Review

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Contemporary Use of Immunotherapy in Treating Patients with Advanced Hepatocellular Carcinoma

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Abstract

Advanced hepatocellular carcinoma (HCC) exhibits a poor prognosis. Immunotherapy has emerged as a major player for both upfront treatment of advanced HCC and disease progression on prior systemic therapies. In the first-line treatment of advanced HCC, immunotherapy demonstrated superior efficacy outcomes compared to tyrosine kinase inhibitors, with a favourable safety profile. Initial treatment strategies of single-agent immune checkpoint inhibitors (ICIs) yielded only limited clinical activity. A better understanding of the hepatic tumour microenvironment and immunotolerance has driven the development of biologically relevant immunotherapy combinations. These combinations, which include anti-angiogenic agents or dual ICIs targeting both programmed cell death protein-1 (PD-1)/ programmed cell death ligands 1 (PD-L1) and cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), are the focus of ongoing research. Recently published clinical trials involving ICI-based combination therapies achieved improved treatment outcomes and continue to reshape the treatment paradigm for advanced HCC. While the enhancement of anti-tumour immunity through different immunotherapy combinations has shown variable triumph, toxicity and costs are inevitably increased. Furthermore, the search for predictive biomarkers remains an unmet challenge in advanced HCC. In this review, we will summarize the notable advances in immunotherapy for the treatment of advanced HCC, discuss the underlying immune microenvironment and rationale for combinations, as well as explore opportunities for novel therapeutic targets beyond conventional immune checkpoints to overcome immunotherapy resistance.

Keywords: immunotherapy; immune checkpoint inhibitors; advanced hepatocellular carcinoma; HCC

1. Introduction

Primary liver cancer is a significant global health burden, ranking the third leading cause of cancer death worldwide [1] Hepatocellular carcinoma (HCC) accounts for 75-85% of primary liver cancer [1] HCC classically develops in the background of chronic inflammation, often with fibrosis and cirrhosis [2] Hepatitis B virus (HBV) infection accounts for the majority of HCC cases in Asia and Africa, whereas chronic hepatitis C virus (HCV) infection is the predominant etiology in patients in HCC across North America, Europe and Japan. Alcohol-related liver disease and non-alcoholic steatohepatitis (NASH) are the other notable risk factors for HCC [3,4] Given the liver's constant exposure to antigens from the gastrointestinal tract, immunotolerance is crucial and is achieved via innate and adaptive immune responses [5,6] The immune microenvironment plays a critical role in the pathogenesis of HCC [2] A shift towards tumor immunotolerance is associated with the development and progression of HCC [7] Evading immune destruction is one of the hallmarks of cancer [8] The cancer-immunity cycle, conceptualized in 2013, elucidated that T cells do not operate independently, but rather within a sequential series of events, some of which extend beyond the confines of the immune system and the tumor microenvironment [9] Contemporary understanding

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of the cancer-immunity cycle acknowledges the essential role of the tumor microenvironment, especially dendritic cells, in modulating and maintaining anti-tumor T cell response which is best illustrated by the cancer-immunity subcycle [10] Immunotherapies aim to overcome the immunosuppressive environment dominated by regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) [2] Programmed cell death protein-1 (PD-1) is a cell surface protein first discovered in 1992. PD-1 is typically expressed on T-cells and functions to downregulate T-cell responses, thereby preventing autoimmune disease. Programmed cell death ligands 1 and 2 (PD-L1 and PD-L2) are the ligands for PD-1. PD-L1 is expressed on antigen presenting cells, immune cells and tumor cells. PD-1 and PD-L1 interaction results in intratumoral T-cell exhaustion and eventually immune invasion. Cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) is another inhibitory receptor expressed exclusively on T cells, and was first discovered in 1987 [11–13] CD80 (B7-1) and CD86 (B7-2) are ligands for CTLA-4, which when bound to CTLA-4 leads to T-cell inhibition. The discovery of PD-1 and CTLA-4 immune checkpoints was a significant breakthrough, ultimately leading to James P. Allison and Tasuku Honjo receiving the 2018 Nobel Prize in Physiology or Medicine [14] While PD-1 and PD-L1 blocking antibodies enhances the activity of cytotoxic T lymphocytes (CD8+ T cells) in peripheral tissues and inside tumors, CTLA-4 blocking antibodies activate CD8+ T cells and suppress the activity of Tregs in the initial T cell activation stage [15,16] Anti-PD-1 antibodies inhibit its interaction with both PD-L1 and PD-L2, while anti-PD-L1 antibodies prevent PD-1 from binding to PD-L1 but allow interaction with PD-L2. Immunotherapies are associated with a distinct spectrum of adverse events known as immune-related adverse events (irAE), and the pathophysiology is postulated to be linked to the function of immune checkpoints in preserving immunological homeostasis [17] Key mechanisms of irAE include T-cell activation, increasing levels of preexisting autoantibodies, cytokine involvement and cross-reactivity [17] In general, CTLA-4 blocking agents are associated with more high-grade immune-related toxicities than PD-1/PD-L1 blockade [18,19]

According to the Barcelona Clinic Liver Cancer (BCLC) staging, advanced HCC refers to BCLC-C characterized by extrahepatic spread or vascular invasion with preserved liver function, or BCLC-B which tumor progressed after locoregional therapies or too extensive to be treated selectively by locoregional therapies [20] In these trials, the eligible patients presented with advanced HCC not amenable to surgery or locoregional therapies, were primarily Child-Pugh A with good performance status. Patients with prior or active autoimmune diseases or history of solid organ transplant are excluded in trials involving immunotherapies.

2. The First Breakthrough:Anti-Angiogenic Agents

Sustained angiogenesis is one of the hallmarks of cancer first described in 2000 [21] Neovascularization has been implied in the pathogenesis of HCC [22] In HCC and other solid tumors, angiogenesis relies on activation, proliferation and migration of endothelial cells. Initial efforts have been focusing on therapeutic inhibition of angiogenesis of HCC. Angiogenesis inhibitors in HCC can be generally divided into an antibody-based treatments and tyrosine kinase inhibitors (TKIs). Sorafenib, an oral TKI targeting RAF, VEGFR, PDGFRβ and RET, emerged as the first targeted therapy for advanced HCC based on the landmark SHARP study in 2008 [15] Median overall survival (OS) was 10.7 months in the sorafenib group, compared to 7.9 months in the placebo group (HR 0.69, P <0.001) [23] Sorafenib was the first systemic therapy approved by the Food and Drug Administration (FDA) for the treatment of patients with advanced HCC. Lenvatinib was another multitargeted oral TKI approved based on non-inferiority to sorafenib in overall survival in the REFLECT study in 2018 [24] Lenvatinib targets VEGFR1-3, FGFR1-4, PDGFRα, KIT and RET [15] It should be emphasized that in the pre-immunotherapy era, achieving non-inferiority was a substantial hurdle [25,26] An unmet need for systemic therapies of HCC persists in view of modest survival benefits and considerable adverse events of TKIs.

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3. The Ame Changer: Evolving Immunotherapy Landscape

A more comprehensive understanding of the pathogenesis, tumor microenvironment and immune system rendered immune checkpoint as attractive therapeutic targets for advanced HCC. The accelerated release from several positive randomized controlled trials has led to a paradigm shift towards immunotherapy-based treatments for advanced HCC in recent years. Immunotherapies for the treatment of advanced HCC generally involve an anti-PD-1/PD-L1 agent as monotherapy, or in combination with either an antiangiogenic agent or an anti-CTLA-4 agent [3] Commonly used immunotherapies for HCC include anti-PD-1 antibody (nivolumab, pembrolizumab, camrelizumab, toripalimab and sintilimab), anti-PD-L1 antibody (atezolizumab, durvalumab and tislelizumab) and anti-CTLA-4 antibody (ipilimumab, tremelimumab). The main efficacy outcomes of selected immunotherapy trials in advanced HCC are summarized in table 1.

Table 1. Selected clinical trials of immunotherapy in advanced HCC.

Trial	Year of First Publication	Phase	Experimental Arm and Control Arm	PFS (Months)	OS (Months)	ORR (%)	G 3-4 TRAEs (%)
1st line							
Anti-PD-1 (monotherapy)							
CheckMate-459	2022	III	Nivolumab vs sorafenib	3.7 vs 3.8 (HR 0.93)	16.4 vs 14.7 (HR 0.85, p=0.075)	15 vs 7	22 vs 49
RATIONALE-301	2023	III	Tislelizumab vs sorafenib	2.1 vs 3.4 (HR 1.11)	15.9 vs 14.1 (HR 0.85)	14.3 vs 5.4	22.2 vs 53.4
Anti-PD-1/PD-L1 and antiangiogenic agents (doublet therapy)							
IMbrave150	2020	III	Atezolizumab plus bevacizumab vs sorafenib	6.9 vs 4.3 (HR 0.65, p<0.001)	19.2 vs 13.4 (HR 0.66, p<0.001)	30 vs 11	43 vs 46
ORIENT-32	2021	II/III	Sintilimab plus a bevacizumab biosimilar (IBI305) vs Sorafenib	4.6 vs 2.8 (HR 0.56, p<0.001)	NR vs 10.4 (HR 0.57, p<0.001)	21 vs 4	53 vs 45
COSMIC-312	2022	III	Cabozantinib plus atezolizumab vs sorafenib	6.9 vs 4.3 (HR 0.74)	16.5 vs 15.5 (HR 0.98, p=0.87)	13.0 vs 5	66 vs 48
CARES-310	2023	III	Camrelizumab plus rivoceranib vs sorafenib	5.6 vs 3.7 (HR 0.52, p<0.0001)	23.8 vs 15.2 (HR 0.64, p<0.0001)	25 vs 6	81 vs 52
LEAP-002	2023	III	Lenvatinib plus pembrolizumab vs lenvatinib	8.2 vs 8.0 (HR 0.87, p=0.047)	21.2 vs 19.0 (HR 0.84, p=0.023)	26.1 vs 17.5	62 vs 57
HEPATORCH	2025	III	Toripalimab plus bevacizumab vs sorafenib	5.8 vs 4.0 (HR 0.69, p=0.0086)	20.0 vs 14.5 (HR 0.76, p=0.039)	25 vs 6	63 vs 61
Anti-PD-1/PD-L1 and anti-CTLA-4 (doublet therapy)							
HIMALAYA	2022	III	Tremelimumab plus durvalumab (STRIDE) vs sorafenib	3.78 vs 4.07 (HR 0.90)	16.43 vs 13.77 (HR 0.76, p=0.0008)	20.1 vs 5.1	50.5 vs 52.4
CheckMate-9DW	2025	III	Nivolumab plus ipilimumab vs lenvatinib or sorafenib	9.1 vs 9.2 (HR 0.87)	23.7 vs 20.6 (HR 0.79, p=0.018)	36 vs 13	41 vs 42
Anti-PD-1/PD-L1 and antiangiogenic agents (triplet therapy)							
CheckMate-040 Cohort 6	2022	I/II	nivolumab, cabozantinib and ipilimumab	22.1	4.3	29	74

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MORPHEUS-Liver	2025	Ib/II	Tiragolumab plus atezolizumab and bevacizumab vs atezolizumab and bevacizumab	12.3 vs 4.2 (HR 0.51)	28.9 vs 15.1 (HR 0.39)	43 vs 11	33 vs 44
2nd line							
KEYNOTE-224	2018	II	Pembrolizumab			17	25
KEYNOTE-240	2019	III	Pembrolizumab vs placebo	3.0 vs 2.8 (HR 0.718, p=0.0022)	13.9 vs 10.6 (HR 0.781, p=0.0238)	18.3 vs 4.4	18.6 vs 7.5
KEYNOTE-394	2022	III	Pembrolizumab vs placebo	2.6 vs 2.3 (HR 0.74, p=0.032)	14.6 vs 13.0 (HR 0.79, p=0.0180)	12.7 vs 1.3	13.3 vs 5.9

3.1. Immune Checkpoint Inhibitor Monotherapy

In the phase II KEYNOTE-224 trial, second-line pembrolizumab showed an overall response rate (ORR) of 17% in patients with advanced HCC, and gained accelerated approval in 2018 [27,28] In the confirmatory Phase III Keynote-240 trial, pembrolizumab as second-line therapy did not achieve statistical significance in progression-free survival (PFS) and OS improvement compared with placebo, although a favourable risk-benefit ratio was demonstrated [29] In contrast, in Asian patients with previously treated advanced HCC, pembrolizumab was associated with statistically significant and clinically meaningful improvement in OS, PFS and ORR compared with placebo in KEYNOTE-394 [30] The design of KEYNOTE-394 and KEYNOTE-240 was identical, except KEYNOTE-394 comprised of an entirely Asian population while KEYNOTE-240 was a global study. Of note, the majority of subjects in KEYNOTE-240 were non-Asians with a non-viral etiology of HCC [31,32] Despite the negative readout of KEYNOTE-240, FDA's Oncologic Drugs Advisory Committee (ODAC) voted to keep the approval for pembrolizumab [33] CheckMate-040 is a Phase I/II study involving six cohorts, investigating nivolumab as a monotherapy or in combination with other agents for patients with advanced HCC [34] Nivolumab monotherapy demonstrated clinical benefit in patients with advanced HCC in CheckMate-040 and led to nivolumab being investigated in the first-line in CheckMate-459 against sorafenib, which was the standard of care at that time [35] CheckMate-459 was the first phase 3 trial to evaluate a single-agent PD-1 inhibitor in the first-line treatment setting. Despite the fact that front-line nivolumab did not achieve a significant improvement in OS when compared with sorafenib (mOS 16.4 months for nivolumab vs 14.7 months for sorafenib, HR 0.85, p=0.075), it showed a favourable safety profile and represented a potential treatment option for patients contraindicated for antiangiogenic agents [35] It is noteworthy that nivolumab demonstrated ORR of 12% and disease control rate of 55% in patients with Child-Pugh B cirrhosis (B7-B8) in Phase I/II CheckMate 040 Cohort 5 [36] In RATIONALE-301, tislelizumab demonstrated non-inferiority to sorafenib in terms of OS [37] Nevertheless, limited efficacy of single-agent immunotherapy for HCC warrants the need for more effective treatment strategies.

3.2. Combining Immune Checkpoint and Angiogenesis Blockade

Antiangiogenic agents are broadly classified into anti-vascular endothelial growth factor (anti-VEGF) antibodies and multitargeted TKIs. IMbrave150 was the first phase III randomized trial to show a significant improvement in overall survival of the combination atezolizumab and bevacizumab over sorafenib and was FDA approved in 2020 [38] In the updated analysis, atezolizumab plus bevacizumab yielded a median OS of 19.2 months, with a 5.8-month improvement over the 13.4 months achieved by sorafenib (HR 0.66, P <0.001), and a nearly three-fold higher overall response rate (30% vs 11%) [39] Bevacizumab is a humanized monoclonal antibody against circulating VEGF-A, which normalizes vasculature, increases T cell infiltration and prevent dendritic cell maturation [22,40] Phase II studies have indicated that bevacizumab as a monotherapy exhibit only minimal activity against advanced HCC, like most other solid tumous [41] It has been

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demonstrated that multiple cell types within the tumor microenvironment, with established immunosuppressive functions, contribute to angiogenesis through the production of a variety of growth factors [42] Angiogenesis inhibitors also downregulate the activity of MDSCs, Treg cells and TAM, shifting the tumor microenvironment from immune suppressive to immune permissive [43] Combining antiangiogenic agents and immunotherapy is thus biologically relevant. Notably, the incidence of upper gastrointestinal bleeding was 7% in the atezolizumab plus bevacizumab group, as compared with 4.5% in the sorafenib group [38] In view of increased bleeding risk associated with bevacizumab, an upper endoscopy within 6 months of initiation of therapy was mandated for patients considered for atezolizumab and bevacizumab. Baveno VI and VII criteria are yet to be validated for HCC patients [44,45] IMbrave150 also excluded patients who are on anticoagulants or have high bleeding risk [38] Vp4 (thrombus in the main trunk of the portal vein or a contralateral portal vein invasion) confers a dismal prognosis to advanced HCC. Exploratory analysis of IMBrave 150 shows consistent benefit of atezolizumab plus bevacizumab over sorafenib in Vp4 subgroup (mOS 7.6 vs 5.5 months, HR 0.62, p=0.104) [46] A further prospective study involving Korean patients found that high levels of antidrug antibodies against atezolizumab at 3 weeks may experience inferior clinical outcomes treated with atezolizumab and bevacizumab [47] ORIENT-32 is a phase II/III study which demonstrated sintilimab plus IBI305 (a bevacizumab biosimilar) was superior to sorafenib in OS, PFS and ORR in all patients in China. Child-Pugh class B7 was allowed in ORIENT-32 [48]

In CARES-310, camrelizumab plus rivoceranib improved OS, PFS and ORR compared to sorafenib in a predominantly Asian population, despite a higher incidence of grade 3-4 TRAE in camrelizumab plus rivoceranib arm than sorafenib arm (81% vs 52%) [49,50] Rivoceranib is a VEGFR2-targeted TKI. VEGFR2 is expressed on endothelial cells and activated upon binding to VEGF-A. [15] In phase III LEAP-002 global study, pembrolizumab plus lenvatinib for patients with treatment-naïve advanced hepatocellular carcinoma improved ORR compared with lenvatinib monotherapy but failed to improve the dual primary endpoints OS nor PFS. Long-term follow-up of LEAP-002 showed that median OS was 21.1 months with pembrolizumab plus lenvatinib and 19.0 months with lenvatinib, HR 0.80. 5-year OS rate were almost doubled for patients receiving the combo versus lenvatinib, 19.7% vs 10.7%. [51,52] LEAP-002 mandated upper endoscopy within 3 months of randomization, and patients with main portal vein invasion were excluded. While the majority of patients in LEAP-002 were non-Asian with 63% patients in the experimental arm having a viral etiology, prespecified subgroup analysis of overall survival showed signals favouring the combination of pembrolizumab and Lenvatinib in patients with HBV etiology [51] In COSMIC-312, cabozantinib (multikinase inhibitor of RTKs including AXL, FIT-3, KIT, MET, RET, VEGFR 1-3) plus atezolizumab improved PFS and ORR over sorafenib but showed no difference in OS. As both PFS and OS were dual primary endpoints, COSMIC-312 can be regarded as a “semi-positive” trial. mPFS was 6.9 months for the combination of cabozantinib plus atezolizumab vs 4.3 months for sorafenib (HR 0.74), ORR 13% vs 5%, OS 16.5 vs 15.5 months (HR 0.98, p=0.87) [53,54] Patients in cabozantinib plus atezolizumab arm experienced higher dose reductions than those in sorafenib arm (62 vs 43%). There was also a notable disparity in post-progression treatment patterns, with an unexpectedly reduced proportion of patients in the cabozantinib plus atezolizumab arm (26%) receiving subsequent therapies compared to sorafenib arm (42%), and the underlying reason including differential liver function at cessation of cabozantinib plus atezolizumab versus sorafenib remains unclear.

It is also noteworthy that the proportion of patients with HBV etiology was less in COSMIC-312 than in IMbrave150, LEAP-002 and CARES-310. Like LEAP-002, subgroup analysis of COSMIC-312 suggested a potential benefit with atezolizumab and cabozantinib in patients with HBV etiology. A meta-analysis of IMbrave150, CheckMate-459, and KEYNOTE-240 showed HBV- and HCV-related HCC derive greater benefit than non-viral HCC from immunotherapy. [55] Recently published HEPATORCH shows that toripalimab plus bevacizumab significantly improves PFS and OS as compared with sorafenib. This combination has been approved in China. Combination of immune checkpoint inhibitor with an anti-VEGF antibody is a promising therapeutic strategy to potentiate anti-tumor immunity for patients with advanced HCC, and further translational studies are needed to characterize patients who benefit most from this combination. Despite the proven efficacy of oral

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multitargeted TKIs sorafenib and lenvatinib in the first-line treatment of advanced HCC, their combination with immune checkpoint inhibitors have yielded mixed results. CARES-310 is the first positive international phase III study with the combination with an immune checkpoint inhibitor and an oral small molecule tyrosine kinase inhibitor. However, rivoceranib exhibits high selectivity for VEGFR2, and is distinguished from other multi-targeted TKI used for the treatment of HCC.

3.3. Combining PD-1/PD-L1 and CTLA-4 Blockade

Combination of PD-1/PD-L1 and CTLA-4 targeting antibodies have shown distinct and complementary effects. CTLA-4 primarily regulates T-cell activation at the priming stage in tumor-draining lymph nodes involving CD28-mediated T cell co-stimulation, while PD-1 inhibition primarily occurs at the tumor site during effector phase. CTLA-4 blockade restores the positive CD28 costimulatory signals through B7 binding and down-regulates immunosuppressive Tregs. On the other hand, PD-1 directly phosphorylates CD28 via SPH2. [56,57] This shared targeting of CD28 represents a functional convergence in their regulatory roles.

The STRIDE regimen consisting of a single dose of tremelimumab with durvalumab every four weeks in patients with unresectable HCC was evaluated in the phase III HIMALAYA study. Patients with main portal vein thrombosis and gastrointestinal bleeding within the last 12 months were excluded. An earlier Phase I/II study showed that a single priming dose of tremelimumab demonstrated a saturable correlation between tremelimumab exposure and CD8+ T cell response, suggesting that further repeated tremelimumab exposure does not necessarily result in further CD8+ T cell proliferation [58] The primary endpoint, OS for STRIDE regimen versus sorafenib was met. mOS was 16.43 with STRIDE as compared with 13.77 months with sorafenib (HR 0.78, p=0.0035). STRIDE also increased ORR compared to sorafenib (20.1% vs 5.1%). However, mPFS was similar between STRIDE and sorafenib. This is in the contrary to COSMIC-312 that PFS was not translated to OS. Updated analysis from HIMALAYA showed a sustained OS benefit of STRIDE versus sorafenib, 5-year OS rate 19.6% versus 9.4%. Among patients who received STRIDE, OS benefit was more pronounced in patients who experienced disease control and any degree of tumor shrinkage [59] FDA approved tremelimumab in combination with durvalumab for unresectable HCC in 2022 [60] In HIMALAYA study, durvalumab monotherapy demonstrated non-inferiority to sorafenib (HR 0.86, 95.67% CI 0.73-1.03; noninferiority margin, 1.08) for patients with unresectable HCC [61] The ongoing SIERRA phase IIIb trial will help to address STRIDE regimen in broader populations with advanced HCC including those with Child-Pugh B cirrhosis (B7-B8), performance status 2 or Vp4 [62]

CheckMate-040 trial cohort 4 is a phase I/II study that randomized patients with advanced HCC to receive the combination of nivolumab 1mg/kg plus ipilimumab 3mg/kg every 3 weeks for 4 doses, followed by nivolumab 240mg every 2 weeks (Arm A), nivolumab 3mg/kg plus ipilimumab 1mg/kg every 3 weeks for 4 doses, followed by nivolumab 240mg every 2 weeks (Arm B), or nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks (Arm C). Patients in arm A achieved the longest median OS of 22.8 months, compared to 12.5 months in arm B and 12.9 months in arm C. ORR was 32% in arm A, 27% in arm B and 29% in arm C. Long-term survival benefit was maintained with a 5-year OS rate is 29% in arm A. Notably, grade 3-4 TRAE was higher in arm A than arm B, 53% vs 29%. [63–65] Higher doses of ipilimumab monotherapy in advanced melanoma and in combination with nivolumab in small cell lung cancer has also been reported to be correlated with improved survival outcomes and higher rates of irAE [66,67] The encouraging result of nivolumab plus ipilimumab in CheckMate-040 supported further investigation of this regimen as first-line treatment option in CheckMate-9DW trial. In CheckMate-9DW, patients with unresectable systemic therapy naïve HCC were randomized to receive nivolumab 1mg/kg plus ipilimumab 3mg/kg for 4 cycles, followed by nivolumab 480mg every 4 weeks or investigator's choice of either lenvatinib or sorafenib. Median OS for patients treated with nivolumab plus ipilimumab was 23.7 months as compared to 20.6 months in the lenvatinib or sorafenib arm (HR 0.79, p=0.018), which is the longest reported OS to date in this setting. OS benefit was consistent across clinically relevant subgroups, regardless of etiology and PD-L1 combined positive score (CPS) status. A post-hoc analysis showed

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that nivolumab plus ipilimumab conferred overall survival benefits versus lenvatinib (HR 0.77) or sorafenib (HR 0.42) individually after propensity score matching. Although PFS was comparable between the two groups, the nivolumab plus ipilimumab group had a numerically higher PFS rates at 18 and 24 months, as well as a more prolonged PFS on next-line therapy of 19.3 months versus 15.4 months in the lenvatinib or sorafenib group, despite the fact that 35% of patients received subsequent immunotherapy. The combination of nivolumab and ipilimumab compared to lenvatinib and sorafenib resulted in a superior objective response rate (36% vs 13%, p<0.0001), alongside longer duration of responses (30.4 vs 12.9 months). For patients who received nivolumab and ipilimumab, achieving complete response or partial response as the best overall response at 24 months is predictive of excellent survival benefits, with the median OS not reached (NR) (95% CI 44.4 months -NR) [68] Grade 3-4 TRAEs occurred at similar rates between ipilimumab plus nivolumab and lenvatinib or sorafenib group (41% vs 42%) [69] Both the STRIDE regimen and nivolumab plus ipilimumab demonstrated a favourable risk-benefit profile for patients with advanced HCC regardless of albumin-bilirubin (ALBI) grade [70–72] Patients with Vp4 were excluded from both HIMALAYA and CheckMate-9DW studies. Based on the results of CheckMate-040 cohort 4, the FDA granted accelerated approval to nivolumab and ipilimumab for HCC patients previously treated with sorafenib, with nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks (4 doses), then nivolumab monotherapy 240 mg every 2 weeks or 480 mg every 4 weeks in 2020 [73] The nivolumab and ipilimumab combination was also approved for first-line treatment of unresectable or metastatic HCC, according to CheckMate-9DW in 2025 [74]

Overall, the robustness of combined CTLA-4 and PD-1/PD-L1 blockade in front-line treatment of advanced HCC was nicely demonstrated in both HIMALAYA with a durable 5-year OS data as well as CheckMate-9DW with deep depth of response. In 2024, FDA approved nivolumab and hyaluronidase-nvhy for subcutaneous injection across all approved solid tumors in adults including HCC. However, nivolumab and hyaluronidase-nvhy is not indicated for concurrent use with ipilimumab. nivolumab and hyaluronidase-nvhy may be used for advanced HCC in substitution of intravenous nivolumab for nivolumab as monotherapy [75] Subcutaneous administration of immunotherapy has the potential benefits of reducing infusion chair occupancy, reducing injection site complications and shortening administration time.

3.4. The Search for Biomarkers

The lack of reliable predictive biomarkers to guide immunotherapy in HCC remains a challenge [76] Although HCC in cirrhotic patients can be diagnosed based on non-invasive imaging such as multiphasic liver protocol CT, tumor biopsy is recommended for patients enrolling in clinical trials to facilitate biomarker development [3]

Current evidence suggests that immunotherapies may confer better outcomes for HCC related to viral hepatitis than non-viral etiologies. This may be explained by the fact that CD8+ T cells in NASH-related HCC fail to mount immune surveillance and paradoxically lead to tissue damage promoting a pro-tumorigenic environment [77] Wnt/TGF-β proliferation subclass in NASH-related HCC has been associated with resistance to immunotherapy [78] On the other hand, abundance of viral antigens and distinct microenvironment may lead to improved outcomes of viral HCC treated with immunotherapy [2,55,79] HBV-related HCC is characterized by upregulation of PI3K-AKT-mTOR, RAS-MAPK, MET and IGF pathways, as well as the frequent TP53 mutations [80] Although the influence of hepatocellular carcinoma etiology on immune checkpoint inhibitor response and survival has been documented in preclinical studies and post-hoc analysis of randomized controlled trials, viral etiology does not always predict treatment outcomes and stratification based on disease viral etiology has not been validated [81,82] We should also be mindful of the heterogeneity of definition of viral etiologies across trials, for instance, variability of HBV and HCV serology test used.

Advancements in multiomic profiling enables molecular reclassification of HCC, which subsequently facilitates development of subclass-specific therapeutic agents [83] Based on transcriptome analysis, HCC can be classified into proliferation and non-proliferation class. The

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proliferation class is characterized by tumors displaying aggressive traits such as poor histological differentiation, vascular invasion and elevated α-fetoprotein (AFP). On the other hand, non-proliferation class HCC are less aggressive, generally well to moderately differentiated with low AFP level [80] Immunogenomic classification divides HCC into immune-active, immune-exhausted, immune-intermediate and immune-excluded subclasses. The abundance of helper T (CD4+) and CD8+ T cell infiltrates in immune-active HCC predicts response to immune checkpoint inhibitors [80,84] CTNNB1 mutated HCC with activating of Wnt/β-catenin pathway have been reported to be resistant to immunotherapy [85]. CTNNB1 mutations in HCC is associated with immune-exhausted phenotype [80,84] However, translation of molecular classifications of HCC from bench to bedside has been lagging behind as compared with many other solid organ tumors. One of the reasons is that most targets identified in HCC e.g. TERT promoter activating mutation, TP53 loss of function, CTNNB1 activating mutation and RAS-PI3K-mTOR pathway alterations are not yet druggable [86,87] Intra- and inter-tumor heterogeneity is another obstacle for molecular classification of HCC [88] The predictive value of PD-L1 expression in advanced HCC is still under investigation.

Currently, all FDA approvals of immunotherapy for HCC have been granted irrespective of PD-L1 expression. A meta-analysis including immunotherapy monotherapy trials shows that positive PD-L1 expression may be associated with higher response rates in advanced HCC [89] CD8+ T cells infiltration does not reliably predict immunotherapeutic response in HCC [86] Tumor mutation burden-high (TMB-H) is an infrequent finding in HCC, and clinical application of TMB as a biomarker in HCC is limited [76] An exploratory analysis of GO30140 phase 1b and IMbrave150 showed that high expression of VEGF Receptor 2 and intratumoral CD8+ T cell density was associated with improved clinical outcomes from the combination, while no correlation was found between TMB and survival benefits [90] A recent study showed that high FGF21 predicts worse survival outcomes in HCC treated with atezolizumab and bevacizumab [91]

Comprehensive molecular profiling, proteomic classification and immunogenomic characterization may be the future direction of HCC in the era of immunotherapy [86]

4. The Future Perspective: Novel Combinations and Targets

Advanced HCC remains a high unmet need with a modest OS of under 2 years. Current research is focused on exploring various combinatorial strategies and identifying novel therapeutic targets to improve treatment efficacy. In the phase I/II CheckMate-040 cohort 6, triplet therapy with nivolumab, cabozantinib, and ipilimumab yielded a mOS of 22.1 months, mPFS of 4.3 months, and ORR of 29% in the first-line treatment of advanced HCC [92] TRIPLET is a phase II/III trial assessing the benefit of adding ipilimumab to the combination of atezolizumab and bevacizumab in patients with advanced HCC treated in the first-line [93]

Novel checkpoint targets such as TIGIT, TIM3 and LAG-3 may define the next wave of immunotherapy development for advanced HCC with the aim to enhance T-cell function and infiltration. T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is another coinhibitory receptor expressed on activated and exhausted T cells and natural killer (NK) cells [94] Patients with HCC have high TIGIT expression in both CD4+ T cells and Tregs. TIGIT plays a critical role in the TIGIT-CD226-PVR axis. TIGIT, CD226, CD96 and PVRIG are expressed on T and NK cells, whereas poliovirus receptor (PVR, CD155) is often overexpressed on tumour cells. Binding of CD226 to its ligand CD155 promotes antitumour immunity response, while TIGIT competitively interacts with CD155 to induce tumour immune invasion. Anti-TIGIT antibodies restores the CD226-CD155 interaction. Innovative combination strategies with anti-TIGIT monoclonal antibody aim to synergize with anti-PD-1/PD-L1 to enhance anti-tumor immunity in a non-redundant manner. [95–97] Tiragolumab is an anti-TIGIT monoclonal antibody.(98) MORPHEUS-Liver is a phase Ib-2 study evaluating the addition of tiragolumab to atezolizumab plus bevacizumab as first-line treatment for advanced HCC. The triplet combination yielded a significantly improved ORR (43% vs 11%), PFS (12.3 vs 4.2 months, HR 0.51) and OS (28.9 vs 15.1 months, HR 0.39). Moreover, addition of tiragolumab did not substantially increase the incidence of treatment-related or immune-mediated adverse events [98] Results of the ongoing phase

 doi:10.20944/preprints202509.1985.v1

III IMbrave152/SKYSCRAPER-14 trial is eagerly awaited [96] Lymphocyte-activation gene 3 (LAG-3) is structurally similar to CD4, found on the surface of helper T cells. With MHC class II as its major ligand, LAG-3 plays a functional role in Treg-mediated immunosuppression [94] RELATIVITY-106 is under way to evaluate the triple therapy nivolumab, relatlimab and bevacizumab in treatment naïve HCC. T cell immunoglobulin domain and mucin domain 3 (TIM-3) found on CD4+ T cells negatively regulates CD4+ T cells through its ligand Galectin-9 [99] TIM-3 is found to be upregulated in patients who develop resistance to PD-1 blockade [15,100] Cobolimab (anti-TIM3 monoclonal antibody) plus dostarlimab have shown efficacy and safety in a Phase II study in the front-line treatment of unresectable HCC [101] Bispecific antibodies (bsAbs) is an emerging therapeutic strategy in haematological and solid organ malignancies. bsAbs are engineered to simultaneously target two different epitopes. Dual checkpoint inhibitor-blocking bsAbs aim to overcome immune checkpoint inhibitor resistance and enhance efficacy by simultaneously blocking two immune checkpoint receptors [102–104] KN046 is a bsAb inhibiting both PD-L1 and CTLA-4. In a phase II trial, KN046 in combination with lenvatinib showed promising efficacy in advanced HCC, and grade ≥3 TRAEs were reported in 47.3% of patients [105] ARTEMIDE-HCC01 is a phase III study evaluating rilvegostomig (anti-PD-1/TIGIT bsAb) plus bevacizumab with or without tremelimumab compared to atezolizumab plus bevacizumab. [106] GEMINI-Hepatobiliary phase II study has a platform design, and includes a HCC cohort testing volrustomig (anti-PD-1/CTLA-4 bsAb) as monotherapy or in combination with bevacizumab or lenvatinib. [107].

5. Conclusions

The introduction of immunotherapies for patients with advanced HCC is revolutionary and have led to groundbreaking results over the past few years. Better understanding of tumor microenvironment and cancer immunity paved the way to rational combination of immunotherapies which generally exhibits superior outcomes than single-agents. The use of single-agent anti-PD-1/PD-L1 should be limited to patients with advanced HCC who are contraindicated to receive immunotherapy-based combinations. Biomarkers for advanced HCC remain an unmet need. There are no head-to-head comparisons between anti-PD-1/PD-L1 in combination with angiogenesis inhibitors and anti-CTLA-4. Cautions ought to be taken during cross-trial comparisons in light of variability in trial designs, demographic factors and eligibility criteria. Given the robust evidence of immunotherapies in advanced HCC, neoadjuvant and adjuvant treatment of early-stage HCC with immunotherapies is being investigated [108] Overall, this is an exciting era for immunotherapies for HCC.

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Abbreviations

The following abbreviations are used in this manuscript:

AFP	α-fetoprotein
ALBI	Albumin-bilirubin
Anti-VEGF	Anti-vascular endothelial growth factor
BCLC	Barcelona Clinic Liver Cancer
bsAbs	Bispecific antibodies
CD4+	Helper T cells
CD8+	Cytotoxic T lymphocytes
CPS	Combined positive score
CTLA-4	Cytotoxic T-lymphocyte–associated protein 4
FDA	The Food and Drug Administration
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
ICI	Immune checkpoint inhibitor
ICIs	Immune checkpoint inhibitors
irAE	Immune-related adverse events
LAG-3	Lymphocyte-activation gene 3
MDSCs	Myeloid-derived suppressor cells
NASH	Non-alcoholic steatohepatitis
NK	Natural killer
NR	Not reached
ODAC	Oncologic Drugs Advisory Committee
ORR	Overall response rate
PD-1	Programmed cell death protein-1
PD-L1	Programmed cell death ligands 1
PD-L2	Programmed cell death ligands 2
PFS	Progression-free survival
PVR	Poliovirus receptor
TAM	Tumor-associated macrophage
TIGIT	T-cell immunoreceptor with Ig and ITIM domains
TIM-3	T cell immunoglobulin domain and mucin domain 3
TKI	Tyrosine kinase inhibitor
TKIs	Tyrosine kinase inhibitors
TMB	Tumor mutation burden
Tregs	Regulatory T cells
VP4	Thrombus in the main trunk of the portal vein or a contralateral portal vein invasion
OS	Overall survival

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