Immune checkpoint therapy in liver cancer

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Abstract. Immune checkpoint therapies have been shown to be extremely successful in the treatment of cancer, with clinical utilization already established for suppressing CTLA-4, PD-1, and PD-L1. The CTLA-4 antibody ipilimumab and the PD-1 antibody pembrolizumab were demonstrated to improve survival in patients with metastatic melanoma. The Food and Drug Administration (FDA) has given approval to certain matching medications for the medical care of various tumors based on these targets. The current study also confirms the potential of these immune checkpoints and inhibitors to treat hepatocellular carcinoma. The objective of ongoing research is to integrate immune checkpoint therapy into hepatocellular carcinoma management, either as a standalone treatment or in combination with other therapeutic modalities, to enhance patient outcomes and survival. In this review, the specific roles and mechanisms of immune checkpoints and inhibitors in hepatocellular carcinoma are presented, as well as summarizing the current progress in their therapeutic use in the treatment of HCC and discussing their directions.

Keywords: Immune checkpoint, hepatocellular carcinoma, immunotherapy.

1. Introduction

Hepatocellular carcinoma (HCC) is the predominant form of primary liver cancer, including around 75% of all cases. According to the American Cancer Society, 41, 210 new cases of liver cancer will be detected in 2023, with approximately 30,000 fatalities [1]. It ranks fourth in the world in terms of cancer-related death, and its rate of incidence rises year after year. Chronic liver dysfunction is substantially linked to the development of HCC, especially in cases of cirrhosis stemming from various causes. Cirrhosis significantly elevates the likelihood of HCC development, emerging as the foremost risk factor for this malignancy. Moreover, it provides an environment conducive to the transformation of hepatocytes into cancerous cells. The progression of chronic liver disease is generally asymptomatic or nonspecific, making early clinical screening and diagnosis for hepatocellular carcinoma relatively difficult. Advanced hepatocellular carcinoma has a high recurrence rate after surgical treatment, despite the fact that common treatments, such as hepatic resection and liver transplantation, can result in a 70-80% 5-year survival rate. Moreover, the provision of surgical treatment mandates a meticulous appraisal of the patient's health status. Additionally, the process of transplantation may encounter obstacles due to factors like shortages of available organs, thereby exerting an influence on the continuum of patient care [2].

Immunotherapy is a treatment that fights disease by modifying, strengthening or improving the patient's immunity. Its main goal is to use and train the individual's immune system to detect, attack, and eliminate abnormal cells, including infectious diseases, autoimmune diseases, and cancer. Immunotherapy has been found to be useful in the treatment of cancer and includes monoclonal antibodies, immune checkpoint inhibitors, cytokines, and therapeutic vaccines. Immunotherapy was being investigated as a systemic therapy to solve the problem of treating advanced hepatocellular carcinoma as well as patients who have failed other treatment methods. Immune checkpoint inhibitor (ICI) therapies have yielded some results in recent years. Immune checkpoints include both stimulatory and inhibiting signals. Immune checkpoint inhibitor (ICI) therapy is said to be efficacy in studies involving the CTLA-4 and PD-1 checkpoints and their ligands. Tremelimumab targets CTLA-4 on activated T cells, which has been shown to be effective as a human monotherapy in preventing disease progression [3,4]. Nivolumab, a PD-1 inhibitor, has been approved by the U.S. Food and Drug Administration (FDA) as a second-line therapeutic drug due to its promising results in terms of both survival and safety for patients with advanced HCC [5]. Additionally, the combined

using of nivolumab and ipilimumab (CTLA-4 inhibitor) for the treatment of advanced HCC patients previously treated with sorafenib improves overall survival and is well tolerated [6]. Another PD-1 immune checkpoint antibody, pembrolizumab, which showed good tolerability in clinical phase II trials was also approved by FDA in 2019 for the treatment of aHCC [7]. Furthermore, numerous immune checkpoint inhibitor, such as mAb of LAG-3 and TIM-3, have been applied to hepatocellular carcinoma [8]. Combining ICI therapies and other cancer-specific treatments is also being investigated.

Immune checkpoint inhibitor (ICI) therapy has introduced a novel therapeutic approach and a more favorable prognosis for patients with HCC. This review will elucidate the precise mechanism of ICI therapy in treating HCC, delineate the ongoing research concerning existing drugs, and deliberate on potential challenges and opportunities in this context.

2. Immune checkpoint inhibitor therapy (ICIs)

2.1. Programmed cell death protein-1 (PD-1) and its ligand (PD-L1)

The CD28 family immune checkpoint receptor programmed cell death protein-1 (PD-1) is upregulated on activated T cells to develop immunological tolerance [9]. In the tumor microenvironment (TME) of HCC, PD-L1 is predominantly expressed in Kupffer cells, whereas PD-1 is predominantly up-regulated on CD8+ T cells. When CD8+ T lymphocytes contact Kupffer cells, they recruit the protein tyrosine phosphatase SHP-2. This recruitment of SHP-2 leads to inhibit the function of effector T cells (including CD8+ T cells). According to research, elevated PD-L1 expression in HCC patients is related to a worse prognosis [10,11]. The presently authorized monotherapeutic antibodies that target PD-1 include pembrolizumab and nivolumab, whereas atezolizumab and durvalumab are among the commonly used monotherapeutic antibodies that target PD-L1 [5, 7, 12]. In 2017, the Food and Drug Administration (FDA) granted licensure to nivolumab (NIVO), a monoclonal antibody that functions by inhibiting the signaling of PD-1 immune checkpoint, for the purpose of serving as a second-line therapy in cases of advanced hepatocellular carcinoma (HCC) that are resistant to sorafenib [5]. In the phase I/II trial, NIVO demonstrated effective tumor control in the treatment of patients with advanced HCC, with an overall objective remission rate (ORR) of 15-20% and six- and nine-month overall survival (OS) of 66% [5]. Meanwhile, the safety and tolerability of NIVO in the treatment of HCC could be demonstrated [5]. In a phase III study comparing Nivolumab (NIVO) to sorafenib as a first-line therapy for unresectable HCC, although statistical significance was not achieved, NIVO showed better results in terms of improving Overall Survival (OS), Objective Response Rate (ORR), and Complete Response (CR) compared to sorafenib [13].

Pembrolizumab has been employed for the treatment of HCC after demonstrating antitumor activity and safety in cancers such as melanoma, Hodgkin's lymphoma, and NSCLC [7]. Pembrolizumab was found to be well-tolerated in a phase II trial involving advanced hepatocellular carcinoma (HCC) patients who had previously undergone sorafenib treatment. The trial showed promising results, with a Disease Control Rate (DCR) of 71%, a 12-month Progression-Free Survival (PFS) rate of 28%, a 12-month Overall Survival Rate (OSR) of 54%, and a median Overall Survival (OS) of 12.9 months [7]. As a result, the FDA granted authorization for pembrolizumab as a second-line treatment for hepatocellular carcinoma (HCC) in 2019 and subsequently initiated the KEYNOTE-240 Phase III study [14]. In the Phase III trial, it was observed that the median Overall Survival (OS) was 11.6 months, and the Progression-Free Survival (PFS) was 2.8 months [14]. While statistical significance wasn't met for the predefined criteria, it was noted that pembrolizumab held meaningful potential for HCC treatment [14]. Furthermore, clinical trials have shown that the combination of atezolizumab and bevacizumab is both safe and effective in treating patients with unresectable hepatocellular carcinoma (HCC) [15]. The results revealed a reduced risk of death, increased 12-month Overall Survival (OS), improved median Progression-Free Survival (PFS), and

better Objective Response (OR) rates and PFS when using the atezo-bev combination compared to sorafenib [15].

Durvalumab has exhibited a well-tolerated safety profile and anti-tumor activity across various cancer types. In 2016, the FDA granted approval for its use in patients with inoperable or metastatic urothelial bladder cancer that is PD-L3 positive and has progressed following chemotherapy [16]. Durvalumab, whether administered as a monotherapy or in combination with tremelimumab for the treatment of advanced hepatocellular carcinoma (aHCC), is currently undergoing Phase I and II clinical trials. The results indicate an Objective Response Rate (ORR) of 10.6% for durvalumab monotherapy and 24.0% for the combination therapy. These treatments were found to be well-tolerated, although they did not exhibit exceptional safety profiles [16]. The Phase III trials are ongoing.

2.2. Cytotoxic T-lymphocyte associated protein-4 (CTLA-4)

CTLA-4 is a CD28 homolog that is abundantly expressed on activated and regulatory T cells (Tregs) [17]. CD80 (B7-1) and CD86 (B7-2) are known as ligands of CD28, and they are typically found on antigen-presenting cells (APCs). CTLA-4 engages in a competitive interaction with CD28 on the antigen-presenting cell (APC) membrane, specifically binding to B7-1/2. This interaction serves to restrict the activation of T cells while promoting the production of regulatory T cells (Tregs) [18]. Ipilimumab and tremelimumab are two common CTLA-4 monoclonal antibodies used to treat HCC [3, 19].

Tremelimumab is a completely human IgG2 monoclonal antibody target CTLA-4, blocking B7 from connecting to CTLA-4 and breaking the inhibitory signal of T cell activation [3]. Tremelimumab demonstrated an excellent safety and tolerability profile as well as antitumor effects when administered as a single drug to patients with HCC, with a DCR of 76.4 percent, a partial remission rate of 17.6 percent, and 6- and 12-month ORs of 64 percent and 43 percent, respectively [3]. Tremelimumab has also been utilized in conjunction with ablative treatment for HCC patients, with a 6 and 12-month PFS of 57.1 and 33.1 percent, respectively, and a median OS of 12.3 months, which is safe and tolerated [4]. Ipilimumab, an IgG1 monoclonal antibody, received FDA approval in 2011 as the first immune checkpoint inhibitor for treating patients with advanced skin cancer [19]. In patients with HCC, the combination of Nivolumab and ipilimumab has also shown therapeutic value [20]. The data indicated an ORR of 31%, with 7 complete remissions (CR), a median DOR of 17 months, a DCR of 49%, a 24-month OS rate of 40%, and an overall well-tolerated NIVO + IPI with an adequate safety profile in SOR-treated patients, with an ORR double that of NIVO alone (14 percent) [20].

2.3. Lymphocyte activation gene-3 (LAG-3)

LAG-3 is an immune checkpoint receptor protein that regulates T cell activity and is found on activated T cells, B cells, and NK cells. LAG-3 was found on CD8+ TILs and Tregs in in situ tumors at a higher level than on lymphocytes in hepatic tissue distant from the tumor and in HCC patients' peripheral blood [21]. In vitro and in vivo, inhibiting LAG-3 boosted the proliferating of CD4+ and CD8+ TILs as well as the generation of mediator cytokines. This shows that LAG-3 is a potential therapeutic target for HCC [21]. Relatlimab is a monoclonal antibody to LAG-3. It has been found that LAG-3, as well as a number of other immune checkpoints, will be upregulated as a surrogate for immune escape when PD1/PD-L1 is blocked, and therefore studies have been conducted on combination therapy of relatlimab with other immune checkpoint blockers [22]. In a trial for the treatment of advanced melanoma, the combination of relatlimab with nivolumab was shown to have a significant improvement over nivolumab alone. However, the combination's safety profile was not outstanding [23]. The combination treatment group had a PFS of 10.1 months and a 12-month PFS of 47.7%, which was a boost in antitumor activity relative to nivolumab's 4.6 months and 36% and was well tolerated [23]. The combination of Ieramilimab (another LAG-3 inhibitor) with spar talizumab (a PD-1 antibody) in phase I/II trials for the treatment of severe solid tumors revealed good

treatment tolerability and a safety profile comparable to that of spartalizumab alone [24]. All of these studies suggest that LAG-3 antibodies are promising in the treatment of HCC.

2.4. T-cell immunoglobulin and mucin domain 3 (TIM-3)

TIM-3 is expressed on several immune cells, such as T cells, NK cells, macrophages, and etc. In the TME of HCC, TIM-3 expression is upregulated in CD4+ and CD8+ T cells and TAM, stimulating the development of HCC [25, 26]. HBV-based studies in patients with HCC revealed that PD1 and TIM-3 levels were higher in liver-infiltrating lymphocytes than in peripheral tissues, and that upregulation was linked with a poor prognosis [26]. In the Phase I/Ib clinical trial, the efficacy and safety of Sabatolimab (MBG453), a monoclonal antibody to TIM-3, and spar talizumab, a monoclonal antibody to PD-1, were examined separately and in combination with the treatment of advanced solid tumors [27]. The finding showed that the combined therapy was quite well tolerated and demonstrated anti-tumor effectiveness, which has implications for further research [27].

3. Combination therapy and novel therapeutic approaches

3.1. ICI combined with tyrosine kinase inhibitor

Tyrosine kinase inhibitor (TKI) drugs like sorafenib and lenvatinib are commonly used for first-line systemic therapy of HCC, while second-line medicines include regorafenib, cabozantinib, and others [28]. As a first-line therapy for HCC, sorafenib, a TKI that mainly targets vascular endothelial growth factor receptor 2 (VEGFR2), platelet-derived growth factor receptor (PDGFR), and c-kit proto-oncogene proteins, has been employed [29]. The relevant combination therapy of sorafenib with ICI has been mentioned previously. Levatinib is another TKI that selectively inhibits VEGFR1-3, fibroblast growth factor receptor 1-4 (FGFR1-4), PDGFR, and c-kit and also performs well in the treatment of HCC [30]. A phase III LEAP-012 trial is underway to evaluate transarterial chemoembolization (TACE) in association with levatinib plus pembrolizumab for the treatment of intermediate-stage HCC [31]. Cabozantinib is a TKI that inhibits VEGFR1-3 and c-Met. Cabozantinib had a 12-week ORR of 5%, a 12-week DCR of 66%, a median PFS of 5.2 months, and a median OS of 11.5 months in a study in patients with HCC, demonstrating its clinical activity in HCC [32]. The phase 1b COSMIC-021 research tested the therapeutic effectiveness of cabozantinib plus atezolizumab in a range of cancer types and assessed the feasibility of this combination therapy as a treatment for advanced HCC [33].

3.2. Oncolytic Virotherapy

Oncolytic virus (OV) is a new type of anticancer drug that selectively infects tumor cells by using genetic mutations within the tumor and other methods to induce an immune system anti-tumor reaction, destroying blood vessels in the tumor so that tumor progression is blocked [34]. Adenoviruses expressing fusion proteins (lysogenic adenovirus, Adv) have been designed, and the recombinant viruses can couple with PD-L1 to enhance cytotoxic T-cell activity, which plays an inhibitory role in HCC [34]. Another replication-competent OV-based adenovirus encoding a bispecific fusion protein (PD1/CD137L) with the extracellular structural domain of PD-1 on one end and CD137L on the other has been created. Tumor-specific CTLs are activated by CD137 pathway signaling, and the PD-1/PD-L1 pathway on CTLs is stimulated and down-regulated, resulting in a significantly increased and extended anti-tumor response [35]. OV combined with molecular targeting for the treatment of HCC has been investigated in many studies, e.g., the combination of sorafenib and OV was demonstrated to boost the anti-tumor activity in mouse HCC tumor models, but there are still many difficulties that need to be overcome. In addition, for the therapy of HCC, OV in combination with ICIs will be a potential therapeutic strategy [34].

3.3. Combination and bispecific formulations (Fusion protein)

The integration of different antibodies directed against a single checkpoint into the same molecule to produce multi-specific antibodies is now available. Compared to single-target drugs, they can improve the specificity for tumors and reduce the side effects of the drugs. Representative bispecific drugs targeting checkpoint inhibitors have been used as immunotherapy for solid tumor treatment in clinical trials, such as PD-1 combined with CTLA-4 (NCT03530397), PD-1 combined with LAG-3 (NCT04140500), and PD-1 combined with TIM-3 (NCT03708328) [36]. However, such bi- or multi-specific combinations can limit the drug's optimum tolerated dosage, and the synthesis of constructed chimeric proteins may lead to more intense resistance in some patients [36].

4. Summary

Resistance to immune checkpoint inhibitors and immune-associated side effects (irAEs) are major challenges for ICIs. Causes of resistance include: (1) intrinsic factors within the tumor cells themselves, such as genetic variation, oncogenic signaling, etc.; (2) antigen presentation defects, such as epigenetic deletion of MHC-I leading to escape of tumor cells from T-cell recognition,; (3) T-cells that are less responsive to neoantigens of the tumors,; (4) the presence of oncogenic signaling pathways that can aid in immune escape, such as Wnt, PTEN/PI3K, and KRAS; and other pathways are associated with reduced T cell numbers, leading to less effective immune checkpoint therapy; and (5) many components of TME interact with each other to cause drug resistance. The mechanisms that lead to irAEs are still unclear, but they can be linked to the function of immune checkpoints in immune system homeostasis, possibly due to elevated levels of inflammatory cytokines and increased inflammatory responses due to the binding of immune checkpoints to their antibodies. irAEs include a number of different species that accumulate in a multitude of organs, such as the gastrointestinal tract, the skin, the liver, etc., and are often individualized from one person to another. Fatal irAEs can be effectively avoided by stratifying patients for precise treatment. Overall, there are still many issues that need to be, and are being, overcome in the treatment of ICIs, and host and tumor microbiome studies have shown the ability to influence immune system activity and thereby modify the therapeutic effects of ICIs.

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