab vs atezolizumab + bevacizumab	Anti-PD-L1 mAb + anti-IL-27 mAb + anti-VEGF-A mAb vs Anti-PD-L1 mAb + anti-VEGF-A mAb	aHCC (first line)	Phase II	134 (R 1:1)[b]	NA Recruitment ongoing
NCT05249569	Bavituximab + axitinib + avelumab	Anti-PD-L1 mAb + mTKI + anti-phosphatidylserine	aHCC	Phase II	29[b]	NA Recruitment ongoing

Notes: [a]More than one arm with different dosages. [b]Estimated number.

Abbreviations: AE, Adverse event; aHCC, advanced hepatocellular carcinoma; GI, gastrointestinal; mAb, monoclonal antibody; mo, month; mOS, median overall survival; mPFS, median progression-free survival; mTKI, multiple tirosin-kinase inhibitor; NA, not available; ORR, overall response rate; wks, weeks, PD-1, Programmed cell death protein 1; PD-L1, Programmed death-ligand 1.

Beyond ICIs

Chimeric Antigen Receptor T Cells (CAR-T) therapy consist of T lymphocytes which are engineered to express a chimeric antigen receptor that specifically recognizes tumor associated-antigens. CAR-T has recently emerged as a promising strategy in the treatment of hematological malignancies and several studies are evaluating its possible application also in the treatment of solid tumors. Glypican-3 (GPC3), NK group 2 member D (NKG2D) and CD147 represent potential targets for CAR-T in HCC of which GPC3 is probably the most studied.[75–77] GPC3 is a heparan sulfate proteoglycan playing an important role in cell proliferation and metastasis which is expressed in approximately 75% of HCC cells, but not in healthy liver tissue.[78] It has been demonstrated in HCC mice models that GPC3-targeted CAR-T cells induced perforin- and granzyme-mediated apoptosis in GPC3-positive HCC cells with also a reduction of Wnt signaling in cancer cells.[79] Despite preclinical studies showing a certain activity of CAR-T therapy alone, its efficacy against solid tumors, including HCC, is still limited due to several obstacles such as the immunosuppressive tumor microenvironment in which PD-1 plays a relevant role.[75] This is particularly true in HCC where the GPC3-targeted CAR-T cells have been suggested to present a reduced cytotoxic effect in PD-L1 positive HCC mice models compared to PD-L1 negative mice, while GPC3-CAR-T cells carrying PD-1 blockade agents showed a significantly increased tumor suppression capacity compared to "classic" GPC3-CAR-T cells.[80,81] These data provide a rationale for combining CAR-T therapy with ICIs.

The rationale for using vaccines in cancer treatment is based on their ability of inducing a tumor-specific immune response by generating new antigen-specific T cell responses and enhancing existing responses. Various peptide vaccines based on defined antigens have been studied in HCC including vaccines targeting alphafetoprotein, multidrug resistance-associated protein 3 and GPC3.[82–84] Particularly, a GPC3-derived peptide vaccine was tested in a phase I trial on 33 HCC patients determining only 1 partial response, even though this treatment induced a relevant GPC3-specific immune response.[83] It has become clear that vaccines alone are not able to exert a satisfactory anticancer response and their association with other agents, such as ICIs, represents a promising strategy.[85] One of the causes of primary resistance to ICIs is the absence of tumor antigens able to effectively prime and activate T cells resulting in a "cold" TME. This is particularly relevant in HCC if we consider high rates of primary progression reported in phase III trials of ICIs monotherapy (nivolumab 37%, pembrolizumab 33%, durvalumab 45.2% and tislelizumab 49.4%)[9–11] [ESMO Congress 2022, LBA36]. Vaccines can produce many neoepitope-specific T cells on which ICIs could exert their stimulatory effect.[86] In addition, several studies showed that vaccines up-regulate the expression of molecules targeted by anti-PD-1/PD-L1 and in some reports were the neoantigen specific T cells, induced by vaccine, which expressed PD-1, thus hypothesizing a reduced vaccine efficacy related to PD-1 up-regulation.[87–89] Cold-Inducible RNA Binding Protein (CIRP) is a toll-like-receptor-4 ligand released under stress conditions which induces the production of inflammatory cytokines. Silva et al developed a CIRP-based vaccine containing GPC3 (CIRP- GPC3 vaccine) and tested it alone and in association with anti-PD-1 and anti-CTLA-4 agents in HCC mice models. The authors found that the association of CIRP- GPC3 vaccine with anti-PD-1 and anti-CTLA-4 antibodies determined an increased immune response, mainly directed against the 522–530 epitope of HLA-A2*01, compared to vaccine alone, without significantly increased hepatic toxicity in mice.[90] Combination of vaccines plus ICIs has already showed promising results in a small series of advanced melanoma patients, and this strategy is receiving growing attention also in HCC.[89,91] Clinical trials investigating the association of CAR-T cell therapy and vaccines plus ICIs in advanced HCC are summarized in Table 4.

Immune-Driven Mechanisms of Epatocarcinogenesis and Tumor Progression

Several factors such as immunity suppression, chronic inflammation, and the decreased recognition of cancer cells have been suggested to play a role in promoting tumor antigen tolerance and epatocarcinogenesis.[92,93] In particular, a number of recent trials have highlighted that the onset of HCC may be favored by alterations in cytokine levels as well as in immune cells' function and number.[94,95] Of note, changes in the expression of immune components cause some shifts in terms of immune response, leading to tumor tolerance and tumor progression. Interestingly, several tumor-related cells, including CD4+ T cells, myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells, regulatory T cells (Tregs), and cytotoxic T cells, are involved in HCC development and tumor progression.[96,97] Disease progression from liver cirrhosis to HCC sees some changes in terms of immune cells' function and regulation; among these, the Tregs'

Table 4 Clinical Trial Investigating the Association of CAR-T Cell Therapy and Vaccines Plus ICIs in Advanced HCC

NCT Number	Strategy	Treatment	Study Type	Primary End Point
NCT03980288	CAR-T + ICIs or mTKI	GPC3-CAR-T cells + mTKI or anti PD-1/PD-L1 antibodies (part 2)	Phase I	DLT, MTD
NCT04251117	Vaccine with plasmid-encoded IL-12 + ICI	Personalized DNA vaccine + INO-9012 + pembrolizumab	Phase I/II	AEs, immunogenicity
NCT05528952	Vaccine + ICI + anti-VEGF agents	UCPVax + atezolizumab + bevacizumab	Phase II	ORR
NCT05269381	Vaccine + ICI + sargramostim	Cyclophosphamide than personalized neoantigen peptide-based vaccine + pembrolizumab + sargramostim	Phase I	AEs
NCT04248569*	Vaccine + ICI	DNAJB1-PRKACA peptide vaccine + nivolumab + ipilimumab	Phase I	AEs, immunogenicity

Notes: *Recruits only fibrolamellar hepatocellular carcinoma.
Abbreviations: mTKI, multiple tirosin-kinase inhibitor; CAR-T, Chimeric antigen receptor T Cells; ICI, Immune checkpoint inhibitor; GPC3, Glypican-3; PD-1, Programmed cell death protein 1; PD-L1, Programmed death-ligand 1; DLT, Dose limited toxicity; MTD, Maximum tolerated dose; AEs, Adverse events; ORR, objective response rate.

recruitment and development is promoted by the differentiation of macrophages into other phenotypes, something that results in a Th2-type immune response.[98] Several studies have suggested a correlation between Tregs and disease progression in HCC patients, and Tregs have been reported to exert a negative effect on other immune-related cells, such as dendritic cells (DCs), NK cells, and T cells, promoting the differentiation of Th17 cells by cytokines with immunosuppressive properties.[99,100] In fact, there is a reduced secretion of Th1 cytokines caused by the loss of the antigen presentation capabilities of DCs as well as a lower cytolytic activity by NK cells. In addition, β-catenin pathway also impairs DCs recruitment and induces resistance to anti-PD-1 agents, thus promoting immune escape.[101]

Based on these premises, it is readily apparent that HCC tumor microenvironment (TME) presents several types of immune cells harboring distinct features, including myeloid cells, NK cells and T cells.[102,103] This "ecosystem" is modified by a number of interactions between tumor and immune cells, with these processes resulting in the exhaustion of pro-inflammatory immune cells and the parallel impairment of anti-tumor response.[104] Interestingly, several recent studies have suggested the presence of some "immune clusters" playing a prognostic role, with some of these clusters being associated with better outcomes.[105,106] In particular, improved survival was reported in HCC patients with low levels of macrophages and high levels of CD8+ T cells; conversely, a more aggressive clinical course was observed in HCC TME with high levels of Tregs, tumor-associated macrophages, and dysfunctional NK cells.[107] Characterization of the tumor immune microenvironment could also represent a promising strategy for the identification of predictors of response to ICIs. Zhu et al analyzed tumor biopsies from 358 patients treated with atezolizumab/bevacizumab, atezolizumab alone or sorafenib in two different clinical trials. They found that an increased Treg to effector T cell ratio was associated with a reduced benefit of atezolizumab/bevacizumab, while an increased expression of CD274, T-effector signature and intratumoral CD8+ T cell density were associated with an improved clinical outcome with atezolizumab/bevacizumab.[108] In a retrospective analysis conducted on tumor samples from the CheckMate 040 trial, PD-L1 ≥1% was associated with improved mOS in HCC patients treated with nivolumab. Despite this, the relatively small sample size does not allow drawing definitive conclusions.[109]

Future Perspectives

Atezolizumab/bevacizumab has revolutionized the treatment of advanced HCC, but it also raised several issues. First, in real-life setting only approximately one third of advanced HCC patients are eligible for atezolizumab/bevacizumab treatment if we consider inclusion criteria of IMbrave 150 trial.[110] In particular, Child-Pugh B patients were excluded and only few data from retrospective studies are available.[111] In this setting, ICIs monotherapy may maintain a role as it has comparable efficacy versus TKIs with a more favorable tolerability profile compared to both TKIs and atezolizumab/

bevacizumab.[10] In the fifth cohort of CheckMate 040 trial, nivolumab was tested in Child-Pugh B patients showing good tolerability and these results are also supported by small retrospective studies.[112,113] Currently, NCCN guidelines consider nivolumab as a therapeutic option for advanced HCC in Child-Pugh B patients; despite this, further studies are needed in order to better understand the real role of nivolumab in this setting and also whether atezolizumab/bevacizumab could be considered in selected Child-Pugh B patients.[114] In addition, orthotopic liver transplant still represents an absolute contraindication to treatment with ICIs, thus excluding patients with recurrent disease, from first-line atezolizumab/bevacizumab. Second, there are no data from prospective studies of subsequent systemic treatment after atezolizumab/bevacizumab. Several small retrospective studies suggest a benefit of treatment with TKIs, and ESMO guidelines recommend TKIs as potential options for second line treatment.[115,116] Currently, several clinical trials are investigating the role of various systemic treatments in this setting (eg, NCT04770896 and NCT05134532). Given the different mechanism of action, treatment with sorafenib or lenvatinib represents, in our opinion, a reasonable option after progression to first-line atezolizymab/bevacizumab.

ICIs-based combinations will probably dominate treatment scenario of advanced HCC in coming years with dual-immune checkpoint blockade being one of the most promising strategies since this therapeutic strategy seems to maintain a survival benefit over TKIs also in nonviral HCC. Despite this, the modest results shown by LEAP 002 and COSMIC-321 trials underline the absence of reliable predictors of response to ICIs treatment and the lack of data on systemic treatment based on HCC etiology as a fundamental unmet need in this setting.[117–119]

Disclosure

The authors report no conflicts of interest in this work.

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