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The potential combinational immunotherapiesfor treatment of hepatocellular carcinoma

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ABSTRACT

The treatment choices available for hepatocellular carcinoma (HCC) are limited and unsatisfactory. Recent improvements in our understanding of the mechanism involving immune checkpoints, including programmed cell death protein 1 (PD1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and also progress in the development of medicines make immunotherapy a promising approach to the treatment of numerous cancers, especially HCC. However, around 40% of patients still suffer from a progressive disease when treated with a monotherapy. Several clinical trials applying a combination therapy including immune checkpoint inhibitors have demonstrated the durable antitumor activity of these approaches in HCC patients. These clinical trials were done with the intent of evaluating the safety of these combination therapies, as well as whether they help improve the overall survival of patients. This study reviewed the recent progress in the use of combination therapies including immunotherapy in treating patients with HCC.

Primary liver cancer (PMC) now ranks as the sixth most commonly diagnosed cancer, and the third leading cause of cancer-related death worldwide.[1] Hepatocellular carcinoma (HCC) is the most common pathological type of PMC, and accounts for more than 80% of cases.[2] However, in clinical practice, the outcomes of HCC treatment are unsatisfactory. Since the early stages of HCC do not necessarily manifest typical symptoms, a considerable number of patients are only diagnosed with HCC in the advanced stages of the disease, and thus miss the opportunity to receive curative treatments, such as hepatectomy or liver transplantation. However, the treatment choices available for unresectable hepatocellular carcinoma (uHCC) are relatively limited. Transcatheter arterial chemoembolization (TACE), molecular targeted agents (MTAs), and local ablation or radiotherapy have been shown to benefit a number of patients, but their ability to improve the overall survival (OS) of uHCC patients is very limited. Therefore, it is vital to explore more effective treatment methods and choose appropriate treatment plans for patients with HCC, especially uHCC.

In recent years, with the development of a more in-depth understanding of the mechanisms involved in immune checkpoints, immunotherapy has shown great potential in the treatment of a wide variety of malignant tumors, such as lung, breast, and gastric cancers. The main mechanism by which an immune checkpoint inhibitor (CPI) exerts its antitumor effects is by reactivating the immune activity of T cells against tumor cells by inhibiting the activity of the immune checkpoint molecules that are overexpressed by tumor cells to avoid being cleared by the human immune system. The future of the application of CPIs in the field of HCC treatment is also promising. The anti-PD-1 (anti-programmed cell death protein 1) monoclonal antibody nivolumab was recognized as a breakthrough therapy by the United States Food and Drug Administration (FDA) in 2014, and has been approved for use in the treatment of a specific range of malignant tumors. Due to the results of a phase II clinical trial for its use in the treatment of HCC (CheckMate-040), nivolumab was approved for use as a second-line agent in the treatment of uHCC patients for whom sorafenib had failed to improve their condition in September 2, 017.[3] Another anti-PD-1 antibody, pembrolicumab, was given priority for trials by the FDA, which was also based on the promising results of a phase II clinical trial (KEYNOTE-224).[4] It was then approved as a supplementary new drug (sND) for use in second-line therapy for the treatment of advanced HCC against which sorafenib was ineffective in 2018. In addition, anti-CTLA-4 (anti-cytotoxic T-lymphocyte-associated protein 4) antibody has also achieved excellent results in HCC-related clinical trials, and is expected to be applied in the treatment of HCC in the near future.

Although immunotherapy has made surprising achievements in several clinical trials against HCC, it should be noted that the response of tumors to immunotherapy alone is usually limited, and the objective

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response rate (ORR) of such monotherapies is still unsatisfactory. About 30–40% of the patients treated in this way suffered from a progressive disease (PD), or moved from a stable disease (SD) state to a PD one. In addition, data from a phase III trial (KEYNOTE-240) showed that treatment with pembrolizumab, plus the best supportive care possible, did not meet its co-primary endpoints of achieving higher overall survival (OS) and progression-free survival (PFS) versus a placebo plus the best supportive care in patients with advanced HCC who were previously treated with systemic therapy. Therefore, similarly to the treatment strategies that were commonly used against other malignant tumors, researchers are now exploring the use of a combination of immune checkpoint inhibitors with other treatments for HCC therapy. Representative examples of such combined therapies include the combination of multiple CPIs (PD-1/PD-L1 (programmed death-ligand 1) antibody combined with CTLA-4 antibody), a CPI combined with molecular targeted agents (MTAs), or a CPI combined with local/systemic therapy. In this review, we summarized recent progress in the development and testing of the use of immunotherapy in combination with other therapies in the treatment of HCC.

1. Immune checkpoint inhibitors combination

Among HCC treatments using combinations of different immune checkpoint inhibitors, the use of anti-PD-1/PD-L1 antibody combined with anti-CTLA-4 antibody is the most common strategy at present. Previous studies have shown that CD8 (cluster of differentiation 8 protein) + T lymphocytes are essential to stimulating immune responses to tumors.[5] Therefore, when there is a lack of CD8 + T lymphocytes in the tumor environment, immune responses to tumors cannot be stimulated, regardless of whether the PD-1/PD-L1 pathway is inhibited.[6] However, the application of anti-CTLA-4 antibody could block the B7-CTLA-4 pathway, resulting in the proliferation of activated CD8 + T cells in the lymph nodes and their infiltration into tumor tissue, thus enhancing the antitumor effect of this treatment.[7] In addition, CTLA-4 is also expressed on the surfaces of the regulatory T cells (Treg cells) that infiltrate the tumor microenvironment, thereby inhibiting the immune system resistance mechanisms of tumors. Anti-CTLA-4 antibody is capable of enhancing the immune activity of Treg cells by inhibiting the expression of CTLA-4 molecules on the surfaces of CD8 + T cells.[8] The results of a phase III clinical trial (CheckMate-067) in patients with advanced melanoma showed that the combination of nivolumab and ipilimumab was more effective in prolonging the overall survival of patients than treatments using nivolumab or ipilimumab alone.[9] The overall three-year survival rate reached 58%, and the combination therapy was immediately approved by the FDA for the treatment of advanced melanoma. At present, several clinical trials of the use of combinations of immune checkpoint inhibitors for the treatment of HCC have been initiated.

1.1. Nivolumab plusipilimumab

At present, the use of nivolumab combined with ipilimumab versus nivolumab alone in the treatment of uHCC is still undergoing clinical trials (part of CheckMate-040), and the announcement of the results of these trials is eagerly awaited. The primary endpoint of that study is the safety and patient tolerance of the combination therapy. There are also two clinical trials targeting patients with resectable HCC or potentially resectable HCC (NCT03222076 and NCT03682276) to explore whether treatment with this combination of inhibitors prior to hepatectomy could improve the disease's prognosis. In addition, a clinical trial is being conducted in Taiwan (NCT03510871) that intends to explore the use of this combination as a neo-adjuvant therapy for uHCC, with the primary endpoint tested being the ORR.

1.2. Durvalumab plus tremelimumab

Durvalumab and tremelimumab are immune checkpoint inhibitors of anti-PD-L1 and anti-CTLA-4, respectively. They have achieved some positive results in the treatment of HCC, and thus show promise for their use in combination therapies. Part of the results of phase I and II clinical trials of the use of the combination of these inhibitors in patients with uHCC were announced at the 2017 annual meeting of the American Society of Clinical Oncology (ASCO).[10] A total of 40 patients were recruited in the phase I clinical trial. Up until the time of the resultant publication, a total of 7 patients that achieved a partial release (PR) from their disease and 23 patients with disease control (complete release + partial release + stable disease) for more than 16 weeks had been identified, which accounted for 57.5% of the total cohort in the study. Meanwhile, no unexpected adverse events occurred. This indicates that the combination of durvalumab and tremelimumab could bring continuous benefits to patients without apparent harm to them. Based on this result, a multi-armed phase III clinical trial (NCT03298451) to assess the use of this combination as first-line treatment for uHCC is currently underway, and is expected to be carried out in 15 countries globally, with a total of 1200 patients enrolled. This trial is named HIMALAYA, which implies the hope of conquering the 'pinnacle' of liver cancer.

2. CPIs combined with MTAs

The main difference between HCC and other malignant tumors is that systemic treatments suitable for use against HCC are extremely scarce. Until 2007, no systematic treatment was recommended for patients with the advanced stages of HCC. After more than 30 years of research, sorafenib has been approved as the first systematic therapeutic medicine for HCC. It has now become the standard treatment for patients with HCC at Barcelona Clinic Liver Cancer (BCLC) stage C, and has been the dominant MTA used as a first-line treatment against HCC for more than 10 years. Lenvatinib, a multi-target inhibitor, has shown anticancer effects comparable to those of sorafinib in phase III clinical trials (REFLECT), and has therefore been approved as the second orally administered MTA for the first-line treatment of uHCC. Nevertheless, the survival benefits of MTAs for HCC patients with advanced stages of the disease have been limited thus far, and sorafenib increased overall survival by only three months compared with a placebo. However, there was no significant difference in the improvement of OS between lenvatinb and sorafenib. When immune checkpoint inhibitors are combined with MTAs, they are expected to play a synergistic role. Previous studies have shown that the VEGF/VEGFR (vascular endothelial growth factor/receptor) pathway can be involved in the regulation of the immune status of the tumor microenvironment.[11] The release of VEGF leads to changes in the immunosuppressive state in the tumor microenvironment, promoting the proliferation and differentiation of Treg cells, inhibiting the maturation of plasmacytoid dendritic cells (pDCs), and ultimately leading to the functional decline of lymphocytes and T cells. In most circumstances, the blood vessels in tumor tissues are abundant and relatively disorganized. Thus, even when the immune system is activated, this may allow the transmission of activated T cells into tumor tissue. When MTAs are combined with PD-1/PD-L1 antibodies, the normalization of blood vessels can be realized by blocking the VEGF pathway, leading to the transmission of more lymphocytes to the tumor site. On the other hand, the immune suppression in the tumor microenvironment should be relieved to achieve better results. Therefore, the combination of immune checkpoint inhibitors and anti-VEGF antibodies or multi-kinase inhibitors (including anti-VEGF) has been a quite promising direction in research on HCC treatment, and some promising research results have continuously attracted the public's interest.

2.1. Pembrolizumab combined with MTAs

At present, the MTAs used in combination with pembrolizumab for treating HCC are lenvatinib and regofenib.

At the 2016 annual meeting of the European Society for Medical Oncology (ESMO), the results of a phase Ib clinical trial of the

combination of lenvatinib with pembrolizumab in the treatment of solid tumors were announced. The trial included 13 patients with different malignant tumors. The results showed that 7 patients achieved PR, as well as an ORR of up to 54%, and none of those patients suffered PD, with a disease control rate (DCR) of 100%. As for HCC, the results of a phase Ib open single-arm multicenter study (KEYNOTE-524) evaluating the tolerance and safety of the use of lenvatinib plus pembrolizumab for treating uHCC patients were published at the ASCO annual meeting in 2018. A total of 30 patients were included in that trial. The results showed that, as of March 2018, 23 patients were still receiving treatment, with an ORR of 42.3% (11/26 patients). The second tumor response assessment was conducted at least four weeks after the initial mitigation, with a confirmed ORR of 26.9% (7/26 patients). The average duration of PFS was 9.7 months. Based on the safety and efficacy evaluations thus far, that trial revised its protocol to include about 94 patients in an expanded cohort for its second part.

The clinical trial of the use of regofenib combined with pembrolizumab as a first-line treatment for patients with HCC is still underway (NCT03347292). This trial will determine the safety and tolerance of regofenib combined with pembrolizumab in patients with advanced stages of liver cancer. In addition, it will explore the anti-tumor mechanisms of the combination of drugs, as well as identify biomarkers associated with disease activity or treatment responses. The results have yet to be further disclosed.

2.2. Nivolumab combined with MTAs

As the first immune checkpoint inhibitor that was approved for HCC treatment in the world, nivolumab has also been widely expected to achieve high success rates in combination therapy strategies. At present, the MTAs used in combination with nivolumab to treat HCC in clinical trials are sorafenib, lenvatinib, and cabozantinib, among others. The results of all relevant studies of these combination treatments have not yet been published.

The clinical trial of the use of sorafenib combined with nivolumab as a first-line therapy for the treatment of unresectable, locally advanced, or metastatic HCC is still in the recruitment stage (NCT03439891). The primary endpoints of this trial will be the maximum tolerated dose (MTD) and ORR.

The feasibility of the use of lenvatinib combined with nivolumab in the treatment of HCC was tested in a phase I clinical trial (NCT03418922). The primary endpoints of this trial were the MTD and the occurrence of adverse effects. Another exploratory, open, single-arm, multicenter, phase II clinical trial is also under way (NCT03841201). The purpose of this trial is to evaluate the efficacy and feasibility (safety and tolerance) of the use of nivolumab combined with lenvatinib in the treatment of advanced multinodular HCC.

Cabozantinib was approved for use in the second-line treatment of advanced HCC by the FDA in 2018. Based on the results of the phase III clinical trial CELESTIAL, the overall survival of patients with advanced HCC who receive cabozantinib after disease progression in combination with sorafenib treatment was significantly improved.[12] The PFS and ORR were also both better compared with those with a placebo. Currently, as part of the CheckMate-040 study, the combination of cabozantinib and nivolumab is mainly being used to evaluate whether it is more effective than treatment with a single medicine; this study is still ongoing. In addition to exploring the efficacy of cabozantinib combined with nivolumab in treating uHCC patients, another clinical trial is underway to assess whether this combination can be used as preoperative neo-adjuvant therapy for locally advanced HCC (NCT03299946). The results have not yet been announced.

2.3. Atezolizumab combined with MTAs

At present, the MTAs that could potentially be combined with atezolizumab are cabozantinib and bevacizumab.

Bevacizumab is a recombinant humanized monoclonal IgG-1 antibody that can efficiently bind to VEGF and prevent it from binding to the receptors (e.g., Flt-1 and KDR) on the surface of tumor vascular endothelial cells. Furthermore, it can accurately inhibit the proliferation of tumor vascular endothelial cells and tumor angiogenesis. HCC happens to involve the formation of vascular-enriched tumors, so the combination of anti-PD-1 antibody and monoclonal antibody is considered to be promising for use in its treatment. The results of evaluations of the safety and clinical activity of atezolizumab combined with bevacizumab in patients with advanced or metastatic HCC in a phase Ib clinical trial were announced at the ASCO meeting in 2018.[13] The patients were treated with atezolizumab (1200 mg) plus bevacizumab (15 mg/kg) every 3 weeks. After a median follow-up period of 10.3 months, release was observed in 15 (65%) of the 23 patients. Release was observed in all subgroups, including those differing in disease etiology (hepatitis B, hepatitis C, or non-viral), geography (Asia (excluding Japan) or Japan/United States), alpha-fetoprotein baseline levels (high/low), or extrahepatic tumor spread (yes/no). Based on the above results, the FDA has approved this combination as a breakthrough therapy for application as a first-line treatment for patients with advanced or metastatic HCC, and a phase III study (IMbrave150) of this treatment approach is currently underway.[14] However, the latest results of that trial were reported at the 2018 ESMO meeting,[15] and among the 103 patients included in that trial, the ORR decreased significantly by either 32% (as assessed by investigators) or 27% (according to independent assessments), which is significantly lower than the ORR of 65% previously reported by ASCO. However, the difference was not statistically significant ($P > 0.05$), even when evaluated according to the mRECIST criteria by independent evaluation. One of the reasons for the decreased response rate in this trial was that the percentage of patients with macrovascular invasion (MVI) and/or extrahepatic spread (EHS) was higher during this trial (88% (ESMO 2018) versus 65% (ASCO 2018)). The results of the ESMO trial showed that the ORR among MVI- and/or EHS-positive (BCLC stage C) patients was 28%, while that among MVI- and EHS-negative (BCLC stage B) patients was 63%. Indeed, patients with advanced-stage HCC were a subgroup with a poor response to systemic therapy compared with that of intermediate-stage patients. However, according to the results published by ASCO, the effective rate was 73% in MVI- and/or EHS-positive patients and 50% in MVI- and EHS-negative patients. Understanding these opposite results might require further follow-up and deeper analyses.

2.4. Camrelizumab combined with apatinib

Camrelizumab (SHR1210) is an anti-PD-1 antibody, and the results of a phase I clinical trial of its use in combination with apatinib, a tyrosine kinase inhibitor selectively acting on VEGFR-2, have been announced.[16] A total of 18 patients with HCC were included in the trial. The ORR was 38.9%, and the median PFS was 7.2 months. The adverse effects were manageable, with treatment only discontinued in one patient because of treatment-related grade 3 hyperbilirubinemia.

3. CPIs combined with local/systemic therapy

Local treatment of tumors is expected to affect the tumor microenvironment and enhance the efficacy of immune checkpoint inhibitors. In addition, it is expected to have improved therapeutic effects by stimulating the release of tumor-associated antigens and new antigens from tumor cells into the blood.[17,18] Local-regional therapies, such as radiofrequency ablation (RFA) and transcatheter arterial chemoembolization (TACE), are often used as standard treatments against tumors, especially in patients with HCC.[19] Many clinical studies have begun to explore the synergistic effects of the use of immune checkpoint inhibitors in combination with such local treatments. For example, in a multivariate analysis by Mizukoshi et al.,[20] it was found that the increased intensity of the interferon-γ specific immune response after radiofrequency ablation

in patients with HCC was the only prognostic factor for this treatment.[20] Once the immune response is stimulated, it may be magnified by immune modulators.

3.1. CPIs combined with RFA/TACE

The results of clinical trials showed that treatment of HCC by radiofrequency ablation combined with a CTLA-4 inhibitor (tremelimumab) could not only effectively control the primary disease lesions, but also increase the aggregation of cytotoxic T lymphocytes in distant, untreated lesions.[21] The objective response of untreated lesions to treatment and the duration of response were also increased. The patients were given tremelimumab every 4 weeks, and local treatment began on the 36th day of treatment. A total of 32 HCC patients were included in the trial, and no dose-related toxicity was observed. Of the 19 evaluable patients, 5 (26.3%) suffered PR outside the ablation- or TACE-treated area. Tumor growth developed in one patient eight weeks after the start of treatment, but then the tumor's growth rapidly subsided. The median PFS was 7.4 months, and the median survival time was 12.3 months.

An early phase I study was initiated to test the safety and feasibility of using nivolumab in combination with drug-eluting bead transarterial chemoembolization (deb-TACE) in patients with liver cancer (NCT03143270).[22] In this study, deb-TACE (loaded with 75 mg of doxorubicin) was administered on Day 0, and then nivolumab was applied at a dose of 240 mg by IV every 14 days for 1 year. The patient recruitment for this trial has been completed, and the follow-up results will be announced in the future.

3.2. CPIs combined with radiotherapy

Through some animal experiments, it has been found that when the tumor area is exposed to a certain dose of radiation, the number of T lymphocytes in the tumor tissue increases. This suggests that radiotherapy can induce tumor-specific T lymphocytes to infiltrate the tumor tissue. The dendritic cells (DCs) can sense the DNA fragments released after the disintegration of tumor cells and produce interferon-β, a substance that guides T lymphocytes to converge on tumor tissue, by autocrine signaling. When the tumor tissue is irradiated, the levels of interferon-β produced by DCs will be increased, which greatly promotes the aggregation of T lymphocytes in and around the tumor tissue. In addition, radiation can also promote the necrosis of immunogenic cells in tumor tissue, which increases the oxygen content and pH value in the tumor tissue and simultaneously induces changes in the tumor vascular system. This provides convenient conditions for the recruitment of immune effector cells (mainly T lymphocytes) to tumor tissue. Therefore, through the influence of radiation on the tumor microenvironment, the immunogenicity of the tumor is enhanced, and the tumor tissue is transformed from the 'cold tumor' state to the 'hot tumor' state, thus improving the response of the tumor tissue to immunotherapy.[23,24]

There is a phase II study underway of the safety and antitumor efficacy of nivolumab after selective internal radiation therapy (SIRT) for the treatment of patients with HCC (NCT03380130). In this trial, SIRT will be performed in a single session using resin microspheres (SIR-Spheres). After 3 weeks, treatment with nivolumab at a dose of 240 mg every 2 weeks will be initiated.

3.3. CPIs combined with chemotherapy

In the past, chemotherapy alone was considered to not be effective enough for the treatment of HCC patients. In later phase II clinical trials, researchers found that oxaliplatin-based chemotherapy could improve the OS and time to progression (TTP) in HCC patients. The Chinese subgroup of a multicenter phase III clinical trial (the EACH study) showed that FOLFOX4 (oxaliplatin, folinic acid, and 5-fluorouracil combination therapy 4) improved local tumor control and the OS in patients with advanced HCC better than a doxorubicin treatment.[25] Thus, the combination of CPIs with oxaliplatin-based chemotherapy is a new strategy to help the immune system fight against cancer. At present, a phase III clinical trial to evaluate the use of camrelizumab (SHR-1210) in combination with the FOLFOX4 regimen as a first-line therapy (in comparison to sorafenib treatment) in subjects with advanced HCC who have never received prior systemic treatment is underway. The primary study hypothesis is that treatment with SHR-1210 combined with FOLFOX4 should improve the response rate and OS of patients compared with those achieved by standard treatments. The follow-up of this combination therapy is worth looking forward to.

4. Challenges and prospects of combined immunotherapy

Since immune checkpoint inhibitors were applied in the treatment of HCC, the enthusiasm of researchers for developing new standard treatments for HCC has been growing rapidly. At present, combined therapies including CPIs have achieved some accomplishments in the treatment of HCC. In some patients, a lasting treatment response is obtained from this type of combination therapy.[26] However, there are still many challenges in the current application of these treatments. First, it remains unclear how to determine the best combined treatment to use. For example, the dose of lenvatinib for the treatment of endometrial cancer, head and neck squamous cell carcinoma, and renal cell carcinoma is 20–24 mg, which is the maximum tolerable dose. However, the prognosis of patients with HCC is relatively poor, and thus lenvatinb is used in them in a relatively cautious manner, and the dose applied was reduced to 8–12 mg in studies of HCC. Therefore, whether the maximum tolerable dose must be used in a combined therapy is still a problem worth exploring, and the most effective dose needs to be constantly searched for in the future. Second, the ORR of the combined therapy can be surprisingly high when applied to other types of tumors, but the ORR becomes very low when the same therapies are applied to HCC. Therefore, methods to identify the group of patients who can most benefit from a particular combined therapy through the use of biomarkers, and even liquid biopsy, should be one of the research directions pursued in the future. Since HCC has only a moderate mutation burden and few excessive mutations exist, mutations are unlikely to be the main determinants of HCC responses to immune checkpoint inhibitors.[27,28] In addition, tumor-specific factors (e.g., immune activation level) and host factors (e.g., intestinal microbiome or human leukocyte antigen (HLA) heterogeneity)[29] might be more important in HCC, and many studies have applied these related factors as integrated biomarkers. In addition to biological correlations, several imaging methods are being developed to help identify such potential markers, including texture analysis and functional T cell imaging.[30] Third, methods should be explored to reduce the incidence of adverse effects of treatment. In general, the incidence of adverse reactions in combined treatments is higher than that in single-medicine treatments. In the previously reported clinical studies, the incidence of grade III to IV adverse effects was between 60 and 70%, which is of great concern. Therefore, the identification of predictive biomarkers is essential for assessing treatment responses and/or patients with poor tolerance. In the future, more efforts need to be directed toward the management of the adverse effects of combined immunotherapy, which is a problem that urgently needs to be solved.

As noted above, combined treatments using immune checkpoint inhibitors could lead to significant innovations in HCC treatment in the near future.[31] As research helps us to further understand the treatment mechanisms of combined therapies, further drug development efforts will greatly improve the prognosis of patients with HCC, especially uHCC.

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