Japanese Journal of Gastroenterology and Hepatology

Review Article

ISSN: 2435-1210 | Volume 10

Immunotherapy for Advanced HCC: Review of Current Applications and Future Perspectives

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Received: 14 Jan 2024
Accepted: 05 Mar 2024
Published: 12 Mar 2024
J Short Name: JJGH

Keywords:

HCC; Immunotherapy; Immune checkpoint inhibitors; CAR-T cell therapy; Tumor microenvironment

Citation:

Braimakis I. Immunotherapy for Advanced HCC: Review of Current Applications and Future Perspectives. J Gastro Hepato. 2024; V10(9): 1-13

1. Abstract

Hepatocellular cancer (HCC) remains a disease with an important health burden worldwide. Early diagnosis in combination with favorable individual factors, including a good performance status and liver function allows the performance of surgical and/or locoregional treatment, which can result in an acceptable expected survival. However, in case of advanced-unresectable HCC, the systemic treatment strategy was only limited to tyrosine kinase inhibitors (TKIs) until 2017. In the past few years, a deeper understanding of the liver and HCC immune microenvironment has led to a breakthrough in the treatment of advanced HCC. New immune-regulating agents, called immune checkpoint inhibitors (ICIs), were found to have a tumor suppressive activity by targeting different molecules called immune checkpoints (such as PD-1, PD-L1, and CTLA-4), while also preserving a manageable safety profile and tolerability. Even though ICIs have gained a place in the current HCC therapeutic algorithms, the treatment results are still not ideal. This phenomenon necessitates the investigation of different novel therapeutic modalities and treatment combinations, aiming for the optimal therapeutic outcomes. This review sheds light on the pathophysiological background of immunotherapy as a treatment modality, the currently established treatment options , as well as the efficacy and the safety data of several combinational therapies, which are currently under evaluation. Additionally, all the current knowledge about several immunotherapeutic modalities including, adoptive cell transfer (ACT) and anti-tumor vaccines will be also described.

2. Introduction

Hepatocellular cancer (HCC) accounts for the majority of the primary liver cancer cases worldwide [1, 2]. The global incidence rate as well as the corresponding mortality rate of HCC have been estimated at 9,3 and 8,5 per 100,000 persons per year respectively [1, 3]. A variation in these rates has been observed, with the incidence rates following an upward trend in North America and Northwestern Europe [4], while a downward trend has been also reported in Asia [1, 5]. A variety of risk factors for HCC development have been identified, with hepatitis B virus (HBV) and hepatitis C virus (HCV) being the most important ones [1]. Nevertheless, the improvement in prevention measures (HBV vaccination) and new treatment options for viral hepatitis [1] as well as the increasing prevalence of Non-alcoholic fatty liver disease (NAFLD) globally, are some of the prevalent factors that have induced a swift change in HCC epidemiologic background, with an increasing part of HCC burden being mainly attributed to NAFLD and the metabolic syndrome [6, 7].

The majority of HCC cases are diagnosed in patients with liver cirrhosis, regardless the etiopathogenesis [8]. The overall prognostication for these cases is not only affected by tumor burden but also by factors regarding liver function [9]. Accordingly, the Barcelona Clinic Liver Cancer (BCLC) staging was created in order to combine tumor

features with liver function and the patient's performance status as a way to guide treatment decisions and determine prognosis [9, 10]. In the 2022 BCLC model update [9], 5 prognostic stages were defined taking the aforementioned factors into account, with different first-line treatment recommendations proposed for each one of them [9]. In particular, for very early stage (BCLC-0) and early stage (BCLC-A) HCC, surgical resection, local ablation such as radiofrequency ablation (RFA), and liver transplantation are among the recommended treatment options, with trans-arterial chemoembolization (TACE) reserved for cases when the aforementioned techniques can't be applied [9, 10]. As for the intermediate stage (BCLC-B), due to its great case heterogeneity, treatment options consist of either liver transplantation, TACE, or systemic therapies [9, 10]. Systemic treatment is also the treatment option of choice for patients that belong in the advanced stage (BCLC-C), while the best supportive care is provided to patients in the terminal stage (BCLC-D) [9, 11].

In 2007, sorafenib, a tyrosine kinase inhibitor (TKI) with the ability to inhibit both angiogenesis and cell proliferation in tumors [12, 13], was first introduced as systemic HCC therapy[14]. This agent inhibits kinases Raf-1 and B-Raf, vascular endothelial growth factor receptors 1, 2, and 3 (VEGFR1, VEGFR2, VEGFR3), and platelet-derived growth factor receptor β (PDGFR-β) [12, 13, 15], while it was proved to prolong survival in Child Pugh A HCC patients [12, 16], as it was evident in two phase III trials, the SHARP trial [12] and an Asia-Pacific trial [16]. Another TKI, lenvatinib, was evaluated in the randomized phase III REFLECT trial [18] and it was proven to be not inferior in terms of the overall survival to sorafenib [17, 18]. Lenvatinib is an angiogenesis inhibitor that inhibits both VEGFRs (VEGFR1, VEGFR2, and VEGFR3) and fibroblast growth factor receptors (FGFRs), suppressing tumor growth signals induced by those molecules [17, 19, 20]. Other inhibitors that were used as second-line treatment options include the multi-kinase inhibitors regorafenib and cabozantinib, as well as ramucirumab [11, 14]. Regorafenib is an inhibitor targeting kinases that promote angiogenesis and tumor growth, such as VEGFR1, VEGFR2, VEGFR3, PDGFR-β, and fibroblast growth factor receptor 1 (FGFR1) [21, 22]. Its efficacy as well as its safety profile were assessed in the phase III RESORCE trial [23] and were proven to offer survival benefits in HCC cases that showed progress while on sorafenib treatment, provided that the HCC patient was able to tolerate sorafenib use [23]. Cabozantinib is another multikinase inhibitor of VEGFRs (VEGFR1, VEGFR2, and VEGFR3) as well as MET and AXL [24, 25, 26]. It was evaluated in the phase III CELESTIAL trial [24], and consequently, it was recommended for its utilization as a second-line treatment option for HCC patients who show progress under sorafenib or do not tolerate sorafenib's adverse events [9, 11]. Additionally, Ramucirumab is a monoclonal antibody that inhibits VEGFR2 [27]. The contribution of the aforementioned agent to the overall survival in HCC patients, was demonstrated in the phase III REACH-2 trial [28], which included HCC patients who had disease progression on sorafenib and whose serum alpha-fetoprotein (AFP) levels were ≥400 ng/ml [11, 28, 29]. A breakthrough in HCC systemic therapy has been the emergence of immunotherapy and its implementation in HCC patients with advanced-stage tumors (BCLC-C and some cases of BCLC-B) [9]. This development was made possible due to the better understanding of the HCC immunosuppressive microenvironment [30] observed in the previous years, which has led to the development of a variety of immunotherapy regimens [31]. This article reviews the current available immunotherapies for the treatment of advanced HCC.

3. Liver and HCC Immune System

3.1. Normal Liver Immune Microenvironment: The liver is the main metabolic organ of the human body that also acts as an immunological organ [32]. The unique blood supply of the liver with both arterial blood via the hepatic artery and venous blood via the portal vein [33] is the reason why it is constantly stimulated by pathogens, microbe-associated molecular patterns (MAMPs), and dietary antigens deriving from the gastrointestinal tract as well as by pathogens from the circulatory system [32, 34, 35]. In order to avoid liver damage and autoimmunity caused by the overactivation of the immune system to those stimuli, a balance between immune response to pathogens and immunotolerance to non-pathological stimuli must be achieved [35, 36, 37]. Hepatic immunotolerance is sustained by an abundance of liver cells, including Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), dendritic cells (DCs), hepatic stellate cells (HSC), and hepatocytes, and their interaction with leucocytes [32, 35], as well as a balance between proinflammatory (IL-2, IL-12, IL-7, IL-15, and IFN-γ) and anti-inflammatory (IL-10, IL-13, and TGF-β) cytokines [35, 37]. Any impairment in this immunological balance can result in liver damage through chronic inflammation and autoimmune pathways and also induce tumorigenesis [34, 35, 38].

3.2. HCC Immune Microenvironment: The current development in immunotherapy for tumors, with HCC being one of them, was put forward after the conceptualization of cancer immunoediting theory [39, 40, 41]. Cancer immunoediting, which is a process initiated after the failure of intrinsic tumor suppressive mechanisms to prevent transformation [42], consists of three stages: elimination, equilibrium, and escape, known as the 3 "Es" [41]. The main concept for this multistage procedure is that the immune system can both restrain cancer growth and inconsistently promote tumors in different phases [39, 41]. The elimination phase, which is also known as immunosurveillance, occurs when cancer cells are targeted before they become clinically evident [40]. This phenomenon is accomplished through an anti-tumor immune response, known as the cancer-immunity cycle [43, 44]. This self-enhancing cycle contains seven distinct steps, starting with cancer antigens being released after cell death due to treatment or other causes [43, 44]. The aforementioned step is followed by the capture of antigens by dendritic cells (DCs), which in turn migrate to lymph nodes [44]. Next, DCs present the antigens to

CD8+ cytotoxic T lymphocytes (CTLs), leading to the activation of effector T cells, which then migrate to liver cancer tissue and infiltrate the tumor [43, 44]. Finally, T cells recognize cancer cells through T cell receptors (TCR) and kill them, releasing more antigens that promote and enhance this cycle [43, 44]. However, the cancer cells that survive this process may enter into a balance-state with the immune system, known as the equilibrium phase. During this phase T cells along with IFN-γ prevent tumor progression but do not achieve cancer cell elimination [41, 42, 45]. A combination of factors, such as genetic mutations [41] and immunogenicity sculpting [42], can lead to the third phase, the so-called escape phase [41]. Furthermore, tumor escape is characterized by the evasion of the immune response to cancer cells [46], which can be achieved either by intrinsic evasion mechanisms, such as flaws in the tumor antigen presentation process [47], or by the creation of an immunosuppressive tumor microenvironment (TME) via the recruitment of immune cells [42].

TME is perceived as an important factor in the process of tumorigenesis and tumor progression. It comprises both a cellular and a non-cellular component[48, 49]. The former, among others, consist of hepatic stellate cells (HSCs) [49], tumor-associated neutrophils (TANs) [48], regulatory dendritic cells (Dregs) [48], regulatory T cells (Tregs) [48], tumor-associated macrophages (TAMs) [50], myeloid-derived suppressor cells (MDSCs) [48, 51], and cancer-associated fibroblasts (CAFs) [49, 52]. The latter comprise cytokines such as IL-6, IL-8, and IL-22 [49], growth factors such as VEGF and PDGF [49, 53], and extracellular matrix (ECM) proteins such as matrix metalloproteinases (MMPs) and proteoglycans [29, 54, 55]. It is well established that chronic inflammation plays a major role in tumor emergence. This is induced not only through the recruitment of inflammatory cells that produce an abundance of factors such as chemokines and cytokines but also through the formation of reactive oxygen species (ROS) as well as the production of reactive nitrogen species [56]. These procedures create favorable conditions for HCC development[56]. What is more, the inflammatory cells drawn into the tumor site [57] subsequently form its TME and by interacting with the tumor cells, they establish an environment that further promotes tumor growth and metastasis [57]. In this context, while a share of the tumor-established immune cells such as dendritic and natural killer cells induce an anti-tumor impact [58], others, such as TAMS, MDSCs and Tregs bring about an immunosuppressive environment leading to HCC escape of the host's immune response [47]. In specific, TAMs recruited in the tumor site are classified into 2 types: the M1 that releases factors with an anti-tumor effect such as TNF-a [44] and the M2 that, on the contrary, releases tumor growth, angiogenic and immunosuppressive factors that result in HCC immune escape and thus enhance tumor progress[44,59,60]. MDSCs have an immunosuppressive impact on TME while also impairing NK cell toxicity against cancer cells [61]. As for Tregs, their increased presence within the HCC results in the suppression of the host's immune system and thus a promotion of HCC progress is occurred [44, 62]. Moreover, tumor development and metastasis are also enhanced by the presence of CAFs in the TEM [63].

Consequently, it is the conjunction of normal liver's immunotolerance on the one hand and the establishment of the immunosuppressive TME on the other hand that elicit immune evasion in HCC cases [65].

4. Immunobiology of PD-1/PD-L1 and CTLA-4

Immune checkpoints are molecules that play an important role in HCC immune escape, with the most studied among them being cytotoxic T lymphocyte protein 4 (CTLA-4), programmed cell death protein-1 (PD-1), programmed death ligand 1 (PD-L1), lymphocyte activation gene 3 (LAG-3), and T cell immunoglobulin domain and mucin domain 3 (Tim-3) [66, 67]. PD-1 is a transmembrane glycoprotein [68] that belongs to the CD28 family and is expressed in a plethora of immune cells, such as activated T cells, B cells, NK cells, and myeloid cells [65, 66, 69]. The main role of PD-1 is to attenuate T cell activity in the peripheral tissues, inducing peripheral tolerance and limiting autoimmunity damage [69, 70]. The expression of this molecule is promoted after T cell activation [71], and upon binding to its main ligands, named PD-L1 (also known as B7-H1) and PD-L2 (also known as B7-DC) [69, 70, 72, 73], an inhibition of T cell receptor (TCR)-mediated lymphocyte proliferation and secretion of cytokines are also induced [69]. More particularly, PD-L1 is not only normally expressed in liver cells such as in liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) but also in HCC cells, resulting in tumor immune escape [32, 65], a phenomenon that worsens HCC prognosis [74].

CTLA-4 is also a member of the CD28 receptor family, while is expressed on activated T cells and T regulatory cells (Tregs) [65, 70, 75]. Both CD28 and CTLA-4 bind to ligands CD80 (B7.1) and CD86 (B7.2) found on antigen-presenting cells (APCs), such as B lymphocytes and dendritic cells [69, 70, 75, 76]. However, while CD28 is a costimulatory receptor [70, 77], CTLA-4 bears the ability to block these signals by binding more tightly to CD80 and CD86 ligands due to its higher affinity for them [70, 75]. As a result, CTLA-4 binding to its ligands attenuates, on the one hand, T cell activation, leading to immune tolerance against self-antigens, but on the other hand, it also promotes T cell proliferation in HCC patients [66, 70, 75].

5. Immunotherapy in HCC

As it is already stated, HCC progression is particularly correlated with tumor evasion of immune system response, with immune checkpoints such as PD-1, PD-L1 and CTLA-4 playing a major role in this evasion mechanism [78]. Consequently, the concept of utilizing monoclonal antibodies to block this evasion, by preventing the interaction and binding of immune checkpoint proteins with their ligands, has been tested [14]. Inhibitors of molecules PD-1, PD-L1 and CTLA-4, wider known as members of immune checkpoint inhibitors (ICI) family, are the main antibodies under evaluation for cancer treatment, with some of them being already part of well-es-

tablished treatment guidelines [9, 11, 31, 79].

5.1. ICI Monotherapy

5.1.1. Nivolumab

ICIs that block PD-1 receptor and its ligand PD-L1 are currently considered the cornerstone of immunotherapy in advanced HCC treatment [9, 80, 81]. The first monoclonal antibody against PD-1, called Nivolumab, was introduced as a probable HCC treatment option in 2017, while it rapidly gained the approval by the Food and Drug Administration (FDA) for its use in advanced HCC patients, who showed progress under sorafenib treatment or after its cessation [31, 82]. Prior to its approval, Nivolumab's efficacy and safety profile were evaluated in the phase I/II multicenter CheckMate 040 study by El-Khoueiry et al. (2017) [83]. In total, 262 HCC patients with Child Pugh B7 or less and Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1 were enrolled in this study, with 48 of them entering a dose-escalation phase and 214 of them entering a dose-expansion phase [83]. The objective response rate (ORR,%) was estimated at 20% and 15% in the dose-expansion and dose-escalation phases, respectively [83]. As for safety, Nivolumab was proven to have a controllable safety profile, with only 25% of the patients in the dose-escalation cohort presenting with grade 3/4 adverse events related to Nivolumab treatment [14, 83]. Moreover, in another study by Yau et al. (2019), nivolumab was found to be equally safe and effective in sorafenib-experienced patients of an Asian cohort and an intent-to-treat (ITT) population, with no regional heterogeneity in terms of ORR [84]. Regarding liver function, a study by Kudo et al. (2021) demonstrated that nivolumab's safety and efficacy were also acceptable in Child Pugh B patients with advanced HCC, suggesting a probable application in this population [85]. To corroborate the aforementioned results, a randomized multicenter phase III CheckMate 459 study was performed by Yau et al. (2022), which compared Nivolumab to sorafenib as first-line treatment options in HCC patients in advanced tumor stages [86]. In this trial, 743 patients with advanced HCC, characterized by Child Pugh A liver function and ECOG performance status ≤ 1, were enrolled and randomly appointed to either the nivolumab (n = 371) or sorafenib (n = 372) arm [86]. Even though the comparison did not point out a statistically significant difference in terms of median overall survival (mOS) (16,4 months vs. 14,7 months in the nivolumab and sorafenib arms, respectively; p = 0,075), the acceptable safety profile (grade 3 or 4 adverse events attributed to treatment were fewer in nivolumab arm in comparison to sorafenib arm) and the clinical activity of nivolumab showcased that it could be a treatment option in selected HCC cases [14, 86]. In (Table 1). we summarize the immunotherapeutic regimens and other systemic agents for HCC (Table 1).

Table 1. Immunotherapy ± other systemic agents for HCC

Regimen	Class	mOS (95% CI), months	Reference number
ICI Monotherapy			
Nivolumab	anti-PD-1	13.8 (11.5–16.6)	80
Pembrolizumab	anti-PD-1	12.9 (9.7–15.5)	87
Camrelizumab	anti-PD-1	13.8 (11.5–16.6)	91
Tislelizumab	anti-PD-1	-	92
Tremelimumab	anti-CTLA-4	15.1 (11.3–20.5)	95
Durvalumab	anti-PD-L1	13.6 (8.7–17.6)	95
Combination Therapy			
Atezolizumab+Bevacizumab	anti-PD-L1+anti-VEGF	19.2 (17.0-23.7)	100
Sintilimab+biosimilar of Bevacizumab	anti-PD-1+ anti-VEGF	-	105
Nivolumab+Ipilimumab	anti-PD-1+anti-CTLA-4	-	106
Durvalumab+Tremelimumab	anti-PD-L1+anti-CTLA-4	18.7 (10.8–27.2)	95
Atezolizumab+Cabozantinib	anti-PD-L1+anti-VEGFR	15.4 (96% CI 13.7–17.7)	104
Pembrolizumab+Lenvatinib	anti-PD-1+TKI	21.2(19.0-23.6)	108
Camrelizumab+Rivoceranib (apatinib)	anti-PD-1+TKI	22.1 (19.1-27.2)	110
Ipilimumab+Atezolizumab+Bevacizumab	anti-CTLA-4+anti-PD-L1+anti-VEGF	-	115

mOS, median overall survival; ICI, immune checkpoint inhibitors; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T lymphocyte protein 4; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptors.

5.1.2. Pembrolizumab

Another monoclonal antibody against PD-1, the so-called Pembrolizumab, was first evaluated as an HCC treatment option in 2018 [87]. A non-randomized, phase II KEYNOTE-224 study was designed and performed, enlisting 104 sorafenib-experienced HCC patients with advanced tumor stage, with Child-Pugh A liver function, and (ECOG) performance status ≤ 1 [87]. The objective response rate (ORR,%) was estimated at 17%, progression-free survival (PFS) at 4,9 months, and mOS at 12,9 months [81, 87]. Regarding safety, grade 3 or 4 adverse events were noted only in 25% of the study population [87]. These results led to the approval of pembrolizumab by the FDA in 2018 as a second-line treatment option for HCC patients who showed progress on sorafenib or could not tolerate it [14]. Further assessment of the anti-tumor efficacy and safety profile of pembrolizumab was conducted via a randomized phase III KEYNOTE-240 study in 2020 [88]. This trial enlisted 413 patients with advanced HCC who had received sorafenib in the past and assigned them to take either pembrolizumab (n = 278) or placebo (n = 135) [88]. The primary endpoints of the study were mOS and median PFS (mPFS), and both improved in the pembrolizumab cohort in comparison to placebo [88]. In particular, mOS was estimated at 13,9 months for pembrolizumab-treated patients vs. 10,6 months for placebo, and mPFS was 3 months and 2,8 months, respectively [88]. However, the predefined threshold for statistical significance was reached for neither endpoint, affecting in a negative way the approval of pembrolizumab monotherapy in other countries except the USA [81, 88]. Yet, in March 2023, a randomized phase III KEYNOTE-394 study by Qin et al. (2023) was published [89]. This trial enlisted 453 patients of Asian origin with advanced HCC, liver function categorized as Child Pugh A, and ECOG performance status ≤ 1, who showed disease progression under sorafenib or chemotherapy (oxiplatin) treatment or were unable to tolerate them [89]. The study population was divided into a group receiving pembrolizumab (n = 299) and a placebo-receiving group (n = 153) [89]. Interestingly enough, in this trial, both primary (mOS) and secondary endpoints (mPFS and ORR) were found to be significantly improved in the pembrolizumab arm. More specifically, mOS was estimated at 14,6 months vs. 13 months in the pembrolizumab and placebo groups, respectively (p = 0,0180), mPFS was 2,6 months in the pembrolizumab arm vs. 2,3 months in the placebo arm, respectively (p = 0,0032), and ORR was found at 12,7% in the pembrolizumab group vs. 1,3% in the placebo group, respectively (p < 0,0001) [89]. Consequently, a contradiction is observed between the KEYNOTE-240 and the KEYNOTE-394 in terms of mOS and mPFS, which can probably be attributed to regional variation [88, 89]. Finally, in a study by Ryoo et al. (2021), the health-related quality of life (HRQoL) was sustained during pembrolizumab treatment in advanced HCC cases [90].

5.1.3. Camrelizumab

Camrelizumab is an anti-PD-1 antibody that is also under evaluation as a treatment option in HCC as well as in other cancer sites [14]. In order to assess its anti-tumor activity, a randomized phase II multicenter study was performed, enlisting in total 217 patients (109 received treatment every 2 weeks and 108 every 3 weeks) with advanced HCC, Child Pugh score ≤ 7, and ECOG performance status ≤ 1 who had experienced sorafenib or chemotherapy treatment prior to Camrelizumab [91]. This trial showed acceptable anti-tumor activity for Camrelizumab, with an ORR estimated at 14.7% and an overall survival rate of 74.4% at 6 months, while the safety profile of it was deemed acceptable, with only 22% of patients facing grade 3 or 4 adverse events attributable to Camrelizumab [91]. Accordingly, Camrelizumab gained approval for use as a second-line treatment after sorafenib or chemotherapy (oxiplatin) from the Chinese Food and Drug Administration [14].

5.1.4. Tislelizumab

Another member of the anti-PD-1 antibodies under assessment for HCC treatment is tislelizumab [81]. Its safety profile and anti-tumor activity as a treatment for solid tumors were first evaluated in a phase IA/IB study by Desai et al. (2020) and were found acceptable [92]. In accordance with that, a randomized phase III RATIONALE 301 trial is currently evaluating the use of tislelizumab as a first-line treatment in patients with advanced HCC in comparison to standard treatment with sorafenib [93].

5.1.5. Tremelimumab

Tremelimumab is a monoclonal antibody against CTLA-4, whose safety and anti-tumor effect in advanced HCC were initially evaluated in a study by Sangro et al. (2013) [94]. The promising results regarding both endpoints, which were observed in the aforementioned study, led to further assessment studies for this agent. Initially, a randomized phase I/II study 22 by Kelley et al. (2021) was organized, enlisting patients with advanced HCC, preserved liver function (Child Pugh score up to A), and ECOG performance status ≤ 1, who were previously treated with sorafenib or were unwilling to do so [95]. Even though this study consisted of 4 cohorts (Tremelimumab 300mg + Durvalumab, Durvalumab monotherapy, Tremelimumab monotherapy, and Tremelimumab 75 mg + Durvalumab), the existence of the monotherapy with the Tremelimumab cohort allowed for some conclusions regarding its use [95]. Concerning anti-HCC activity, the Tremelimumab monotherapy cohort showed the lowest ORR (7,2%), the greatest disease control rate (DCR) (49,3%), a mOS estimated at 15,11 months that only came second after the Tremelimumab 300mg + Durvalumab cohort's mOS at 18,73 months, and the highest median duration of response (DOR) at 23,95 months [14, 95]. As for the security profile, adverse events probably attributed to the applied treatment were the highest in the tremelimumab cohort, both those regarding all grades (84,1%) and those with grades ≥ 3 (43,5%) [95]. These results highlight the more favorable anti-HCC and safety profile of the combination treatment (tremelimumab 300mg + Durvalumab) in comparison to monotherapy [95].

5.1.6. Durvalumab

Durvalumab, an anti-PD-L1 antibody, was indirectly evaluated as a monotherapy treatment option for HCC in a number of studies. Accordingly, in the aforementioned phase I/II study 22 by Kelley et al. (2021), one of the arms under comparison was monotherapy with durvalumab [95]. In this study, durvalumab monotherapy was second in terms of ORR (10,6%) and third in terms of mOS (13,57 months) between the different treatment options [95]. Moreover, in the HIMALAYA phase III study by Abou-Alfa et al. (2022), among other endpoints, durvalumab as a single-agent first-line therapy for HCC was evaluated and found to be non-inferior to sorafenib in terms of overall survival [96]. Nevertheless, in both trials, the combination therapy of durvalumab with tremelimumab was qualified as the one with the best profile of benefit-risk [95, 96].

6. Combination Therapy

Since the monotherapy treatment approach didn't bring about the expected results, a swift in combination therapies evaluation has been observed during the previous years [81]. Accordingly, a combination of ICIs with different agents such as anti-VEGF antibodies, TKIs, and locoregional therapies, or even concomitant administration of 2 different ICIs, have been put forward.

6.1. Combination of ICIs with Anti-VEGF Antibodies: The importance of the anti-angiogenic effect of different treatment agents such as sorafenib [12, 13, 15], lenvatinib [17, 19, 20], and regorafenib [21, 22] in HCC therapy is well established [14]. Bevacizumab is another monoclonal antibody against VEGF with a known anti-angiogenesis impact on advanced HCC [97]. Atezolizumab, a monoclonal antibody targeting PD-L1 [32], was initially compared as monotherapy in unresectable HCC vs. the combination atezolizumab with bevacizumab in the same context in a multicenter phase Ib GO30140 study, which showed a more extended PFS in the combination treatment group [98]. The atezolizumab-bevacizumab combination was also tested against sorafenib in the randomized, multicenter phase III IMbrave150 study [99]. In this trial by Finn et al. (2020), 501 patients with advanced HCC and Child Pugh score A, ECOG performance status ≤ 1, who haven't experienced any other systemic HCC treatment before were enrolled in the study [99]. The study population was randomized in an atezolizumab + bevacizumab cohort (n = 336) and in a sorafenib cohort (n = 165) [99]. The combination treatment showed prolonged overall survival with a 42% lower risk of death, 2,5 months higher mPFS (6,8 months in the combination cohort vs. 4,3 months in the sorafenib group), and an increased ORR of 27,3% in comparison to 11,9% observed in the sorafenib group [81, 99]. Moreover, in terms of safety, grade 3 or 4 adverse events were of similar rate in both arms (56.5% vs. 55.1% in the atezolizumab + bevacizumab and sorafenib arms, respectively) [99]. In 2022, a study by Cheng et al. (2022) was published with updated data regarding the IMbrave150 study after an additional 12 months of follow-up [100]. The mOS and mPFS remained significantly increased in the atezolizumab-bevacizumab arm in comparison to the sorafenib arm. More specifically, mOS was found to be 5,8 months higher in the combination treatment group (19,2 months vs. 13,4 months in the atezolizumab-bevacizumab and sorafenib arms, respectively), and mPFS was found at 6,9 and 4,3 months in the two arms, respectively [100]. Moreover, real-life data from Asia regarding the efficacy and safety of the atezolizumab-bevacizumab combination corresponded to the aforementioned results [101, 102]. Finally, in a most recent retrospective study by Casadei-Gardini et al. (2023), atezolizumab plus bevacizumab vs. lenvatinib as a first-line treatment in patients with advanced HCC were compared, with no statistically significant difference in terms of OS being noted [103]. Consequently, the combination of Atezolizumab and Bevacizumab is currently regarded as the first-line systemic treatment in advanced HCC cases [9, 11].

Another combinational treatment that is conceivably effective against HCC is that of atezolizumab (an anti-PD-L1 antibody) plus cabozantinib. In a randomized phase III COSMIC-312 study, the combination atezolizumab plus cabozantinib was tested against sorafenib as a first-line systemic treatment in patients with advanced HCC, ECOG performance status ≤ 1, and liver function Child Pugh A [104]. In total, 837 HCC patients were enlisted and separated in the atezolizumab-cabozantinib arm (n = 432), in the sorafenib arm (n = 217), and in the cabozantinib arm (n = 188). From the primary endpoints of this study, mPFS was significantly increased in the combination arm (6,8 months vs. 4,2 months in the atezolizumab-cabozantinib group and sorafenib group, respectively; p = 0,0012), while mOS was not improved in the combination arm (15,4 months vs. 15,5 months in the atezolizumab-cabozantinib group and sorafenib group, respectively; p = 0,44) [104].

Finally, another combinational regimen, which is under assessment is that of sintilimab (an antibody targeting PD-1) with a biosimilar of bevacizumab, called IBI305 [105]. Accordingly, a randomized phase II/III ORIENT-32 trial was performed in order to compare this combination to sorafenib as a first-line systemic treatment in cases of unresectable HCC [105]. Both OS and PFS were found to be significantly increased in the sintilimab-IBI305 arm, rendering this combination a probable treatment option [105].

6.2. Dual ICIs Therapy: As immune checkpoints (PD-1, PD-L1, and CTLA-4) affect tumor development through different mechanisms, the combined use of different kinds of ICIs is a matter of research in several studies. The co-administration of nivolumab (an anti-PD-1 antibody) and ipilimumab (an antibody against CTLA-4) in patients with advanced HCC as a second-line treatment after sorafenib exposure was evaluated in a randomized phase I/II CheckMate 040 study by Yau et al. (2020) [106]. In this trial, 148 HCC patients were enlisted and divided into 3 arms with different doses of nivolumab and ipilimumab in each of them. Among them, arm A (every 3 weeks administration of 4 doses of nivolumab 1 mg/kg + ipilimumab 3 mg/kg and following that every 2 weeks nivolumab 240

mg) showed an ORR of 32% and an acceptable safety profile [106], and as a result, the FDA approved the use of this combination [81, 106]. Further appraisal of the nivolumab-ipilimumab combination as a first-line therapy in comparison to TKIs (sorafenib and lenvatinib) is expected from the phase III CheckMate 9DW ongoing study [82].

Another dual ICI therapy assessed is the administration of durvalumab (an anti-PD-1 antibody) with tremelimumab (an anti-CTLA-4 antibody). In the phase I/II study 22 by Kelley et al. (2021) previously described, the Tremelimumab 300mg plus Durvalumab arm was the one that showed the greatest anti-tumor efficacy, with an estimated ORR of 24% and a mOS of 18.73 months, as well as an acceptable safety profile [95]. What is more, in the HIMALAYA phase III study, the combination durvalumab plus tremelimumab was tested as first-line therapy in unresected HCC [96]. In this study, OS for the combination therapy was improved significantly in comparison to the sorafenib arm, while the ORR of this combination was the highest between the different arms, estimated at 20.1% [96]. Accordingly, durvalumab plus tremelimumab is recommended as a first-line treatment option in advanced HCC cases [9].

6.3. Combination of ICIs with TKIs: The combination of pembrolizumab (an anti-PD-1 antibody) and lenvatinib was assessed in terms of efficacy and safety in a phase Ib multicenter trial, enlisting 104 patients with unresectable HCC and no previous exposure to systemic therapy [107]. The ORR was 46.0% per mRECIST and 36.0% per RECIST v1.1, and the mPFS was 9.3 mo per mRECIST and 8.6 mo per RECIST v1.1 [107]. Moreover, mOS was 22 months, while the safety profile was acceptable [107]. Based on these encouraging results, a phase III LEAP-002 study is currently comparing the pembrolizumab-lenvatinib combination against lenvatinib monotherapy [81]. However, in the published primary results of this study [108], the primary endpoints did not reach the predetermined statistical significance threshold. More specifically, mOS at final analysis was 21,2 months in the pembrolizumab-lenvatinib arm and 19 months in the lenvatinib arm (p = 0,0227), and mPFS at interim analyses was 8,2 months in the pembrolizumab-lenvatinib arm and 8 months in the lenvatinib arm (p = 0,0466) [108].

Another combination under evaluation is that of camrelizumab (an anti-PD-1 antibody) plus rivoceranib (TKI targeting VEGFR2, also called apatinib) [109, 110]. Initially, the anti-tumor activity and safety profile of this combination were assessed in a nonrandomized phase II RESCUE trial, enrolling 190 patients (70 in the 1st line treatment arm and 120 in the second line treatment arm) with unresectable HCC, ECOG performance status ≤ 1, and liver function Child Pugh A [109]. This combination, when used as a first-line therapy, showed an ORR of 34.3%, an mPFS of 5.7 months, and a 12-month survival percentage of 74.7%, while when used as a second-line therapy, the aforementioned endpoints were 22,5%, 5,5 months, and 68.2%, respectively [109]. For both arms, the safety profile was deemed acceptable [109]. Following that study, the combination between camrelizumab plus rivoceranib was compared against sorafenib as a first-line treatment in a randomized phase III CARES-310 study [110]. In the CARES-310 study, 543 patients with unresectable HCC and without exposure to systemic therapy in the past were allocated to a camrelizumab-rivoceranib (n = 272) and a sorafenib (n = 271) arm [110]. Both mPFS (5,6 months vs. 3,7 months in camrelizumab-rivoceranib and sorafenib groups, respectively; p<0,0001) and mOS (22.1 months vs. 15,2 months in camrelizumab-rivoceranib and sorafenib groups, respectively; p<0,0001) were found to be statistically improved in the combinational treatment, while serious adverse events associated with treatment were found in 24% of the combination treatment cohort and 6% of the sorafenib cohort [110]. Thus, camrelizumab-rivoceranib is found to be an effective first-line treatment option for advanced HCC.

6.4. Triple Therapy Combinations: In order to further improve treatment outcomes, especially in advanced HCC evaluations of different treatment combinations is currently in progress. In a phase II TRIPLET study by Zhang et al. (2023), triple therapy with concomitant use of camrelizumab (an anti-PD-1 antibody), rivoceranib , and hepatic arterial infusion chemotherapy (HAIC) using the FOLFOX combination (oxaliplatin, fluorouracil, and leucovorin) was evaluated for use in advanced HCC (BCLC-C) [111]. The ORR was estimated at 77.1% and mPFS at 10.38 months, with the most common adverse events being a reduction in lymphocyte (37.1%) and neutrophil count (34.3%) [111].

In another phase II study by Lai et al. (2022), HAIC-FOLFOX was combined with lenvatinib and toripalimab (an anti-PD-1 antibody) in 36 patients with advanced HCC [112]. In this trial, the triple therapy showed promising anti-tumor efficacy with mPFS at 10.4 months and mOS at 17.9 months, with an acceptable safety profile [112].

What is more, a combination of TKIs (sorafenib, regorafenib, and lenvatinib) with camrelizumab (an anti-PD-1 antibody) and transarterial chemoembolization (TACE) (epirubicin hydrochloride and oxaliplatin) was found to have acceptable anti-tumor activity and safety profiles in advanced HCC cases [113]. In particular, the mPFS was 10.5 months and the ORR was 71.3% per mRECIST and 35.6% per RECIST version 1.1 [113].

TACE in combination with atezolizumab and bevacizumab was also evaluated in a retrospective study by Zhao et al. (2023) and found effective against unresected HCC cases with an mPFS of 7,03 months and a 12-month OS of 75.4% [114].

With a glance into the future of HCC immunotherapy, the triple combination of ipilimumab (anti-CTLA-4 antibody), atezolizumab (anti-PD-L1 antibody), and bevacizumab (anti-VEGF antibody) is currently under evaluation in a randomized phase II/III TRIPLET-HCC study in comparison to an atezolizumab-bevacizumab arm [115]. Apart from that, the combination TACE + lenvatinib + pembrolizumab is being tested against TACE monotherapy in a phase III LEAP-012 trial [116].

7. Other Immunotherapies

7.1. Adoptive Cell Transfer (ACT): Another approach to HCC treatment is the utilization of the so-called ACT. It is a type of therapy in which immune cells have been expanded ex vivo and then re-administered to the patient in order to target cancer cells [117]. The cells that are usually activated and used in this setting are NK cells, tumor-infiltrating lymphocytes (TIL), chimeric antigen receptor (CAR) T lymphocytes, and cytokine-induced killer (CIK) cells [80, 117]. The use of activated CIK as adjuvant immunotherapy was tested in a phase III trial by Lee et al. (2015) against a control group that didn't receive any adjuvant therapy and resulted in higher survival (recurrence-free and overall) for the arm using CIK [118]. CAR-T cell therapy has already been proven to have efficient antitumor activity against lymphoid malignancies [119], and as a result, its efficacy against HCC is already being tested in several studies. Most of them are still in an early phase, but some data about activity and safety are already available. In the majority of CAR-T cell therapies, the targeting molecule is glypican-3 (GPC3), which is increased in HCC [120, 121, 122]. In particular, in the phase I studies by Shi et al. (2020), CAR-GPC3 T-cell therapy was found to be safe and bear anti-HCC activity [123]. Moreover, another phase I study by Fang et al. (2021) assessed the efficacy of CAR-GPC3 T cell therapy in previously treated HCC patients [124]. The ORR was estimated at 16.7% and the mPFS at 4.2 months, with acceptable safety, rendering it a treatment option in previously treated HCC patients, especially when combined with TKIs [124].

7.2. Tumor Vaccines: Antitumor vaccines are also an area of research for their possible HCC treatment potential. The logic behind this approach is that antigens derived from tumor cells can be used to prompt anti-tumor immune responses [125]. These tumor-associated antigens (TAA) can be peptides that are presented by APCs to T cells, while other categories of vaccines are DC-based and oncolytic-virus-based ones [82]. Based on these observations, a DC-based vaccine was manufactured [126, 127] and used in a phase I trial by Rizell et al. (2019) [128]. In this study, the so-called ilixadencel was assessed as monotherapy or in combination with a TKI (sorafenib), showing a manageable safety profile as well as some anti-tumor activity in advanced HCC [128]. The safety and efficacy of a peptide-based vaccine were evaluated in a phase I study by Sawada et al. (2012), where a GPC3 peptide vaccine was used in 33 patients with advanced HCC, proving to be well-tolerated with some anti-tumor activity [129]. As for oncolytic virus-based vaccines, JX-594 (Pexa-Vec) was assessed in a phase II trial by Heo et al. (2013), showing a dose-dependent oncolytic action [130]. However, in a phase IIb TRAVERSE trial, Pexa-Vec didn't result in improved OS in patients with advanced HCC who were previously exposed to sorafenib [131]. What is more, a phase III Phocus trial by Abou-Alfa et al. (2023), which compared Pexa-Vec combined with sorafenib against sorafenib monotherapy, was terminated early as the mOS in the combination arm was worse than in the sorafenib monotherapy arm, thus failing to show clinical benefit in advanced HCC [132]. In (Figure 1) we present a schematic presentation of several immunotherapeutic modalities and their targets.

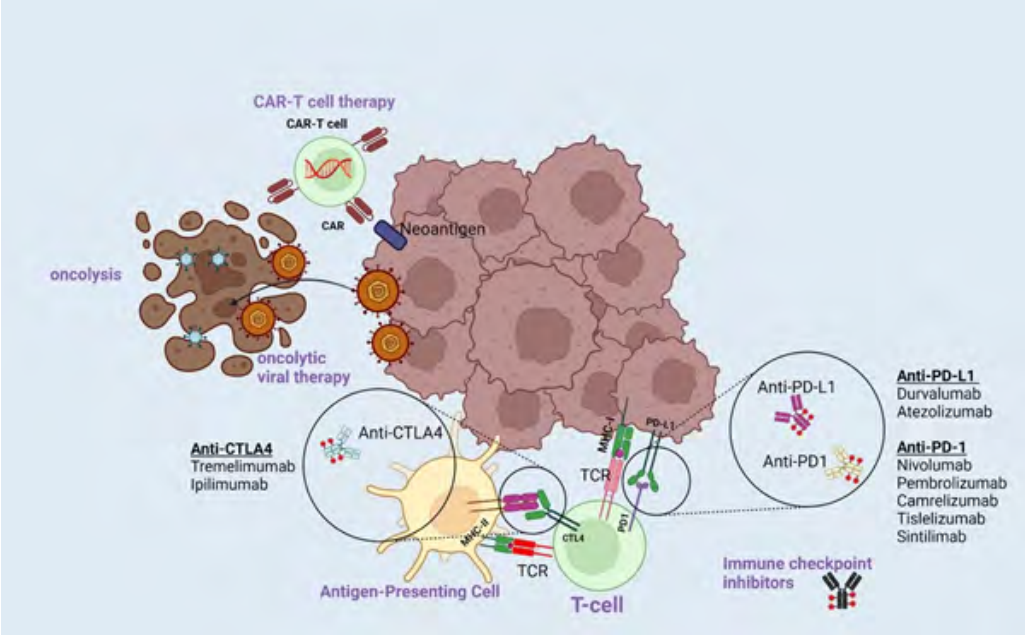

Figure 1: A schematic presentation of several immunotherapeutic modalities and their targets. There is a wide variety of immunotherapeutic modalities that are introduced in HCC management, including immune checkpoint inhibitors, chimeric antigen receptor (CAR)-T cell therapy, adoptive cell transfer, cancer vaccines, and/or combinations of all the aforementioned. This figure was created with BioRender.com (agreement number DJ263BLC83, accessed on 13 November 2023)

8. Conclusion

The progress in understanding the HCC immune microenvironment allowed for immunotherapy to arise as a promising treatment option, especially in HCC cases not amendable by resection and/or locoregional therapies. The many different immune-regulating mechanisms identified are being used as targets for inhibitory molecules in an attempt to direct and enhance the host's immune response against tumor cells. An abundance of immunotherapy agents has been evaluated either as monotherapy or in combination with other treatment options such as a second immune response-inducing agent, a TKIs, or an anti-VEGF agent. Some of these combinations are currently considered first-line treatment options in advanced HCC cases as they are proven to offer adequate survival benefits with a manageable safety profile. Nevertheless, since the existing treatment combinations are still far from being the ideal therapy for advanced HCC, more complicated combinations are currently under assessment, while cellular immunotherapy and anti-tumor vaccines are also under research. In this struggle, the best possible combination of high anti-tumor activity and good drug tolerability is the main goal that all future trials regarding HCC systemic treatment should try to meet.

9. Declarations

9.1. Authors' Contributions

Authors contributed equally to this manuscript. Conceptualization, I.B., N.P. and M.D.; validation, I.B., N.P. and M.D.; investigation, I.B. and E.-M.T.; resources, I.B.; data curation, I.B.; writing—original draft preparation, I.B. and E.-M.T.; writing—review and editing, N.P., P.A and M.D.; visualization, I.B.; supervision, P.A and M.D. All authors have read and agreed to the published version of the manuscript.

9.2. Availability of Data and Materials

Not applicable.

9.3. Financial Support and Sponsorship

None

9.4. Conflicts of Interest

All authors declared that there are no conflicts of interest.

9.5. Ethical Approval and Consent to Participate

Not applicable.

9.6. Consent for Publication

Not applicable.

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