

REVIEW



Potentiating cancer vaccine efficacy in liver cancer

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ABSTRACT

Hepatocellular carcinoma (HCC) is the most common liver malignancy with a poor prognosis and an overall 5-year survival rate of approximately 5–6%. This is due because standard of care treatment options are limited and none of them shows a sufficient efficacy.

HCC is an "inflammation-induced cancer" and preliminary preclinical and clinical data suggest that immunotherapeutic approaches may be a good alternative candidate for the treatment of HCC patients improving the dismal prognosis associated with this cancer.

However, recent findings strongly suggest that an optimal immunotherapy in HCC requires the combination of an immune activator with immune modulators, aiming at compensating the strong liver immune suppressive microenvironment. One of the most promising strategy could be represented by the combination of a cancer vaccine with immunomodulatory drugs, such as chemotherapy and checkpoint inhibitors. Very limited examples of such combinatorial strategies have been evaluated in HCC to date, because HCC easily develops resistance to standard chemotherapy, which is also poorly tolerated by patients with liver cirrhosis. The present review describes the most update knowledge in this field.

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the ten most frequent solid cancers and the third leading cause of cancer-related death worldwide and its incidence is still rising in many Countries. A wide range of therapies are used in the management of HCC according to the extent and severity of liver disease, however, the overall prognosis for HCC patients is poor.^{1–3}

The major risk factors for HCC are viral hepatitis chronic infections, which are responsible for persistent inflammation in the liver. Consequently, HCC can be classified as an "inflammation-induced cancer" and the immune-based therapies may be valid alternative treatments, as suggested by preliminary preclinical and clinical data.

However, despite developing in a context of chronic inflammation, HCC is characterized by an immunosuppressive tumor microenvironment. Indeed, liver is considered "an immunological organ" characterized by a strong intrinsic immune tolerance, strong innate immunity and poor adaptive immune response.⁴ In the context of the healthy adult liver, the resident immune cell populations play central roles in regulating inflammation and maintaining organ homeostasis, resulting in a balanced microenvironment between tolerance and inflammation.

The HCC tumor microenvironment (TME) includes different cell types which, as whole, contributes to the favourable

tissue context to the tumor growth. Three distinct subsets of phagocytic cells are responsible for the intra-hepatic tolerogenicity: liver sinusoidal endothelial cells (LSECs), Kupffer cells and liver dendritic cells (DCs) (reviewed in⁵) and even hepatocytes, which have been shown to drive T cells toward an anergic cytotoxic phenotype and a clonal deletion.^{6,7} The two principal immunosuppressive cell populations are present, namely myeloid-derived suppressor cells (MDSCs)^{8,9} and CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Tregs).^{10,11} Both immune regulatory cells mediate their suppressive activity through the production of the immunosuppressive cytokines IL-10 and TGFβ which inhibit the T cell activation and proliferation.

Several studies demonstrated that the accumulation of these immunosuppressive cell populations within the tumor are correlated with disease progression and poor prognosis and can regulate the response of cancer cells to chemotherapy.¹² In particular, Tregs play a role in the development of cirrhosis, the transformation of cirrhosis to HCC, and the progression and metastasis of HCC. Higher level of Tregs in the peripheral blood and/or tumor sites is correlated with a poorer prognosis in HBV-related liver conditions. Further, the findings of Kobayashi et al suggest that the prevalence of CD8⁺ tumor-infiltrating lymphocytes decreases significantly during hepatocarcinogenesis and is inversely correlated with the one of infiltrating Tregs.¹³

Moreover, the immune suppressive TME of HCC is further enhanced by the expression of immune checkpoint molecules (i.e. programmed death ligand-1 PD-L1) on liver sinusoidal endothelial cells, interacting with programmed death 1 (PD-1) molecules on T cells and leading to the induction of antigen-specific T cell tolerance.^{6,14}

All the components of the HCC intra-tumoral immunosuppressive environment lead to tumor escaping, inefficient immune mediated control and, consequently, the unsatisfactory results observed in cancer immunotherapy clinical trials.¹⁵

As a result, there is growing need for new therapeutic strategies in HCC to improve induction of anti-tumor immune response by counteracting the immune suppressive TME. Combinatorial protocols combining specific immunotherapy approaches (i.e. cancer vaccine) with immune modulator strategies (i.e. chemotherapy and anti-checkpoint molecules) would be highly effective (reviewed in¹⁶), providing better results than individual treatments (reviewed in^{17,18} (Figure. 1)

2. Immunomodulatory approaches in HCC

The efficacy of anti-tumoral immune response can be enhanced by contrasting the different components of the immunosuppressive TME using different immunomodulatory treatments, represented by **conventional or low-dose**

chemotherapy and/or **anti-immune checkpoint inhibitors**. The first type of treatment aims at reducing the suppressor immune cells or at increasing the tumor mutational burden. On the contrary, the second type blocks the inhibitory pathway mediated by immune checkpoint molecules.

2.1. Conventional chemotherapy in HCC treatment

Chemotherapy agents may enhance antitumor immunity by several mechanisms. Indeed, when administered at standard or low-dose metronomic regimens, they have been shown to selectively kill immunosuppressive cell populations. Moreover, they induce an immunogenic cell death in cancer cells with the release of danger signals able to polarize DCs towards a pro-inflammatory phenotype driving an anti-tumor T helper 1 (Th1) response.^{19,20} Furthermore, they can modulate the expression of tumor antigens and immune checkpoint molecules as well as molecules directly involved in antigen processing and presentation (i.e. MHC class I molecules). Finally, they may counteract the immune tolerance and suppression to promote antigen-specific immunity.²¹ Overall, these mechanisms would generate a favorable tumor immune environment and may potentiate effects of anticancer vaccines (reviewed in²² and²³).

Conventional chemotherapy is based on the pharmacological maximum tolerate dose (MTD), the highest effective single dose usually administered at defined intervals (3 weekly,

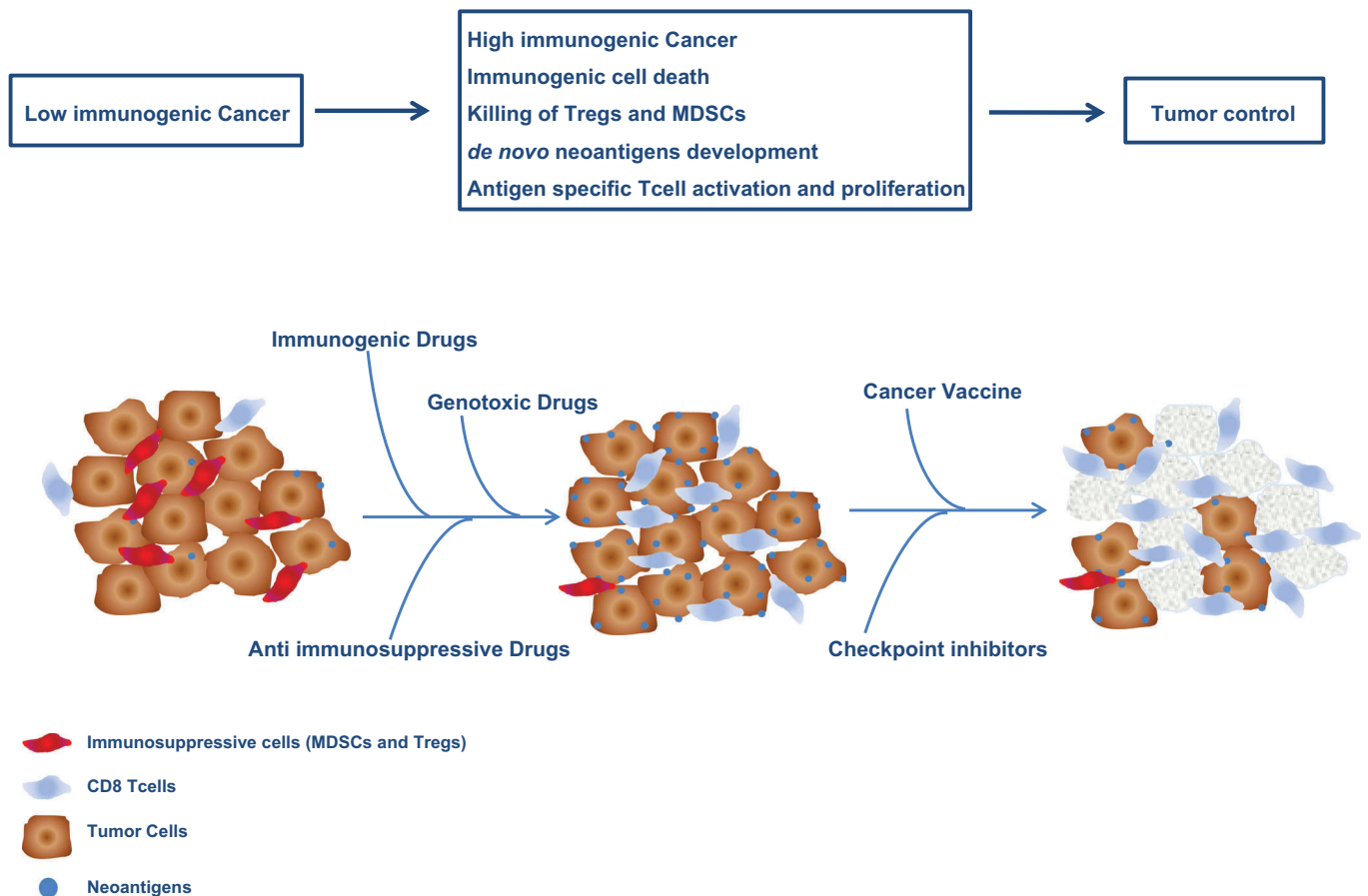


Figure 1. Combinatorial strategies with immunogenic, anti immunosuppressive and genotoxic chemotherapy to enhance efficacy of cancer vaccine and checkpoint inhibitors.

fortnightly, weekly). MTD has a strong anti-angiogenic effect on the tumor and mainly targets replicating tumor cells, including immune cells, causing a long-term toxicity and massive lymphodepletion.^{24–27} As reviewed by Shurin et al., the main immunosuppressive effect of MTD chemotherapy is the depletion of T lymphocytes, while restoration of peripheral CD4+ cell levels takes significantly longer than CD8+ cells.^{28–30}

However, in the last years, many studies have reported that standard chemotherapy is not fully immunosuppressive. Indeed, after the initial T cell depletion, it would induce a rebound replenishment of various immune cell subsets, leading to positive effects on anticancer immune response.³¹ Numerous evidences indicate the benefits of chemotherapy on T-cell-mediated immune responses. Mice vaccinated with doxorubicin- or cisplatin-treated ovarian cancer cells show an enhanced antitumor immunity, and prolonged survival largely dependent on CD4 T-cell-mediated immune responses.³²

Moreover, several clinical studies demonstrate that chemotherapy can interact positively with immunotherapy treatments improving their efficacy by several means and, in particular, potentiating the effects of anticancer vaccines (reviewed in²²).

More recently it has been demonstrated that cytotoxic agents may increase the anti-tumor immunity by making more immunogenic the tumor, inducing a higher number of tumor-specific mutated neo-antigens. In particular, the Temozolomide drives the inactivation of the DNA mismatch repair (MMR) machinery promoting a dynamic increase in the number of mutations and *de novo* neoantigens in cancer cells. The induction of high numbers of tumor-specific non-self antigens results in a strong antitumor immune response and improved efficacy of immune-checkpoint inhibitors.³³

All these observations indicate that immunomodulatory chemotherapeutic agents may be good candidates also for combination with different immune-based therapeutic approaches such as cancer vaccines.

However, although this can be proposed for many cancer types, MTD chemotherapy is not feasible in HCC. Indeed, systemic chemotherapy has been used for palliative treatment in advanced HCC, but severe toxicities have been reported compared to other cancers, due to the liver cirrhosis and the intrinsic chemoresistance of hepatocytes.^{24,34} For such a reason, systemic chemotherapy is proposed only for patients with good performance status and preserved hepatic function.³⁵ However, single agents such as doxorubicin, cisplatin and fluorouracil give a 10% response rates which increases to 20% in combination regimens, without any impact on survival.^{36–38} Therefore, using a full therapeutic dose of cytotoxic agents in HCC will not be considered ethical until their immunological effects will be assessed as more relevant than their cytotoxic effects.

A valid alternative should be the low dose metronomic chemotherapy which has been shown to have anti-immunosuppressive effects without toxicity and potentiate the efficacy of cancer vaccines.

2.2. Metronomic chemotherapy in HCC

Metronomic chemotherapy refers to a regular administration of a chemotherapeutic drug for a long time with no extended

drug-free breaks.³⁹ Multiple repeated low doses of chemotherapy drugs administered at short intervals were originally reported to inhibit tumor neo-angiogenesis and significantly suppress tumor growth. More recently, it has been shown that metronomic chemotherapy exerts the anti-tumor effect via additional mechanisms (reviewed in⁴⁰). In particular, as for the full dose, metronomic chemotherapy induces an *immunogenic cell death* (ICD), generating a favorable tumor immune environment (reviewed in¹⁹). Indeed, low-dose cyclophosphamide is toxic to immunosuppressive Treg cells improving the anti-tumor T cell response.^{41–43} Similarly, gemcitabine selectively kills myeloid-derived suppressor cells (MDSCs) *in vitro* and *in vivo*.⁴⁴ Finally, docetaxel has been reported to modulate different cell subsets, enhancing CD8⁺ function and deleting Tregs.⁴⁵ Overall, these effects on the tumor microenvironment can significantly improve immunotherapy approaches, including cancer vaccines.

In this framework, we have previously reported in preclinical studies a novel combinatorial strategy, based on peptide vaccine and a metronomic chemotherapy including taxanes and alkylating agents. This combinatorial strategy showed to significantly delay tumor growth and prolong animal survival. Such anti-tumor biological effects were shown to be directly correlated with induction of immunological cell death (ICD), enhanced T cell response and reduction of the immune suppressive Tregs cell population.^{20,46}

To date, more than 50 clinical trials of metronomic chemotherapy have been reported in patients affected by different cancers showing an enhancement of anti-tumor immunity.²³ However, only four clinical trials evaluating metronomic chemotherapy in HCC have been registered to date. Treiber et al. reported no effects in terms of time to progression and overall survival.⁴⁷ Hsu et al. reported that metronomic tegafur/uracil (UFT) could be safely combined with Sorafenib, enhancing its antitumor efficacy without additional severe side effects.^{48,49} Woo et al. reported that combination of metronomic epirubicin with cisplatin and 5-FU is a potentially useful treatment for HCC patients with portal vein thrombosis.³⁵ In addition, Shao et al. reported a modest activity of a metronomic UFT plus thalidomide, sorafenib, or bevacizumab in patients with advanced HCC.⁵⁰ Very recently a phase II clinical trial evaluating metronomic capecitabine in advanced hepatocellular carcinoma patients showed efficacy in treatment-naive patients as well as in those previously treated with Sorafenib.⁵¹ Moreover, two case reports provided encouraging results in individual HCC patients treated with metronomic capecitabine.^{52,53}

Overall, results of the metronomic chemotherapy in HCC patients are encouraging and motivate the design of large randomized phase II/III trials to confirm its efficacy.

2.3. Checkpoint inhibitors in HCC

Immune checkpoints are a wide range of membrane molecules expressed in cancer cells as well as in different cell types involved in the immune response, including B and T cells, natural killer (NK) cells, DC, tumor associated macrophages (TAM), monocytes, and myeloid-derived suppressor cells (MDSC). These molecules inhibit T cell activation and

proliferation during the immune response against non-self antigens.^{54–56} In particular, high expression of programmed cell death protein 1 (PD-1) and PD-L1 in liver cancer tissue is correlated to poor prognosis in HCC patients as well as to more aggressive tumor characteristics.^{57,58} There are other inhibitory membrane molecules which are expressed on HCC cells, including cytotoxic T-lymphocyte protein 4 (CTLA-4), the lymphocyte activation gene 3 protein (LAG-3), T-cell immunoglobulin and mucin-domain containing (TIM-3) and B and T lymphocyte attenuator (BTLA).^{59–61} However, only CTLA-4 and PD-1/PD-L1 pathways have been pursued for HCC in clinic evaluation, and the anti-CTLA-4 inhibitor Tremelimumab, was the first molecule to be clinically evaluated in HCC^{62,63} (Table 1).

The study by Sangro et al. involved HCC patients not eligible for surgery or locoregional therapies. The authors reported a good safety profile, with a 17.6% partial response rate and 76.4% disease control rate. Time to progression was 6.48 months (95% CI 3.95–9.14). Antitumor activity induced by Tremelimumab appeared promising in all measured outcomes, including objective tumor responses, durable stabilizations and long time to progression (TTP).⁶²

In a very recent clinical trial by Duffy et al. was described that the efficacy of Tremelimumab can be improved when combined with tumor ablation, enhancing a specific tumor immune response and leading to the accumulation of intratumoral CD8⁺ T cells.⁶³

As regards the PD-L1/PD-1 pathway, results of a very recent clinical trial has been reported on the use of the anti PD-1 inhibitor Nivolumab in HCC patients.⁶⁴

The trial was conducted in HCC patients after treatment with Sorafenib. Treatment was well tolerated without any significant side effects. Convincing signs of efficacy were reported and, in particular, tumor responses were observed in about 20% of patients. The responses were meaningful and durable for a median of 17 months. An additional 45% of patients had stable disease lasting more than 6 months in most cases. Response rates were similar across different etiologies, and both in Sorafenib-naïve and Sorafenib-exposed patients. These results support Nivolumab as a viable second-line therapy following Sorafenib in HCC patients.

3. Active immunotherapy approaches for HCC

Several passive immunotherapies, based on immune cells transfer, have been tested in early phases clinical trials in HCC with limited clinical benefit for patients and none of them has been moved in phase III efficacy clinical trials. Active immunotherapy approaches may provide more promising outcomes.^{5,65,66}

Table 1. Published and ongoing HCC cancer vaccines based on peptides alone or in combination with chemotherapy, evaluated in human clinical trials.

Antigen	Vaccine strategy	Combination	Ref
Telomerase	Single Peptide	No	73
AFP	Single Peptide	No	75
GPC3	Single Peptide	No	76
Telomerase	Single Peptide	Low-dose cyclophosphamide	93
HEPAVAC	Multiple Peptides	Low-dose cyclophosphamide	NCT03203005

3.1. Preventive vaccine for HCC

Hepatitis B virus (HBV) infection is one of the main etiologic factors of hepatocellular carcinoma (HCC).⁶⁷ The universal preventive hepatitis B vaccination for infants has been launched in the late '80s – early '90s in most Countries all over the World with high incidence of HBV infection. As direct consequence of prevention from HBV infection, the HBV vaccine is a successful preventive vaccine for HCC occurrence. Indeed, reduced incidence of liver cancer in children and adolescents have been reported in studies performed in different Countries.^{68–72}

3.2. Cancer vaccines in HCC

Over the past 15 years, a number of tumor-associated antigens (TAAs) have been identified in HCC, some of which elicit tumor specific immune responses.⁷³ Unfortunately, only a limited number of such TAAs have been used because most of them are not specific to HCC, such as telomerase reverse transcriptase – TERT;^{74,75} Wilms' tumor 1 – WT-1;^{76,77} alpha fetoprotein – AFP;⁷⁸ glypican 3 – GPC3;^{79,80} MAGE-A, SSX-2, NY-ESO-1.⁸¹

Among such TAAs, only a limited number of vaccines based on HLA class-I restricted epitopes derived from AFP, TERT and GPC3 have been tested so far in human clinical trials with limited results.^{82–84} A vaccine based on an AFP-derived peptide was evaluated in the first clinical trial conducted in the early 2000's, showing the induction of an AFP specific T cell response in six patients.⁸⁴ More recently, GPC3 peptides were proven to induce specific CD8⁺ CTLs in the tumor microenvironment (TME).⁸⁵ A phase II clinical trial testing the GPC3-based vaccine as adjuvant therapy for patients after surgery or RFA is currently ongoing (UMIN-CTR: 000002614). An open label phase II clinical trial based on telomerase peptide did not lead to any complete or partial responses in advanced HCC patients.⁸² A very innovative strategy is currently pursued for identification of shared "off-the-shelf" HCC-specific antigens within the HEPAVAC project (www.hepavac.eu).⁶⁵ In particular, novel HCC-associated antigens have been identified and a multi-epitope, multi-HLA peptide vaccine has been produced. A phase I/II clinical trial is currently ongoing to assess safety and immunogenicity in early-intermediate stage HCC patients undergoing surgical and/or loco-regional treatments (NCT03203005). The vaccination protocol will include also an actively personalized vaccine (APVAC) in a subset of vaccinees, based on patient-specific HCC-specific neo-antigens.

3.3. Enhancing cancer vaccine efficacy in HCC by combinatorial strategies

As discussed above, immunomodulatory treatments such as standard as well as metronomic chemotherapy and checkpoint inhibitors, may significantly improve the efficacy of anticancer immune responses.^{86–89} Consequently, cancer vaccines may result more effective when combined with such treatments, due to the sum of different biological effects

such as potentiating the effector functions of activated T cells and NK, control of the suppressive activity of Tregs, enhancement of antibody-dependent cellular cytotoxicity.¹⁷

Indeed, clinical trials have shown that the combination of chemotherapy and cancer vaccines induces in cancer patients a better clinical outcome than individual treatments.^{90,91} Chemotherapy can improve anti-tumor effects of cancer vaccines not only by overcoming the immune-suppression, but also by enhancing cross-presentation of tumor antigens as well as increasing the number of effector cells in the tumor microenvironment.^{20,46,92–94}

Combination of low-dose metronomic chemotherapy and cancer vaccines could represent a potentially attractive option for HCC patients, given the strong immunosuppressive microenvironment in the liver for the presence of several immunosuppressive cells such as CD4⁺CD25⁺ regulatory T cells,⁹⁵ which are known to suppress the function of antigen-specific T cell responses and are increased in patients with HCC.⁹⁶

Greten TF et al. have demonstrated that low-dose cyclophosphamide systemic treatment, but not the full dose, decreases the frequency and suppressor function of circulating CD4⁺CD25⁺Foxp3⁺ regulatory T cells in peripheral blood and unmasks α -fetoprotein-specific CD4⁺ T-cell responses in patients with advanced HCC. Moreover, in such patients affected by high tissue damage due to liver cirrhosis, Authors demonstrated that low-dose metronomic chemotherapy is safe without any significant hematologic side effects.⁹⁷ Afterwards, the same Authors investigated the effect of a cancer vaccine based on hTERT peptide (GV1001) in combination with a low-dose cyclophosphamide treatment in a single arm phase II trial. Unfortunately, the combination did not show antitumor efficacy in respect to tumor response and time-to-progression.⁸² No additional attempts have been done subsequently in such a tumor setting.

Alternatively, combination of checkpoint inhibitors and cancer vaccines could represent an additional attractive option for HCC patients.⁹⁸ Several pre-clinical studies have investigated combination strategies including cancer vaccines and checkpoint inhibitors, all of them showing significant enhancement of cancer vaccine effects associated with increased infiltration of effector CD8⁺ T cell.^{99–101} All such pre-clinical data have provided the rationale for designing and conducting several clinical trials for testing the efficacy in different cancer settings of such combinations. All such clinical trials are currently recruiting patients and results are expected to be available in the coming months (review in.¹⁰²) None of such combinatorial strategies have been evaluated in HCC yet, but positive results reported in clinical trials evaluating checkpoint inhibitors in liver cancer¹⁰³ strongly suggest that cancer vaccine and checkpoint combinations may show better efficacy also in HCC. Instead, the combination of checkpoint inhibitors with co-stimulatory molecules such as CD137 (4-1BB), CD134 (OX40), glucocorticoid-induced tumor necrosis factor receptor (GITR) or CD40 has been evaluated in a preclinical study using an aggressive transgenic hepatocellular carcinoma mouse model. This study demonstrated that combinatorial strategy is effective to potentiate the effector

functions of activated T cells and to decrease the density of immunosuppressive cells in tumor microenvironment.¹⁰⁴

4. Conclusions

HCC is a tumor with very high medical need. The current lack of an effective therapy, especially in more advanced stages, makes urgent the need for developing alternative treatments such as immunotherapies.

However, the immunosuppressive liver tumor microenvironment must be counterbalanced by combinatorial strategies, in order to improve the efficacy of such immunotherapies. Full dose chemotherapy is not applicable, due to drug resistance of HCC and toxicity. Therefore, the only treatments possibly available for combination with cancer vaccines are low-dose chemotherapy and checkpoint inhibitors.

The first option has been evaluated in several clinical trials with unsatisfactory results. The second one has not been yet evaluated and represents a priority goal. The recent favorable results from the CheckMate 040 clinical trial, showing safety and efficacy of anti-PD-1 in HCC patients, opens a new horizon in this setting.

Furthermore, cancer vaccines for HCC needs to be improved, identifying more specific target antigens. To this aim, results from the currently ongoing Hepavac-101 clinical trial (NCT03203005) will hopefully provide promising results. In addition, personalized HCC cancer vaccines based on patient-specific mutated neo-antigens represent the ultimate frontier and is yet an unexplored field.

All such considerations suggest that the field of HCC immunotherapy will see a blossom of pre-clinical and clinical evaluations of combinatorial strategies in the coming years.

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