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Research Article

Immunotherapy in the Context of Hepatocellular Carcinoma: Advances and Challenges

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Abstract

Hepatocellular Carcinoma (HCC) is a malignant neoplasm that affects the main cells of the liver, the hepatocytes. It is cancer that causes a mutation in these cells, being extremely aggressive and with high morbidity rates. New treatment alternatives are needed to increase the survival of patients undergoing resection. Immunotherapy is a biological treatment with great efficacy, and with the main purpose of boosting the immune system against certain infections and cancer, and has become an important method of these adjuvant treatments, as it represents a way to reduce the incidence of HCC in the population. Using the Pubmed database, available at https://pubmed.ncbi.nlm.nih.gov/, with the keywords, in English, "immunotherapy" and "hepatocellular carcinoma", 30 articles from clinical trials were selected, completely performed exclusively in humans. Immunotherapy as a treatment for HCC proved to be effective and safe, not presenting serious risks to patients. These are therapies that have been studied for many years, expanding techniques, and unveiling new methods, however, few can obtain this type of treatment, mainly due to its cost. Every day these are the hope of patients struggling with HCC.

Key words: Carcinoma; Hepatocellular; Immunotherapy; Treatment

Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer, being an important medical problem; affects the main cells of this organ, the hepatocytes. It is classified as the sixth most common neoplasm, with 782,000 diagnosed cases, and became the third leading cause of cancer death with 746,000 deaths in 2012. The development of HCH has been closely related to the presence of chronic liver disease, and this type of cancer is recognized as one of the main causes of death in patients with cirrhosis and chronic infection caused by hepatitis B or C viruses [1-3]. Its development and progression are related to chronic inflammation, and once the tumor settles in the tissue, mutual interactions between tumor and immune cells present during inflammation, can make a favorable environment for the survival of tumor cells [4,5] . This disordered progression can also occur by external agents, such as hepatitis virus, or by excessive cell multiplications, such as chronic regeneration in hepatitis, which causes a significant increase in the duplication of genes of these cells The risk of HCC appears when cirrhosis is established, and increases as progressive impairment of liver function [2,3]. It is aggressive, and if diagnosed only in the symptomatic phase, the individual has little life expectancy, because he has a poor prognosis, his treatment is limited, especially for patients with advanced disease who could not perform curative hepatectomy or liver transplantation. Only a minority of Cases of HCC are subjected to a potentially curative intervention. Immune response mechanisms during tumor progression have been investigated for decades; the efficacy and safety of immunotherapeutic methods are also being tested in the clinical treatment of malignancies [1-3,6] . The HCC has no specific symptoms in the initial phase and is presented in an advanced stage, such as abdominal pain, in about 40 to 60% of the cases, palpable right tumor in 23%, abdominal distension and lack of appetite in 45%, jaundice, and ascites, being 16 to 26% of cases, respectively. Other symptoms include slimming 29%, general malaise 60%, signs of hepatic encephalopathy, drowsiness to coma, and finally, gastrointestinal bleeding in 7% of reported cases [3]. Data pointed out by the World Health Organization (WHO) highlight that in Brazil, the highest incidence of HCC cases is in the states of Espírito Santo and Bahia. HCC rarely affects people under the age of 40 years, with 70 years of age being the highest incidence of the disease [3]. In the 1980s, the first studies on immunotherapy were initiated to use it in the treatment of HCC; is an immunotherapy that stimulates the immune system as an adjunct treatment for cancer, with the recognition of cellular receptors that stimulate the body's defenses against tumor cells. The immunotherapy technique has been studied and improved, as it presents significant progress [7]. The main objective of this therapy is to signal tumor cells, allowing the immune system to create a response mechanism that does not harm healthy cells, but that reaches only tumor cells. This method is becoming an expressive tool, presenting therapeutic advantages; several institutions have invested in this technique, as it can enable treatments for cancers that have been intractable [8]. However, because many surgical and chemical methods do not present as much efficiency in treatment, immunotherapy may be a new path to decreasing the incidence of HCC in the population. Great advances have been made in prevention, detection, diagnosis, and treatment. This study aims to conduct a literature review showing the advances and challenges of immunotherapy for the treatment of HCC. For this, articles on clinical cases were used from the "PubMed" database from 2011 to 2020.

Methodology

This is a bibliographic review constructed from the active search for scientific articles indexed in the Pubmed database,on the theme

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of the use of immunotherapy applied in patients with hepatocellular carcinoma. Articles that comprised a time range between 2011 and 2020 were selected using the keywords, in English, *immunotherapy, and hepatocellular carcinoma*. Only complete clinical trials conducted exclusively in humans were selected. The review articles were disregarded. Thus, after the insertion of the keywords, 2,899 articles were initially found, additionally applying the time criterion (2011 to 2020). Then, the research was refined, limiting the work performed only on humans; 1,938 articles were selected. Of these, 866 were review articles, which were subtracted, leaving 1,072 studies. Subsequently, focusing on full texts in clinical trials, 39 articles remained. After analyzing the content of each study, we observed that 9 addressed the theme of this literature review because they addressed other cancers, such as ovarian and head cancer. Finally, we obtained 30 articles, which underscored our study objective.

Results and Discussion

The selected articles (APPENDIX 1) were clinical cases of patients with HCC, submitted to immunotherapeutic methods with advanced stages of the disease. Patients treated with local regional therapies, including Radio Frequency Ablation (RFA), surgical resection, and Transcatheter Arterial Chemoembolization (TACE) were prospectively included in the study.

Use of Immunotherapy in Hcc

Durvalumab and Ramucirumab

Durvalumab is an anti-PD-L1 IgG1 monoclonal antibody that prevents the binding of PD-L1 (programmed death-ligand 1) under the PD-1 (programmed death receptor) and CD80 receptors expressed by T cells; this method removes PD-1/PD-L1 from the injunction pathway, where it is usually activated in the microenvironmental tumor, which is a type of protein designed to recognize and bind to a particular target substance in the body. It can help the immune system fight cancer because it is a therapeutic protein and consequently has a potential for immunogenicity. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay [9]. Ramucirumab is a fully-humanized monoclonal antibody that inhibits receptor-2 of vascular endothelial growth factor and is considered a standard treatment option after progression in first-line therapy for advanced or metastatic disease. Many clinical trials are underway to assess the combination function of ramucirumab with new agents, whether in metastatic tumors and/or in perioperative environments [10]. During a clinical trial, the combination of ramucirumab and durvalumab for the treatment of HCC was analyzed, where the method presented a safe profile and demonstrated antitumor activity. Both were administered in patients with HCC with a dosage of 750 mg durvalumab and 8 mg of ramucirumab. It is possible to observe that this method developed about 11% of the antitumor response; recurrence-free survival (RFS) of mean disease and overall survival (OS) were 4.4 and 10.7 months for patients with HCC presenting manageable safety with antitumor activity in the study phases [11].

131 I-metuximab

Radioimmunotherapy (RAIT) is a promising treatment modality for tumors due to the specificity of antibodies and cancer eradication, resulting in proven clinical efficacy and with few side effects [12]. An anti-HCC radiotherapeutic method of immunological agent I-metuximab generated by iodine labeling for treatments of unresectable HCC was approved; metuximab, a type of fragmented iodine-labeled monoclonal antibody used against HCC associated with HAb18G/CD147, an immunoglobulin obtained from HCC tissues; the CD147 family member (inducer of extracellular matrix metalloproteinases) was highly expressed on HCC cells. I-metuximab, found to focus on tumors, was found to be safe, effective for treatment, and did not develop life-threatening toxic effects [13]. Treatment with I-metuximab associated with RFS and OS was significantly higher than no adjuvant applied in patients with HCC and positive expression of CD147; metuximab recognizes and specifically binds to the CD147 antigen. Patients aged 18 to 75 years were applied in this study, with application of transarterial doses of 27 - 75 mg/kg 4 to 6 weeks after hepatectomy [14]. This therapeutic method significantly improved RFS at 5 years after hepatectomy for HCC tumors in 43.4% of patients. Adverse events were observed in the first 4 weeks after application of l-metuximab, where all were properly treated within 2 weeks. This treatment was well tolerated by the patients, however, further studies are suggested to verify its results [14].

Mogamulizumab, Nivolumab and Anti-CC Antibody

Mogamulizumab is a humanized IgG1 immunoglobulin that selectively binds to CCR4, a CC chemokine G-protein-associated receptor that is involved in lymphocyte trafficking to various organs, including the skin, resulting in target cell depletion. CCR4 is expressed on the surface of some cancer cells, including cell malignancies. [15] Nivolumab is a fully human immunoglobulin G4 monoclonal antibody that binds to PD-1 and blocks its interaction with PD-L1 and PD-L2, stimulating the immune system to attack and destroy cancer cells. This drug does not act directly on cancer, it works by stimulating the immune system cell. This defense cell, when activated, seeks out and destroys the tumor [16]. Combination therapy of nivolumab and mogamulizumab is able to block several mechanisms involved in suppressing antitumor immunity and results in increased antitumor immune responses compared to either agent alone. It is a safe, well-tolerated combination with clinical benefits [17]. In a study of combination therapy with mogamulizumab and nivolumab in patients with advanced or metastatic solid tumors, patients received 3.0 mg/kg of nivolumab along with mogamulizumab at doses ranging from 0.3 to 1.0 mg/kg every two weeks with a 1.0 mg/kg increase in the dose in expansion, until progression or unacceptable drug toxicity; no limiting toxicity was observed on the dose escalation part [18]. Adverse events were observed in treatment grade 3/4 (TRAEs) in 29% of patients. Over the course of treatment, populations of effector Tregs (CD4+, CD45RA - FoxP3 high) decreased, and CD8+ T cells in tumor-infiltrating lymphocytes increased [18]. The association of anti-PD-1 antibody with nivolumab and Treg-depleting anti-CCR4 antibody with mogamulizumab provided acceptable safety, along with antitumor activity, opening doors to be an effective method in cancer immunotherapy [18].

Antigen Monitoring in HCC

IFN-γ+

IFN-γ is a cytokine that acts on CD4 T cells, promoting the differentiation of lymphocytes with a Th1 profile and inhibiting the differentiation of Th2 lymphocytes. It promotes B cell differentiation, inducing isotype switching to IgG. It has effects on the maturation of TCD8 lymphocytes, in effector cells with cytotoxic activities. IFN-γ is produced by all lymphocytes and natural killer (NK) cells after activation or in response to IL-2 and IL-12 [19]. Treatment with sorafenib may delay tumor growth and metastases in HCC patients, not only by

inhibiting angiogenesis, but also by suppressing immunosuppressive cells, subsets with concomitant activation of IFN-γ and granzyme B (GrB) produced by cytotoxic T lymphocytes (CTLs). Their findings implicate sorafenib-mediated blockade of VEGF/VEGFR/flt-3 signaling, including activation of mitogen-activated protein (ERK) in immunosuppressive cells, as a key molecular event in the immune system [20]. Sorafenib is a chemically synthesized drug that acts as a multiple kinase inhibitor. Its mechanism of action is by blocking intracellular and cell surface kinases. Several of these kinases are involved in tumor cell signaling mechanisms, angiogenesis and apoptosis. Thus, the action of sorafenib tosylate promotes the reduction of cell proliferation, inhibiting the growth of cancer cells [21]. The central role of CD8+ T cells in this targeted therapy for liver cancer suggests that CD8+, Ki67+ and IFN-γ+ cells may be a viable immune biomarker for the effectiveness of sorafenib treatment in HCC patients. Furthermore, a high baseline CD4+Teff/Treg ratio may be a biomarker of prognostic significance in HCC. [20] Biomarkers that help reduce exposure to toxic therapies can have a significant effect on cost, quality of life, and patient survival. These agents can potentially synergize to rekindle antitumor in HCC patients and improve the magnitude and durability of antitumor responses. Appropriate biomarkers can aid in the selection of the therapy sequence and be a paradigm shift for this disease [20].

Thymosin α1

The thymus is an important organ for the differentiation and maturation of T lymphocytes; epithelial cells are capable of secreting many peptides that regulate the development of different phenotypic markers and the functions of lymphocytes. Thymosin alpha 1 (Tα1) is a thymic peptide that obtains immunoregulatory activities, used in the treatment of some malignancies, infections and immunodeficiencies [22]. Tα1, a biological response modifier (BRM) increases the immune response of specific lymphocytes, also stimulating the production of lymphokines (IFN-c, IFN-a, IL-2 and macrophage migration inhibitory factor (MIF)). Tα1 also acts as an antagonist to CD4+ and CD8+ apoptosis, induced by dexamethasone (DEX), and the decrease in the values of thymus and spleen indices induced by hydrocortisone (HC). It alone or in combination with other BRMs or chemotherapeutics has good effects; in decreasing tumor burden and progression, prolongs survival and reduces adverse reactions from radiotherapy and chemotherapy [22]. Studies have shown that Tα1 is able to upregulate the major histocompatibility complex l (MHC l) in infected cells, as well as having the ability to influence the differentiation and expression of antigen in different tumor cells capable of affecting lymphoid cells [23]. A clinical trial study with Tα1 initiated on the day of TACE surgery was performed. The proportion of CD4+/CD8+ T cells increased significantly, indicating that the use of Tα1 after TACE can improve T cell function. Compared to the control group, the percentage of CD3+, CD4+, CD8+ T cells and the proportion of CD4+/CD8+ increased at 1, 4 weeks and 3-month follow-up [24] The study results have certain limitations as they reflect on the distribution of T cell subsets and peripheral blood mononuclear cells (PMCs) in patients with advanced liver cancer. The level of autophagy was altered, but autophagy inhibitors were not used to block it, and the role of autophagy on Tα1 in regulating immune cell activity was analyzed. The number of cases in this study is small and the follow-up time is short. It is necessary to increase this number to fully confirm the effect of Tα1 on immune cell autophagy in patients with advanced liver cancer [24].

Glypican-3

Glypican-3 (GPC3) is an onco-fetal antigen, expressed specifically in HCC tissues, placenta and embryonic liver in normal tissues. The GPC3-derived peptide vaccine proved to be safe, with development of antitumor effects and immune response [24]. GPC3 has strong immunogenicity and is mediated by GPC3-specific T cells. Immunotherapy is suitable for adjuvant therapy against HCC by inducing tumor-specific immune response on markers including α-fetoprotein (AFP) and vitamin K- or antagonist II-induced protein (PIVKA-II), and GPC3-specific CTLs in PBMCs before and after treatment [25]. GPC3 peptide-specific CTLs appear in the blood periphery, and many CD8-positive T cells infiltrate tumors after GPC3 peptide vaccination; patients with GPC3-specific CTLs had a longer survival, however, there were no significant differences in the clinical origins of patients with GPC3-specific CTL frequencies [24]. In a phase I clinical study, the GPC3-derived peptide vaccine was administered to 30 patients with advanced HCC. Among them, 16 patients had the human leukocyte antigen gene, which encodes the HLA gene A (HLA-A24) 24 times, and 14 had the HLA-A2 gene (encodes it twice). Three important points were highlighted: (I) HLA-A2- restricted GPC3 peptide is immunogenic at an advanced stage in patients with HCC; (ll) dose-dependent effects of GPC3 tide vaccine; (lll) establishment of CTL clones showing not only high avidity but also natural antigen-specific killing activity against HCC cells. The GPC3 peptide was previously identified as an HLA-A restricted peptide. In addition, the peptide can also bind to HLA-A molecules [26]. Although the CTL clones established by Dextramer assay that they could react to HLA-A+, GPC3+ and HCC from cell lines, these clones did not react to HLA-A+, GPC3+ from melanoma cell lines expressing GPC3 mRNA and proteins at a lower level than CHC cell lines. They established CTL clones that are more reactive to the active tumor and with greater avidity than CTL clones established by dextramer assay. Tumor reactivity was established by CD107 through the mobilization assay; these clones can also react in patients with metastatic melanoma [26]. However, tumor-infiltrating lymphocytes with high antitumor avidity can only be generated from a few melanoma patients. Substantial evidence was provided that CTLs not only show high avidity, but also natural antigen-specific killing activity against HCC cells, which can be induced in patients through vaccination [26]. GPC3-specific CTLs have been identified before and during pretreatment in some patients. This detection of CTLs is favorable for anticancer immunotherapy, as the specific T cell-mediated immune response antigen can be detected without stimulation *in vitro* [25]. Locoregional therapies including RFA, surgical resection and TACE were compared. Part of the patients after RFA or TACE showed an increase in GPC3-specific CTLs, which may have been induced by the treatment, and more than half of the patients after resection showed a decrease. This suggests that RFA induced a stronger GPC3-specific immune response compared to surgical resection. RFA destroys tumor tissue and causes local necrosis followed by release of tumor-associated antigens, while all tumor-associated antigens must be completely removed after resection. They did not obtain any other favorable data on the immune response after TACE [25]. Although further investigation is needed, TACE, which is also a necrosis-inducing treatment, can induce an antigen-specific immune response, even though the correlation between antitumor immune response and clinical response is controversial. Tumor-infiltrating CTLs are insufficient to suppress cancer recurrence despite massive infiltration. The study further reports that RFA has a stronger effect on the immune system when compared to resection surgery [25]. Immunohistochemical staining

of GPC3 (IHC), induction of CTLs and GPC3 from postoperative plasma were screened for patients who received the vaccine, and unvaccinated patients who underwent surgery. Positive vaccine efficacy was observed with plasma concentration of GPC3; the relapse rate was one year after surgery, reduced by approximately 15%, and the five- and eight-year survival rates were improved by approximately 10 and 30%. High concentrations increased the five-year survival rate to 75%. They concluded that vaccination was positive for GPC3 IHC staining and GPC3 plasma induced CTLs in order to significantly improve the long-term prognosis [27].

α-fetoprotein-derived induced peptides

AFP is a serum protein expressed by the fetal liver, at about 1 mg/mL, but is transcriptionally repressed soon after birth, within 4 to 6 months of life. It is positively expressed in HCC patients, produced by 50 to 80% of tumor cells; its measurement has become an important role in diagnosing and monitoring responses to treatment. [28] T cells can recognize AFP-derived peptide epitopes by MHC 1; despite high exposure to plasma levels of this oncofetoprotein during embryonic development, some AFP-specific T cells are not eliminated during ontogeny [29]. A comparative analysis of the various tumor-associated antigens, the tumor-associated immunogenic antigen (TAA)-derived peptides, AFP and AFP peptides were frequently recognized by PBMCs in HCC patients, which induce AFP-specific CTLs. Thus, vaccination with AFP peptides is a promising candidate therapy for HLA-A AFP-producing HCC patients. Investigated the safety and immunogenicity as a vaccine, where notable clinical benefits were noted for some patients, including the disappearance of intra and extrahepatic tumors, and a decrease in serum AFP. This suggests that there is induction of robust tumor-specific immune responses after immunization with this peptide. [30]The unique aspect of the selection of AFP-derived peptides among many different peptides was observed; it was possible to note specific CTLs of AFP-derived peptides induced after HCC treatment, including RFA or TACE, which can induce tumor necrosis or apoptosis. Furthermore, induction of CTLs was increased by transfusion of DCs into the tumor site after treatment. But as AFP is not expressed in all HCC tissues, the effects of vaccines may be limited to patients with positive serum AFP. [30] The TCR-α and TCR-β chains of individual cells were analyzed, as well as their functions within a short period. AFP-specific TCR genes were amplified without establishing T cell clones; 14 types of TCRs were cloned. TAAs have been shown to correlate well with the operating system [30]. AFP-specific CD8 T cells and T cells were not strongly induced prior to vaccination. Therefore, TCR analysis cannot predict the results of the AFP-derived peptide vaccine in advance of treatment. Patients immunized with antitumor drugs strongly induced by immune responses can be good sources for efficient TCRs, as such TCRs have greater functionality in relation to transduction and expression efficiency, cytotoxicity and cytokine production [30]. The highest grade 2 toxicity was observed by the Common Terminology Criteria for Adverse Events (CTCAE), and the treatment-related adverse event was a skin reaction at the injection site. Clinical outcomes of HCC are related to specific TCR functionalities induced by AFP-derived peptide vaccine treatment [30].

Supplementary Strategies

Vaccines

Dendritic Cells: Due to high relapse rates, the development of therapeutic modalities has increased significantly over the years, with the likelihood of a potentially curative treatment. [31-34] DCs have been widely used in clinical studies to obtain or amplify antitumor immune responses. It is known that DCs are potentially effective antigen-presenting cells (APCs), capturing antigens through endocytosis and phagocytosis in peripheral tissues, in their immature format, which soon after become mature, and are able to stimulate and regulate the response of T lymphocytes. Normally, they are extremely sensitive to danger signals. During a tumor process, they reach the compromised tissues through blood circulation, where they generate stimuli that trigger their maturation. During this maturation process, DCs lose their ability to phagocytose so that cytokines and chemokines can be produced, making changes in the expressions of chemokine receptors, costimulatory molecules and MHC molecules; the antigen that will be present at these receptors will be recognized by the T lymphocyte receptor [35,36]. Because they have a unique ability to modulate immunity and tolerance, they become an extremely attractive target for the development of new immunotherapeutic strategies, with a potential antitumor response. [35].

Adjuvant therapy with pulses of dendritic cell tumor antigens: This method was based on the development of adjuvant therapies in order to reduce the chances of relapse. DCs play an essential role in tumor anti-immunogenicity, mainly in tumor proliferation of specific CTLs, however, the number and function of DCs on the tumor are suppressed or dysfunctional. Thus, vaccination of DCs produces an appropriate antitumor effect; this adoptive immunotherapy has been tested in clinical trials for several malignancies, including HCC. According to the data in question, DCs were considered a potent cellular adjuvant therapy in the production of specific tumor vaccines [37-40]. Natural killer (NK) cells were observed to be a promising new strategy for adjuvant adoptive immunotherapy; NK cells are a mixture of T lymphocytes, expanded in vivo with cytokines, with the main effector cells CD3, CD56 and CTLs. These cells have a high rate of proliferation, potent antitumor effects and a low degree of toxicity to normal cells. NK cell immunotherapy increased RFS after tumor resection, showing its ability to kill tumor cells in an in vitro study [39]. In order to evaluate the efficacy and safety of a new treatment with low side effects, a vaccination modality of radiation therapy combined with immunotherapy consisting of vaccination of personalized peptide (PPV), DCs and CTL (PPV-DC-CTL) was developed. In conducting a phase l clinical trial in 9 patients with unresectable HCC, this regimen was well tolerated without serious reporting and achieved good disease control. Radiation therapy can successfully immunize some patients against cancer, converting the irradiated tissue into an in situ vaccine and providing the host with a set of powerful new tools to control systemic disease. Radiotherapy can be used as an “immune response modifier”, in general, a new tool to be added to the arsenal of immunotherapeutic agents, varying its response according to the administered dose [41]. They observed that DCs pulsed with autologous tumor lysate with CD3-activated T-cell (CAT) adoptive immunotherapy improved the RFS and OS of patients with invasive HCC after surgery in an adjuvant setting; this combination induced specific tumor immunity [42]. In most cases, HCC leads to a decrease in lymphocytes and leukocytes, specifically monocytes. Authors reported that the infusion of CATs can improve the immune status of the host in order to allow it to acquire a vaccine-induced immune response in patients with advanced cancer who show a reduced number of lymphocytes. Some cytokines such as TGFβ, IL-4, IL-10 or regulatory T cells are critical factors in suppressing the immune system. The PD-L1 22 antibody and the anti-CTLA-4 23 antibody are likely to be able to improve the T cell immune response. The efficacy of CATs can

be enhanced by DC vaccines [42]. The need for an adjuvant therapy with effective methods and without toxicity was idealized for patients with HCC who also had HVB, undergoing surgical resection. This therapy induces long-lasting endogenous immune responses against tumor cells responsible for recurrences and metastases. They observed that vaccination using autologous DCs pulsed with tumor stem cells derived from an autologous tumor cell line (DC-TC vaccine) is a promising strategy, as it demonstrated early evidence of safety and tolerability [43]. The success rate was 100%, showing that they can be reliably established in the short term. Participants in this study did not experience any worsening in parameters related to liver inflammation. The concern of collaborators in developing this immunotherapeutic method would be that this anticancer vaccine could distract the immune system's responses against HVB, which could result in liver inflammation or a possible increase in liver dysfunction. Throughout the study, they identified that there was little safety information, as the toxicity was minimal; there were limitations in the number of cells that could be cultured, making it difficult to increase the final dosages. A Phase II study is needed to expand on safety observations [43].

Immunotherapy by autologous dendritic cell vaccine: A number of methods have been applied in order to increase the effectiveness of the DC vaccine. Initially, 3 antigens expressly more common in HCC were used (AFP, GPC3 and melanoma associated with antigen 1 [MAGE-1]), these antigens were detected more frequently in the tumor matrix. The vaccine was administered subcutaneously (SC) in the thigh, close to the inguinal lymph nodes to increase the number of DCs, aiming to reach the regional lymph nodes; Toll-like receptor 7 (TLR-7) agonist, applied at the injection site, known to facilitate the migration of DCs to regional lymph nodes. They also used recombinant TAA proteins to restrict HLA. For efficient delivery of antigens into the cytoplasm of DCs, transduction mediated by duction protein (CTP) was used; DC vaccine pulsed with CTP-conjugate triggered an immunity mediated by Th1 and antigen-specific CTLs [44-47,36] These therapeutic cancer vaccines target the cellular arm of the immune system to initiate a CTL response against tumor-associated antigens. They can process and present antigens to T cells and therefore generate TCD8-specific tumor cells. DCs can be pulsed with tumor-specific antigens (TSA) that are capable of stimulating the immune system to antitumor responses[38,48]. Based on a set of experiments performed using HCC-derived DCs, the result of the vaccine's antigen uptake capacity was very high, being evaluated by the FITC-dextran uptake assay. Topical application of imiquimod, a TLR7 ligand, has also been used to increase antitumor immunity in synergy with the vaccine. Imiquimod is a new type of treatment in the category of drugs known as immune response modifiers, and is indicated for the treatment of condyloma acuminata [36]. In the preclinical study, the immunogenicity of in vitro TAA-pulsed mature DCs (mDCs) prepared with PBMCs from three healthy patients were evaluated. Therefore, the autologous DC vaccine was developed from PBMCs from 12 patients with HCC; TAA-pulsed mDCs were sufficient to stimulate antigen-specific CD8 T cells and CTLs. DCs were stimulated via blood monocytes [49,44,39]. Injection of DCs depends on the ability to uptake and transport antigens from tumor cells to draining lymph nodes, where they are presented to T cells [50,51]. In one study, 12 patients received doses of the vaccine over 24 weeks, where they observed that the main results were safety and stimulation of TAA-specific cellular immunity. The immunity mediated by TAA-specific cells that were induced by the vaccine was evaluated by IFNy linked to the ELISPOT enzyme with proliferation of lymphocytes observed through the blood samples of the patients at weeks 4, 10, 18 and 24. After application of the vaccine, no adverse events were observed, except in patients 7 and 9 who had grade 3 hematologic problems; leukopenia, neutropenia and lymphopenia in patient 7 and thrombocytopenia probably related to HCC progression in patient 9 [39]. Through flow cytometry results, they observed that DCs express high levels of HLA. With regard the MHC, responses were expressed by HLA-ABC receptors in MHC l and HLA-DR in MHC ll, and the stimulator molecules CD86, CD80, CD40 and CD83 [39] . Most patients showed increased TAA-specific immune response after vaccination, leading to tumor inhibition; the degree of immune response was of clinical significance, as they could correlate with clinical efficacy results. Among the TAAs, AFP and MAGE-1 demonstrated greater reactivity compared to GPC3, which showed moderation in inducing effector T cell responses. The preliminary results were encouraging for more clinical trials to be developed, as the limited number of patients made the study, for the authors, speculative [39]. Pulsation of DCs with lysed tumor cells prepared from the responses of human hepatocarcinoma cells (HepG2) were used, obtaining promising results in the possibility of breaking tolerance to an AFP similar to a self protein; most patients developed lymphadenopathy after vaccination, due to the migration of DCs to the lymph nodes, causing proliferation of lymphocytes and their increase in volume. After vaccination, they observed a significant improvement in liver functions and increased immunity [52,53]. The median survival of patients after treatment was 6 months; tolerability was excellent, with minimal side effects. However, optimizing the source of DCs, the charge/pulsation and the vaccination dose are prerequisites for a better immunological maneuver in the treatment of HCC [52] .

Dendritic cells transfected with heat shock protein 70 (HSP70) mRNA related to HCV: Infections caused by the hepatitis virus are the most important risk factors, accounting for 80% of HCC cases worldwide; this association between HCC and the hepatitis virus, HBV or HCV, causes liver dysfunction, which leads to restriction of chemotherapy [33,54]. HSP70 is a member of the heat shock protein 70 family, highly expressed in HCV-associated HCC tissues. The expression of HSP in tumor cells is considered important in immune responses against the tumor, as it is a possible way to increase immune recognition. In the process of cancer formation, HSP levels are altered; HSP expression may reflect the intensity of oncogenicity under malignant cells, providing important information for their behavior. The association between HCC and HCV can be upregulated by HSP70 [55,56] . About 92% of HCCs express HSP70 by immunohistochemical staining. DCs pulsed with DNA or RNA of an antigen can lead to prolonged presentation of this antigen to develop a high-affinity tumor reactive CTL response. DCs transfected with RNA, which encode specific TAA, can induce potent tumor antigen-specific T cells with responses directed against multiple epitopes. Researchers suggest that the introduction of HSP mRNA to DCs may be useful for the treatment of patients with HCV-related HCC [33]. DCs loaded with RNA cells from HepG2 tumor cells were also able to generate anti-HCC T cells. Packing of DCs with HSP70 complexes derived from HCC cells resulted in maturation of DCs which, in turn, stimulated proliferation of HCC-specific CTLs. [52] DCs transfected with HSP70 mRNA (HSP70-DCs) were used in 12 patients with inoperable or recurrent tumors. It is believed that HSP70 has been shown to be functional for endogenous danger signals that can increase the immunogenicity of tumors and induce CTL responses, as well as activate innate immunity. HSPs can send TAAs to APCs through MHC l and ll, activating the antitumor cytotoxic mechanism and helper T

cells. In a mouse model, HSP70 is able to stimulate the immune system through CD40 effectively. Based on this, they decided to use the HSP70 method as a treatment for HCV-related HCC [52]. No serious adverse effects were observed during treatment, other than the formation of a liver abscess, which upon analysis, was not developed by the treatment, as its location was different from the tumor site [52]. Through ELISPOT assays, it was observed that immune responses were developed by this immunotherapy; innate and adaptive immunity were evaluated, in which there were significant numbers of INF-y+, TCD8+ and NK cells. It is believed that the INF-y response in the ELISPOT assay was developed from TCD8+ and NK cells, as a positive representation of the applied method. They reported that GrB-expressing CD8+ T cells were detected in the lesions, indicating that this method specifically activated adaptive immunity resulting in an effective antitumor response [52]. HSP70 is a physiologically essential and highly conserved protein in prokaryotes and eukaryotes, which perform a variety of vital intracellular actions. Therefore, HSP70-DC immunotherapy for HCV-associated HCC was shown to be effective and safe, with the ability to induce mainly adaptive immunity [57][52].

Personalized peptide vaccination: Personalized peptide vaccination (PPV) is a novel immunotherapeutic approach based on a specific pool of peptides. The peptide pool includes all information about HLA 1A and the host's existing pre-immunity prior to vaccination. The vaccine contains "personalized" antigens with pre-existing immunity that can trigger antigen-specific memory T cells to produce rapid and strong secondary immune responses. Furthermore, an important feature of PPV is that it activates CTLs, which have stronger antitumor cytotoxicity, greater proliferative capacity, and more cytolytic activity than lymphokine-activated NK in vitro. This new therapeutic strategy for patients with advanced HCC is well tolerated, safe, feasible and effective. [58] Another feasibility study of PPV was performed for patients with HCC refractory to locoregional therapy. CTL responses to the HLA-match peptide combination in PBMCs pre-vaccination of patients refractory to locoregional therapies, and those refractory to locoregional and systemic therapies were observed in 22 and 25% of patients, respectively. PPV increased the response of CTLs to HLA-match peptides in PBMCs of 57% of patients and increased specific immunity against the vaccine. These immunological features may warrant further clinical trials of PPV for patients with HCC. Therefore, despite PPV being efficient and well tolerated during the treatment of patients with HCC, it is necessary to develop new clinical trials to verify and confirm the benefits of this new immunotherapeutic strategy[41][59]. An immunotherapeutic approach using GPC3-targeted PPV and treatment with ipilimumab (anti-CTLA4), a humanized monoclonal antibody, showed promising results; The patients' pre-existing immunity was analyzed to select 4 of 31 pooled peptides derived from 15 different TAAs, then administered as a vaccine. PPV prolongs the OS of patients with advanced tumors that did not match standard chemotherapy. They associated PPV with the derivation of HCV peptides against the development of HCC with HCV; the HCV peptide core at positions 35-44 (C-35 peptide) were able to induce CTLs of different types of HLA l. Thirty-six patients with positive HCV and advanced HCC were included in this study [60]. At the end of the study, they reported that 19 of the 36 enrolled patients had specific IgG responses, and increased viral-derived CTL peptide responses; there were no serious adverse events after vaccination. PPV may be useful in the treatment of HCV in patients with advanced HCC who have not responded to locoregional or systemic treatments, from the point of view of safety and immune responses [60].

Protein 3-derived vaccination associated with multidrug resistance

Multidrug Resistance-associated Protein 3 (MRP3) is a transport protein belonging to the ABC transporters that transport substances against the Adenosine Triphosphate (ATP) concentration gradient, and its enhancement is observed in tumor cells. MRP3 is a tumor rejection antigen recognized by CTLs, and may be useful as a target antigen in immunotherapy against HCC [61,62]. In one study, 12 patients with HCC underwent hepatic arterial infusion chemotherapy (HAIC) along with the administration of MRP3-derived peptide. Elevated levels of MRP3 in HCC tissues were considerably higher compared to normal tissues. MRP3-specific CTLs showed toxicity against HCC cells that overexpress MRP3. The results showed that therapies using the peptide vaccine with conventional chemotherapy can increase the antitumor effects of the peptides, inducing MRP3-specific immunity in 72.7% of patients [63]. This adjuvant treatment demonstrated safety and even a possible prolongation of OS time, but it should be evaluated later with further studies, as the number of patients included was limited, and there is a need to confirm these results [63].

Pexa-Vec (JX-594)

JX-594 (also called Pexa-Vec; Jennerex Inc.) is a virus with disruption of the thymidine kinase (TK) gene for cancer selectivity and insertion of human granulocyte-macrophage colony stimulating factor (hGM-CSF) and β-galactosidase transgenes for stimulation of the immune response and evaluation of replication. JX-594 has direct oncolytic activity and mediates tumor cell death through induction of innate and adaptive immune responses [64]. In a study where JX-594 was administered, objective responses were observed with tumor shrinkage and contrast enhancement in injected and non-injected subjects; absolute concentrations of neutrophils and eosinophils increased substantially within the first few days after treatment in most subjects with quantifiable hGM-CSF in their blood. The development of mediated complement-dependent cytotoxic (CDC) antibody was evidenced in the serum of subjects over time after treatment. Radiographic evidence included near-complete progressive tumor, hypovascularity, and necrosis associated with a marked increase in non-injected tumors that develop over 3 to 4 months [65]. In the evaluation of cellular immunity, cytotoxic T cells were induced for vaccine peptides. Replication and expression of the hGM-CSF transgene were associated with three mechanisms of action: acute vascular disruption in tumors, tumor oncolysis and necrosis of antitumor immunity. The dose of JX-594 has anticancer efficacy, actively inducing functional antitumor immunity, being an important determinant in the duration of OS in individuals with carcinoma, given that oncolytic viruses replicate in tumor cells [65].

FANG™

Autologous Whole Tumor Cell Immunotherapy (FANG™) has been shown to be a safe, single method immunological induction that has three modulatory components (it is an immunotherapy derived from autologous tumor cells): 1. An autologous whole tumor cell complex, which provides a specific tumor; 2. immune activation via local-regional expression of the GM-CSF protein; 3. inhibition of TGFβ 1 and 2 action by the furin proprotein convertase using bi-shRNAi technology (bifunctional RNA interference) [66]. The FANG method uses the GM-CSF DNA, which has immunological recognition, and the bi-shRNAi, which has an active immunological suppression against TGFβ 1 and 2. These last two mechanisms, when

exposed to a more complete antigenic action, allow reaching a (re) education and/or reversal of tolerance. The immunomodulatory activity was developed to monitor the tumor for the development of therapeutic methods, as well as in adjacent environments; randomized trials were designed to determine the effectiveness of immunomodulatory treatment. Monitoring antigen targeting is critical for successful cancer control through immunomodulation [67]. The liver has immunological tolerance capabilities, presenting in abundance APCs, specifically Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs) and DCs; the first two are constitutively expressed through the anti-inflammatory cytokines IL-10 and TGFβ. One of the means of escape for tumor cells is the involvement of DCs, and the immunosuppressive factors that inhibit their maturation are released by tumors. DCs have a tumor reduction function in the way of stimulating T cells, and their inhibition is associated with tumor survival. Presence of CD4+, CD25+ and FoxP3+ were detected at high levels in the peripheral blood of these patients. These Tregs impair the cytotoxic function of the HCC [66]. It is concluded that the preliminary clinical and immunological results, as well as the long-term follow-up in HCC patients through FANG immunotherapy provided a basis for a more specific exploration, which justifies further testing in a phase ll study. [66].

Radiation

Proton Beam Radiotherapy (PBT)

Proton beam radiotherapy (PBT) is a category of external beams using charged particles; these beams increase the energy deposit, with maximum penetration depth, in order to form the Bragg peak. Proton beams allow a rapid increase in dose at the end of the beam's range, they deposit energy to a specified depth without an exit dose. No dose is deposited in normal tissue, as the objective is to target tumor cells, protecting normal tissues. The range of particles is determined by their energy input [68-70]. There are some data on the action of immunomodulatory effects of radiation associated with antitumor immunity, with potential synergistic effects, however, radiation, provided in prolonged use, can develop a rapid reduction in the populations of circulating lymphoid cells (CLCs); this can vary significantly between individuals being treated and the type of CLCs [71]. Although they recognize that there is a differential impact on fractionation and dosage of radiotherapy on immune system cells, little is known about the effects of proton therapy under tumor cells [71]. Radiotherapy has been observed to increase the effects of immunotherapeutic approaches, even if to become an ideal treatment, it still needs to determine some approaches to immunotherapy-radiotherapy that are effective in this disease. This method triggered hypotheses that generated interest in deeply investigating these cellular biomarkers, as they are important to seek radiation combinations with specific immunotherapy approaches [71]. To test the effectiveness of the "in situ vaccination approach", a clinical trial was performed using hydroxiapatite (HA; $CA_{10}(PO_4)_6(OH)_2$). The injected adjuvant not only supplied recurrence at the primary tumor site, but also the formation of the tumor, and may induce a systemic immune response called the Abscopal Effect when the AFTV immunoadjuvant is doubled to the in vivo tumor site [72]. Ionizing radiation positively regulates immunological cell surface molecules such as mucin-1 in in vitro human cancer cells. In addition, X-ray radiation increased cell immunogenicity. The PBT is effective in the local control of HCC due to excellent dose in accordance with the target cell, preserving the environment in normal fabric. These characteristics may suggest that they also have an advantage of preserving the immune reaction in the local tumor environment [72]. The immunoadjuvant composition used for AFTV delivered directly to the tumor tissue and linked to the denaturation of tumor cells after PBT, the coated immunoadjuvant is expected that tumor cell fragments will become an in situ tumor vaccine. Similar effects can be expected for patients with HCC treated by local ablation, such as necrotic or apoptotic tumor tissue left in situ, available as antigens tumor. However, this treatment is not suitable for patients undergoing surgery as tumor tissue has been removed [72] Hydroxyapatite was used as a moderately soluble bacillus carmette-guérin (BCG) carrier to maintain bioactivity at the injection site in this study. Adjuvant of precipitated calcium phosphate in solution with a 1.0 Ca/P molar ratio was also used in humans as immunoadjuvant for many years [72]. CalTUMP safety (a recently developed immunoadjuvant consisting of BCG extract connected to hydroxyapatite and microparticulate tuberculin, following local HCC PBT) was tested by the injection of three different doses (1/10, 1/3, 1/1). Although it was shown that the 3 doses were tolerable, the occurrence of adverse effects was not clearly dependent on the dose, requiring further analysis. CalTUMP doses used in this test did not induce specific inflammatory responses, such as transient fever or increased levels of C-reactive protein (PCR), and lack of these responses were associated with a significant bad prognosis [72]. This comparative analysis revealed a trend for prolonged progression free survival in patients receiving CalTUMP. In this study, survival rates were examined and the progression time of the last PBT followed by the injection of CalTUMP, considering less of the previous treatments performed before the last PBT. The genuine efficacy of this in situ vaccine for HCC recurrence should be examined in the future by a randomized clinical trial with better patient selection criteria. However, this immunotherapy treatment was considered viable and safe [72]. In this study, patients received 15 fractions of a dose of proton beam, equivalent to 67.5 GY. Dose reduction was allowed to maintain an average dose of 24 GY in the liver. CD3+PLC fractions, CD8+, CD25+, CD56+, CD127+TCD4, CD3+CTLs, CD8+, CD25+, CD25+, and NK cells in blood samples collected from 1, 8 and 15 were evaluated [71]. Effective lymphocytes and key suppressors were investigated during liver radiotherapy. The results showed that immune responses can occur at the beginning and during radiotherapy, associating with survival. These data provide new searches for rational combinations of radiological immunotherapy in liver cancer [71].

Microwave ablation therapy

Thermal ablation with the use of different energy sources such as microwave has gained preference in recent decades as a minimally invasive management technique for the treatment of HCC, due to minimal damage to the liver, presenting relative lack of complications and low mortality as well as results promising clinicians [73]. Microwave ablation is a very effective method of thermal ablation, as microwave energy is not limited by carbonization and fabric desiccation, and is less affected by the heat dissipating effect of local blood vessels, providing great potential for ablation in HCC [74]. In a study conducted in patients with HBV HCC after percutaneous microwave ablation, they showed that there was an increase in peripheral lymphocyte percentage after the application of multiple immunocytes in the groin lymph nodes and the abdominal cavity under the precise guidance of ultrasound and sleep. Such a form that the tumor load is significantly reduced, as tumor immunosuppression is removed and antigen is released with death and apoptosis of tumor cells after ablation therapy [75]. Immature DCs exhibit powerful phagocytosis and immigration, infusing themselves in the place of ablation. The foster infusion of

DCs and effective cells improve the local and systemic immune system, eliminating tumor cells. This results in reduced viral HBV load in patients, but there is an increase of this viral load 6 months after the end of treatment. This treatment is evidenced by an active recruitment of infiltrating tumor lymphocytes that display cytolitical antitumor activities in the presence of interleucines (ILs), but do not kill tumor cells. DCs can capture and process the present antigens. Cytokine-induced NK cells prove to be a heterogeneous population, from which most express both CD3 cell marker and CD56 cell. NK cells and cytotoxic T lymphocytes are specific to antigen and effective in the death of a non -specific tumor [75]. The results of this study also demonstrated that percutaneous microwave ablation (PMWA) stimulated the response of the local immune system, but only for a short time, suggesting that immunotherapy after ablation therapy should be performed. The tumor load is temporarily reduced after HCC treatment ablatherum, which represents the best opportunity to perform immunotherapy. During therapy, clinical manifestation of autoimmune reaction was observed in patients [75].

Icaritin: Small immunomodulatory molecule

Medicinal herbs are of increasing interest in scientific exploitation, in the hope that produce new compounds with properties that can overcome currently available drugs. Plant -based compounds are low cost and high availability [76]. Epimedium, *Herba Epimedii*, is a genus with approximately 52 species of herbaceous flowers. In recent years, they have significantly increased the number of anticancer research, with the main active compound effects of *Herba Epimedii* within 52 species is icaritin. These are compounds that seem to be promising, with anticancer activities against numerous types of cancer cells [76,77]. Small molecule immunological modulator agents can be particularly suitable for the treatment of advanced HCC patients, because the dysfunctional liver is vulnerable to limited therapeutic tolerability, and the HCC tumor microenvironment is particularly an immune tolerogenic [78]. Icaritin is a single molecule with purity greater than 98% used for immunological modulation. Icaritin treatment associated with anticancer and immunomodulation activities have been demonstrated in cells, as well as immune cells, including NK cells, TCD8+ IFN-γ producing cells and immunosuppressive myeloid suppressing cells (MDSCs). Icaritin displays antiproliferative activities in both cancer cells and cancer stem cells [78]. Icaritin demonstrated safety profiles and preliminary survival benefits in advanced HCC patients who were correlated with their immunological modulation activities, great potential in anti-inflammation and cancer immunotherapy. These results suggest the potential of icaritin as a new oral immunotherapy for advanced HCC [78].

Natural Killer Cells

Natural killer cells represent 5 to 20% in humans, having an effective function that occurs through the release of substances that lead to the induction of death by target cell apoptosis. Due to their immune role, these cells have been extending within immunotherapy as a very effective method in the treatment of HCC [79]. The antitumor activity developed by these cells in various types of tumors is of remarkable conservation, suggesting that murder cells can detect common modifications to cell metabolism or expression of a gene that is shared or induced by many oncogenic processes. This ability makes this cell considered a promising therapeutic tool in cancer immunotherapy [80]. A phase II study -induced by lymphocyan-activated killer cells (LAK) in HCC treatment demonstrated great antitumor efficacy in malignant tumors, resulting in rapid proliferation and elevation of immunological function safely. The combination of LAK and IL-2 has proven to be cytotoxic for both tumors and autologists, which are reactive and comprise a rare population of cells on the normal periphery. These are probably the most important cells for the anticancer effect of cytokine-induced killer treatment (CIK). [81].

Cytokine stimulation with IL-2 increases the density of surface expression of activation molecules and consequently frequent cytotoxicity of murder cells and stimulating overexpression of these molecules through cytokine gene transduction increase cytolitic activity [79] . CIK treatment indicates that HCC cells are a type of immunosable cancer and suggests that patients benefit a lot from this treatment strategy. CIK cells show rapid in vitro proliferation, stronger antitumor efficiency, and their use in treatment demonstrates lower adverse effects, prolonged survival and improvement of quality of life for patients with a variety of neoplastic and immunosensitive tumors. In contrast, CIK treatment has its limitations according to the CHC stage, although CIK treatment shows a significant benefit to OS [81-85].

Conclusion

After analysis of the articles, they were clinical cases of patients with HCC, undergoing immunotherapeutic methods at an advanced stage of the disease, and patients who were already under locoregional therapies, including RFA, surgical resection and TACE. The research has demonstrated efficacy and the safety of immunotherapy in the treatment of these patients with HCC, and this type of cancer is one of the most difficult to treat due to frequent recurrences and because they have limited treatments. Globally, to obtain the best prognosis, patients had long-term follow-up, applying tested immunotherapy methods, where most have achieved their goal positively. Even with the application of various therapies, methods and research analyzed have shown that many of them develop similar answers, and therefore tend to complete themselves. Most use GPC3 peptide vaccine that is well tolerated and induces measurable immune responses and antitumor efficacy. It is important to highlight that the GPC3 antigen, along with GPC IHC color, showed CTL and postoperative plasma GPC3 induction. Patients who were positive for GPC3 color in the IHC were more predisposed to have induced CTLs and positive relationship with plasma GPC3 concentration. High concentrations increased the survival rate from 5 years to 75%. On the other hand, DC vaccines with pulsed AFP linked to cytoplasmic transduction peptide, and GPC3 recombinant fusion proteins and melanoma antigen 1 also presented a good result in relation to treatments. Elevation of TCD8 and INFy were observed after vaccination and improvement in patient survival.The application of the vaccine after surgery and the transfer of activated T cells can also be a viable and effective treatment in the recurrence of patients with HCC, assisting in long-term survival. Moreover, the application of HSP70 together with HCC DCs related to HCV has proved to be safe and viable.ASI with DCs presenting TC strain antigens proved to be a promising treatment for long-term survival in metastatic cancer. ASI may benefit patients with high-risk primary HCC although they do not include patients with hepatitis virus. In the application of CIK vaccines, they observed low risk rates and global recurrence-free survival. Patients with non-adequate HCC for surgical procedures also demonstrated the prolonged and RFS. Another effective treatment for HCC is PPV vaccination based on pre-existing immunity. PPV-DC-CTL provides a new therapeutic strategy for patients with advanced HCC. None of the patients have developed side and liver side effects, only one patient has developed

II-grade bone marrow suppression. This therapeutic method was well tolerated, safe, viable and effective.

Finally, there are many reasons to believe that immunotherapies are more effective, safe and specific methods regarding patients who seek to extend their days of life, especially individuals with advanced disease HCC. These are therapies that have been studying for many years, expanding techniques, unraveling new methods, but few get this type of treatment, especially for its cost. Each day these are being the hope of patients fighting against HCC.

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Appendix

	Year	Authors	Title
1	2011	(ZHOU et al., 2011c)	Phase I clinical study of combination therapy with microwave ablation and cellular immunotherapy in hepatocellular carcinoma.
2	2011	(YOSHIKAWA et al., 2011b)	HLA-A2-restricted glypican-3 peptide-specific CTL clones induced by peptide vaccine show high avidity and antigen-specific killing activity against tumor cells.
3	2012	(SAWADA et al., 2012)	Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival.
4	2012	(TADA et al., 2012)	Phase I/II study of immunotherapy using tumor antigen-pulsed dendritic cells in patients with hepatocellular carcinoma.
5	2012	(NOBUOKA et al., 2012)	Radiofrequency ablation for hepatocellular carcinoma induces glypican-3 peptide-specific cytotoxic T lymphocytes.
6	2013	(HEO et al., 2013)	Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer.
7	2013	(EL ANSARY et al., 2013)	Immunotherapy by autologous dendritic cell vaccine in patients with advanced HCC.
8	2013	(ABEI et al., 2013)	A phase I study on combined therapy with proton-beam radiotherapy and in situ tumor vaccination for locally advanced recurrent hepatocellular carcinoma.
9	2014	(SHIMIZU et al., 2014)	Postoperative dendritic cell vaccine plus activated T-cell transfer improves the survival of patients with invasive hepatocellular carcinoma.
10	2014	(YU et al., 2014)	A randomized phase II study of autologous cytokine-induced killer cells in treatment of hepatocellular carcinoma.
11	2014	(NEMUNAITIS et al., 2014)	Summary of bi-shRNA/GM-CSF augmented autologous tumor cell immunotherapy (FANG™) in advanced cancer of the liver.
12	2015	(LEE et al., 2015c)	Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma.
13	2015	(SAWADA et al., 2015)	Programmed death-1 blockade enhances the antitumor effects of peptide vaccine-induced peptide-specific cytotoxic T lymphocytes.
14	2015	(MAEDA et al., 2015)	Dendritic cells transfected with heat-shock protein 70 messenger RNA for patients with hepatitis C virus-related hepatocellular carcinoma: a phase 1 dose escalation clinical trial.
15	2015	(LEE et al., 2015a)	A phase I/IIa study of adjuvant immunotherapy with tumour antigen-pulsed dendritic cells in patients with hepatocellular carcinoma.
16	2015	(WANG et al., 2015)	Phase I trial of active specific immunotherapy with autologous dendritic cells pulsed with autologous irradiated tumor stem cells in hepatitis B-positive patients with hepatocellular carcinoma.
17	2015	(YUTANI et al., 2015)	Phase II Study of Personalized Peptide Vaccination with Both a Hepatitis C Virus-Derived Peptide and Peptides from Tumor-Associated Antigens for the Treatment of HCV-Positive Advanced Hepatocellular Carcinoma Patients.
18	2015	(JIANG et al., 2015)	A phase I clinical trial utilizing autologous tumor-infiltrating lymphocytes in patients with primary hepatocellular carcinoma.
19	2015	(MIZUKOSHI et al., 2015)	Phase I trial of multidrug resistance-associated protein 3-derived peptide in patients with hepatocellular carcinoma.
20	2017	(NAKAGAWA et al., 2017)	Association Between High-Avidity T-Cell Receptors, Induced by α-Fetoprotein-Derived Peptides, and Anti-Tumor Effects in Patients With Hepatocellular Carcinoma.
21	2017	(YUTANI et al., 2017)	Feasibility study of personalized peptide vaccination for hepatocellular carcinoma patients refractory to locoregional therapies.
22	2017	(FANG et al., 2017)	Effect of transcatheter arterial chemoembolization combined with thymosin alpha 1 on the autophagy of immune cells from advanced hepatocellular carcinoma.
23	2017	(SHEN et al., 2017)	Phase I clinical study of personalized peptide vaccination combined with radiotherapy for advanced hepatocellular carcinoma.
24	2018	(GRASSBERGER et al., 2018)	Differential Association Between Circulating Lymphocyte Populations With Outcome After Radiation Therapy in Subtypes of Liver Cancer.
25	2019	(FAN et al., 2019)	First-in-class immune-modulating small molecule Icaritin in advanced hepatocellular carcinoma: preliminary results of safety, durable survival and immune biomarkers.
26	2019	(DOI et al., 2019)	A Phase I Study of the Anti-CC Chemokine Receptor 4 Antibody, Mogamulizumab, in Combination with Nivolumab in Patients with Advanced or Metastatic Solid Tumors.
27	2019	(KALATHIL et al., 2019)	Augmentation of IFN-γ+ CD8+ T cell responses correlates with survival of HCC patients on sorafenib therapy.
28	2020	(BANG et al., 2020)	Ramucirumab and durvalumab for previously treated, advanced non-small-cell lung cancer, gastric/gastro-oesophageal junction adenocarcinoma, or hepatocellular carcinoma: An open-label, phase Ia/b study (JVDJ).
29	2020	(LI et al., 2020)	Adjuvant 131I-metuximab for hepatocellular carcinoma after liver resection: a randomised, controlled, multicentre, open-label, phase 2 trial.
30	2020	(TANIGUCHI et al., 2020)	Peptide vaccine as an adjuvant therapy for glypican-3-positive hepatocellular carcinoma induces peptide-specific CTLs and improves long prognosis.

Appendix 1: Articles selected after active search in database.

HERALD

Advances In Industrial Biotechnology | ISSN: 2639-5665

Advances In Microbiology Research | ISSN: 2689-694X

Archives Of Surgery And Surgical Education | ISSN: 2689-3126

Archives Of Urology

Archives Of Zoological Studies | ISSN: 2640-7779

Current Trends Medical And Biological Engineering

International Journal Of Case Reports And Therapeutic Studies | ISSN: 2689-310X

Journal Of Addiction & Addictive Disorders | ISSN: 2578-7276

Journal Of Agronomy & Agricultural Science | ISSN: 2689-8292

Journal Of AIDS Clinical Research & STDs | ISSN: 2572-7370

Journal Of Alcoholism Drug Abuse & Substance Dependence | ISSN: 2572-9594

Journal Of Allergy Disorders & Therapy | ISSN: 2470-749X

Journal Of Alternative Complementary & Integrative Medicine | ISSN: 2470-7562

Journal Of Alzheimers & Neurodegenerative Diseases | ISSN: 2572-9608

Journal Of Anesthesia & Clinical Care | ISSN: 2378-8879

Journal Of Angiology & Vascular Surgery | ISSN: 2572-7397

Journal Of Animal Research & Veterinary Science | ISSN: 2639-3751

Journal Of Aquaculture & Fisheries | ISSN: 2576-5523

Journal Of Atmospheric & Earth Sciences | ISSN: 2689-8780

Journal Of Biotech Research & Biochemistry

Journal Of Brain & Neuroscience Research

Journal Of Cancer Biology & Treatment | ISSN: 2470-7546

Journal Of Cardiology Study & Research | ISSN: 2640-768X

Journal Of Cell Biology & Cell Metabolism | ISSN: 2381-1943

Journal Of Clinical Dermatology & Therapy | ISSN: 2378-8771

Journal Of Clinical Immunology & Immunotherapy | ISSN: 2378-8844

Journal Of Clinical Studies & Medical Case Reports | ISSN: 2378-8801

Journal Of Community Medicine & Public Health Care | ISSN: 2381-1978

Journal Of Cytology & Tissue Biology | ISSN: 2378-9107

Journal Of Dairy Research & Technology | ISSN: 2688-9315

Journal Of Dentistry Oral Health & Cosmesis | ISSN: 2473-6783

Journal Of Diabetes & Metabolic Disorders | ISSN: 2381-201X

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Journal Of Environmental Science Current Research | ISSN: 2643-5020

Journal Of Food Science & Nutrition | ISSN: 2470-1076

Journal Of Forensic Legal & Investigative Sciences | ISSN: 2473-733X

Journal Of Gastroenterology & Hepatology Research | ISSN: 2574-2566

Journal Of Genetics & Genomic Sciences | ISSN: 2574-2485

Journal Of Gerontology & Geriatric Medicine | ISSN: 2381-8662

Journal Of Hematology Blood Transfusion & Disorders | ISSN: 2572-2999

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Journal Of Internal Medicine & Primary Healthcare | ISSN: 2574-2493

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Journal Of Neonatology & Clinical Pediatrics | ISSN: 2378-878X

Journal Of Nephrology & Renal Therapy | ISSN: 2473-7313

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Journal Of Nuclear Medicine Radiology & Radiation Therapy | ISSN: 2572-7419

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Journal Of Ophthalmology & Clinical Research | ISSN: 2378-8887

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Journal Of Pharmacology Pharmaceutics & Pharmacovigilance | ISSN: 2639-5649

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