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Specific immunotherapy in hepatocellular cancer: A systematic review

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Abstract

Background and Aim: In recent years, several novel immunotherapeutic approaches were developed and investigated in patients with hepatocellular carcinoma (HCC). We designed this systematic review, to evaluate clinical efficacy of specific immunotherapy in patients with HCC, according to the guidelines of Border of Immune Tolerance Education and Research Network (BITERN) and Cochrane collaboration.

Methods: We searched Medline, Scopus, CENTRAL, TRIP, DART, OpenGrey, and ProQuest through the 9th of December 2015. One author reviewed and retrieved citations from these seven databases for irrelevant and duplicate studies, and two other authors independently extracted data from the studies and rated their quality. We collated study findings and calculated a weighted treatment effect across studies using Review Manager.

Results: We found 12144 references in seven databases of which 21 controlled studies with 1885 HCC patients in different stages were included in this systematic review after the primary and secondary screenings. Overall, patients undergoing specific immunotherapy had significantly higher overall survival than those in control group (HR = 0.59; 95% CI = 0.47–0.76, $P < 0.0001$). There was a significant difference in recurrence-free survival between patients undergoing specific

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Systematic Review Protocol.

Appendix S2. Tool for assessing risk of bias in clinical trials.

immunotherapy and patients in control groups and patients in immunotherapy groups overall had less recurrence than control group (HR = 0.54; 95% CI = 0.46–0.63, $P < 0.00001$).

Conclusions: Results of this systematic review based on the available literature suggest that overall specific immunotherapeutic approaches could be beneficiary for the treatment of patients with HCC. This further supports the current and ongoing evaluations of specific immunotherapies in the field.

Keywords

active immunotherapy; clinical outcomes; hepatocellular carcinoma; immunotherapy; meta-analysis; passive immunotherapy; systematic review; vaccines

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related mortality worldwide.¹ Multiple therapeutic modalities can be applied in patients with HCC depending on factors such as the degree of liver function, tumor burden, and associated comorbidities. Curative treatment options for early stage HCC are surgical resection or orthotopic liver transplantation (OLT).² Other methods for early but non-resectable HCC include minimally invasive approaches such as percutaneous ethanol injection (PEA) and radiofrequency ablation (RFA).^{2,3} Trans-arterial chemoembolization (TACE) is currently considered as first line therapy in patients with intermediate-stage disease.³ However, most patients with HCC presents with advanced disease.² Multi-drug resistance (MDR) genes are reported to be highly expressed in HCCs; therefore, HCC is extremely chemo-resistant.⁴ In addition, due to underlying cirrhosis and impaired drug metabolism in most patients with HCC, chemotherapy results in devastating adverse effects.

There are several reports of spontaneous regression in patients with HCC, for which anti-tumor immunity has been known to play a leading role.^{5,6} The observation of spontaneous regression of HCCs, as a consequence of the host immune response, paved the way toward development of novel passive and active immunotherapeutic approaches in the management of advanced HCC.^{5,6} HCC is a typical inflammation induced cancer, which makes it possibly more susceptible to an immune based approach. Unlike, nonselective effects of conventional treatments, immunotherapy, theoretically, could selectively target and destroy malignant cells with minimal side effects.⁷⁻¹¹ Passive immunotherapy involves administrating components of immune system such as immune cells or antibodies to patients to provide immunity against tumor cells.⁷⁻¹¹ On the other hand, active immunotherapy stimulates host immune responses to recognize and destroy malignant cells.⁷⁻¹¹

We designed this systematic review to evaluate clinical efficacy of specific immunotherapies in patients with HCC, according to the guidelines of Border of Immune Tolerance Education and Research Network (BITERN) and Cochrane collaboration.

Methods

We conducted this systematic review in accordance with general methods recommended by Cochrane collaboration and Border of Immune Tolerance Education and Research Network guidelines.

Inclusion criteria for considering studies for this review.

In this systematic review, only controlled clinical trials (irrespective of blinding and randomization) investigating clinical efficacy of specific immunotherapies (irrespective of modes of administration, dosage, frequency and duration) in patients with primary HCC (irrespective of disease stage, age, gender or race) according to inclusion criteria stated in the protocol were included (Appendix S1). According to the protocol, phase 1 clinical trials without control or studies investigating non-specific immunotherapies such as immunomodulatory antibodies and immune checkpoints inhibitors and cytokine therapies were not included in this systematic review, considering the early nature of their development and their declaration of a new era that is currently being studied further.

Literature search strategy for identification of studies.

The primary search process was conducted in Medline, Scopus, TRIP, and CENTRAL databases (as databases for journal articles) and DART, OpenGrey, and ProQuest (as databases for grey literature) according to the search strategies described in the protocol (Appendix S1) on 18 May 2014. We updated the search process in Medline, Scopus, CENTRAL, ProQuest, DART, and OpenGrey databases up to the 9th of December 2015.

One of the authors imported citations from all databases into an Endnote library (version X6, Thomson Reuters, USA), removed duplicate citations using the “Find Duplicates” feature of Endnote software and then removed obviously irrelevant studies by screening title and abstract of the remainders of search results. We then retrieved full texts of remainder of citations for further screening and data collection process and quality assessment.

Data extraction and quality assessment.

Two of authors carefully read full texts of articles and independently included eligible articles based on the inclusion criteria stated in the protocol (Appendix S1). Disagreements between two authors were resolved by discussion or consultation with a third author and a final consensus.

Two authors independently extracted data including study title, corresponding author name, population information (sample size, age, and gender of participants), type of study, intervention, inclusion criteria, outcomes, and bias assessment from full texts of articles and entered them to pre-designed data extraction forms in Microsoft Excel spreadsheets (version 2010, Microsoft Corporation, USA).

Two reviewers independently evaluated studies for risk of bias in six domains of random sequence generation (selection bias for controlled trials), allocation concealment (selection bias for controlled trials), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias)

and selective reporting (reporting bias) according to the “Risk of bias” tool (Appendix S2) and attributed each domain to be of “low risk” of bias, “high risk” of bias, or “unclear risk” of bias for each article. Any disagreement between two authors regarding quality of studies was resolved through discussion or consultation with the third author.

Evidence synthesis.

We did evidence synthesis using systematic approaches like tabulation and textual description, and we converted results from each study into a common rubric using Review Manager (Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014). For missing data, we used statistical methods to calculate missing data using data presented in the articles or in some cases we contacted the corresponding author.

We used Review Manager to do meta-analysis and calculate a weighted treatment effect across studies. We evaluated between-study heterogeneity by the chi-square statistic and its P value and I^2 statistic. We considered a P value < 0.1 and $I^2 > 40\%$ as indicating significant between-study heterogeneity. In case of significant statistical heterogeneity or clinical heterogeneity, a random-effects model was used to do meta-analysis. In addition, we used sub-group analysis in case of clinical heterogeneity. We expressed the results of meta-analysis in hazard ratio (HR) with 95% confidence intervals (CI) for time-to-event outcomes and risk ratio (RR) with 95% CI for dichotomous outcomes.

Results

Description of included studies.

We found 12144 references by applying search strategy in four databases of journal articles and three databases of grey literature. We then discarded 1847 duplicate citations using Endnote software and 10084 articles due to obvious irrelevancy of their topics in primary screening (Fig. 1). In secondary screening of 213 full texts, we excluded 192 articles, and finally included 21 controlled trials with 1885 HCC patients in this systematic review (Table 1).

Overall, six studies with 254 patients addressed two active immunotherapeutic approaches including autologous formalin- fixed tumor vaccine (AFTV)^{12–15} and dendritic cell (DC)-based vaccines.^{16–18} Twelve studies with 1459 patients investigated passive immunotherapeutic approaches including autologous cytokine-induced killer (CIK) cells,^{19–27} auto lymphocyte therapy (ALT),²⁸ autologous lymphokine-activated killer (LAK) cells^{7,26,29} and radio immunotherapy (RIT).^{30–33} The clinical efficacy of pulsed DCs, an active immunotherapy, with CIK cells, a passive immunotherapy was studied in three controlled trials with 172 patients concurrently.^{34–36} All immunotherapeutic approaches reported in these studies were administered systemically.

Clinical outcomes investigated in these studies included overall survival, progression-free survival, recurrence-free survival, and toxicity.

The overall quality of studies was fair. There was high risk of selection bias in studies of Shimizu et al. and Hao et al. as they did not apply randomization methods to allocate

patients to treatment arms (Table 2).^{25,34} In addition, the randomization method was not described in majority of studies and therefore, the risk of selection bias was unknown. Majority of studies did not use placebo or blinding methods to prevent performance bias. There was no risk of attrition bias for studies (Table 2).

Overall survival.

Overall survival and mortality were reported in 17 studies of which data on hazard ratio could have been retrieved from 15 studies.

Considering the presence of moderate statistical heterogeneity and clinical heterogeneity between results of studies ($\text{Tau}^2 = 0.08$; $P = 0.05$; $I^2 = 40\%$), we employed subgroup analysis and random effects model to perform a meta-analysis on results of these 15 studies. Overall survival was significantly higher in patients who underwent specific immunotherapy comparing to patients in control groups (HR=0.59; 95% CI=0.47–0.76, $P < 0.0001$) (Fig. 2).

Active immunotherapies.—Gao et al. and Ansary et al. reported that treatment with DC-based vaccines had a significant beneficiary effect on overall survival of HCC patients (Table 1).^{16,17} However, Nakamoto et al. reported that vaccination with immature DCs did not have any beneficial effect on overall survival (Table 1).¹⁸

Kuang et al. reported that in subgroup analysis for tumor size in 39 patients, AFTV therapy significantly improved overall survival in patients with tumor size ≤ 50 mm.^{12,13}

Passive immunotherapy.—In subgroup meta-analysis of studies on passive specific immunotherapy, patients undergoing passive immunotherapy had significantly higher overall survival than those in control group (HR = 0.6; 95% CI = 0.44–0.8, $P = 0.0006$). In subgroup meta-analysis of five studies on CIK therapy, CIK cell transfusion significantly prolonged overall survival (HR = 0.5; 95% CI = 0.35–0.71, $P = 0.0001$).

Combined immunotherapies.—In subgroup meta-analysis of studies investigating combined immunotherapeutic approaches (autologous DC-CIK cell therapy), patients in experimental groups had higher overall survival than those in control groups although this difference was not significant (HR = 0.58; 95% CI = 0.31–1.09, $P = 0.09$).

Recurrence-free survival and progression-free survival.

Recurrence-free survival was reported in 13 studies including four studies on active immunotherapy and eight studies on passive immunotherapy and one study on combined immunotherapy with 94 patients reported data on hazard ratio of recurrence between experimental and control groups.

Although there was not any statistical heterogeneity between results of these 13 studies, considering the presence of clinical heterogeneity, we employed subgroup analysis and random-effects model to conduct meta-analysis. Overall, patients undergoing specific immunotherapy had significantly higher recurrence-free survival than patients in control groups (HR = 0.54; 95% CI = 0.46–0.63, $P < 0.00001$) (Fig. 3).

Active immunotherapies.—In subgroup meta-analysis of four studies on active immunotherapy with 194 patients, patients undergoing active immunotherapy had significantly higher recurrence free survival (HR = 0.59; 95% CI = 0.40–0.85, $P=0.005$). Although, three studies on AFTV did not report any significant difference in recurrence rate between patients in AFTV and control arms, in pooled analysis, AFTV significantly improved recurrence free survival in patients with HCC (HR = 0.55; 95% CI = 0.35–0.87, $P = 0.01$). In addition, Gao et al. reported a significant lower recurrence rate after 1.5 years in patients undergoing DC-based vaccination than controls (Table 1).¹⁶

Kuang et al. reported that in subgroup analysis for tumor size in 39 patients, AFTV therapy significantly improved recurrence-free survival in patients with tumor size ≤ 50 mm.^{12,13}

Passive immunotherapies.—In subgroup meta-analysis of eight studies on passive immunotherapy with 1018 patients, recurrence-free survival was significantly higher in patients undergoing passive immunotherapy (HR = 0.5; 95% CI = 0.40–0.62, $P < 0.00001$). In subgroup meta-analysis of three studies investigating CIK therapy, hazard ratio of recurrence was significantly lower in patients in experimental groups (HR = 0.41; 95% CI = 0.23–0.76, $P = 0.004$).^{19,20,27} In addition, Hao et al. and Yu et al. both reported beneficial effect of CIK cell therapy in patients with HCC on progression free survival (Table 1).^{25,26}

Lee et al. reported that in subgroup analysis for etiology of HCC in 186 patients, CIK cell therapy significantly improved recurrence-free survival in patients with HBV but this improvement in recurrence-free survival was not statistically significant in patients with HCV or other etiologies.¹⁹ In subgroup analysis for a number of Clinicopathophysiologic characteristics, Bian et al. reported that Licartin therapy significantly improved recurrence-free survival in patients with BCLC stage 0–A ($n = 94$), tumor size ≤ 30 mm ($n = 49$) and single lesion ($n = 66$)³⁰

Combined immunotherapies.—Shimuzu et al. reported that patients in combined specific immunotherapy arm had higher recurrence-free survival than those in control arm however this difference was not significant.³⁴

Safety.

Overall, 20 studies reported safety parameters in patients undergoing specific immunotherapy. Overall, specific immunotherapies were safe and comparing to standard treatments resulted in even less serious adverse events although minor shortterm side effects as fever (mostly), allergic reactions, headache and pain were reported (Table 1).

Discussion.—We used a comprehensive search strategy in seven databases to retrieve controlled clinical trials investigating specific immunotherapy and presented and analyzed results of 21 retrieved articles with 1885 HCC patients in a comprehensive and systematic manner. The retrieved studies were heterogeneous in regard of the immunotherapeutic approaches they reported and also their study populations. Therefore, we collected and presented details of each study in our systematic review. In addition, we used random effects and sub-group meta-analysis based on different approaches of immunotherapy used in these studies.

Active immunotherapy.

Overall, two active immunotherapeutic approaches including AFTV and DC-based vaccination were reported in 6 clinical trials.

This systematic review showed positive effect of AFTV, an autologous tumor vaccine fixed with formalin, on recurrence-free survival. The patient's immune system can recognize tumor antigens present in autologous fixed tumor lysates and therefore can elicit a specific antitumor response.^{12,14,15} In addition, sustained release of GM-CSF and IL-2 from microparticles, administered along with AFTV, persistently activates antigen presenting cells (APCs) and cytotoxic T lymphocytes (CTLs) and induces longterm stimulation of the immune system and persistent antitumor immunity.^{12,14}

On the other hand, AFTV contains some normal tissues in addition to malignant cells. Antigens from these normal liver cells can be uptaken by APCs and result in an autoimmune response against normal cells of liver.¹⁴ However, none of studies reported an autoimmune disease in patients under treatment with AFTV.^{12,14}

Our systematic review showed that vaccination with antigen-preloaded DCs, one of the novel modalities of immunotherapy in cancer, had a beneficiary effect on overall survival and recurrence-free survival.^{16,17} DCs, as the most potent APCs, express high levels of the major histocompatibility complex (MHCs) and various costimulatory molecules and cytokines and therefore can effectively absorb and process tumor antigens to effector immune cells and elicit strong anti-tumor immune response.^{8,9,16,17,37} However, infusion of immature DCs did not improve overall survival or recurrence-free survival which might be due to insufficient antigens to be taken up by DCs after the infusion.^{7,10,18,37}

Although DC vaccination was effective in terms of overall survival and recurrence free survival, these approaches are too cumbersome and costly.^{12,15}

Passive immunotherapy.

Overall, four passive immunotherapeutic approaches including autologous CIK cell therapy,^{19–27} ALT,²⁸ autologous LAK cell therapy^{7,26,29} and RIT^{30–33} were reported in twelve clinical trials.

Our systematic review showed that CIK therapy is effective in terms of prolonging overall survival, recurrence-free survival and progression-free survival. CIK cells are non MHC-restricted cytotoxic lymphocytes with the characteristic CD3⁺–CD56⁺ phenotype.^{38,39} They can be generated by addition of various cytokines including IL-2, IL-1, and IFN- γ to peripheral blood monocytes.^{8,20,38} CIK cells can traffic efficiently to tumor sites and produce a strong anti-tumor response.^{21,25} In addition, they can shift the immune response toward a TH1 direction by producing effector cytokines (such as IL2, IL6, and IFN γ) and chemokines.^{8–10,21,25} Lee et al. also found significant improvement in recurrence-free survival in patients with Hepatitis B but not in group of patients with hepatitis C, although lack of significant effect in this group might be due to few numbers of patients in this subgroup. However, CIK cells might reduce tumor recurrence by suppressing the replication of HBV.¹⁹

Results from this systematic review revealed that LAK cells are effective in prolonging recurrence free survival in patients with HCC. LAK cells consist of a diverse population of cells including primarily cytotoxic natural killer cells and T cells.^{7,26,29,40,41}

In ALT, lymphokine activated autologous lymphocytes depleted from suppressor cells are transfused back to the patients.^{28,39,42}

RIT is one of the more attractive approaches to cancer treatment in which radioactive tumoricidal agents are delivered to tumor cells using radio-conjugated monoclonal antibodies (mAbs) against tumor antigens.^{7-10,31} Licartin (¹³¹I-metuximab injection), one of novel radio-conjugated mAbs, is produced by labeling metuximab with iodine-131.^{30,33} Metuximab is a murine mAb against CD147 (HAb18G), an extracellular matrix metalloproteinase inducer associated with hepatocarcinogenesis and HCC grading.^{30,33} Results from our systematic review suggest that Licartin had a beneficial effect on overall survival and recurrence-free survival of patients with HCC.

Combined immunotherapy.

Patients with HCC often have a compromised immune system due to liver cirrhosis, decreased level of lymphocytes, high levels of TGF- β , IL-4, IL-10, or regulatory T cells. Therefore, applying passive immunotherapy can improve anti-tumor immune responses induced by active immunotherapy.^{8,10,39} Three studies investigated combined immunotherapeutic approaches including ATVAC (autologous tumor lysate-pulsed dendritic cells plus activated T cell transfer) and autologous DC-CIK cell therapy in patients with HCC and only ATVAC improved overall survival of patients. Activated T cells administered in ATVAC can improve the induction of antitumor response by autologous tumor lysate-pulsed DCs particularly in patients with HCC who have a compromised immune system.^{8,11,34,41}

α -Galactosyl epitope is an antigen which is absent in normal human cells and therefore, natural antiGal antibody is abundant in serum.³⁶ In the approach used by Qiu et al., DCs were pulsed with lysate tumor cells expressing α -Gal epitope which resulted in insitu binding of the patient's natural anti-Gal IgG and consequently DC opsonization and enhanced uptake of tumor antigens by DCs.³⁶ Then these pulsed DCs were co-cultured with CIK cells to induce tumor-specific CIKs from naïve CIKs.³⁶ In addition, in this study, newly differentiated CIKs from bone marrow stem cells were used instead of circulating T cells (which are compromised due to the overall immunocompromised state of patients).³⁶ However, this approach did not result in any significant beneficial effect on survival in patients with HCC.

Limitations.

This meta-analysis had some limitations. First, there were considerable clinical heterogeneity in designs, interventions and populations (regarding stage and etiology of HCC and race of patients) of studies included in this systematic review. To address this issue, we collected details of each study and presented these details in a homogenous format in a table. In addition, we used random effects and sub-group meta-analysis based on different approaches of immunotherapy used in these studies. However, we did not use a

subgroup meta-analysis based on study populations considering that few number of studies would be included in each sub-group.

Another source of heterogeneity was the designs and methods used in each study and consequently quality of studies. Therefore, we rated quality of studies using systematic methods of Cochrane collaboration and employed subgroup meta-analysis and randomeffects model.

Second, some outcomes in some studies were not reported properly so we could not include them in our meta-analysis. Therefore, we converted their results to the most interpretable outcome measures. For example, we have converted results of Kaplan-Meyers survival curves to HR or raw data of survival to risk ratio or median differences of survival time. In addition, some clinical outcomes like recurrence-free survival were not reported in some studies. We tried to address this in quality rating of studies under selective reporting domain in Table 2. Third, the risk of bias was high in some studies and enough information was not reported to allow judgment of bias in particular domains in several articles. Fourth, number of included studies and sample sizes were small for some immunotherapeutic approaches like autologous DC vaccines studied by Ansary et al, Gao et al. and Nakamoto et al. which limited statistical power of detecting a significant difference between experimental groups and controls.

Conclusions.

Immunotherapy had extensive and varied use in the treatment of HCC. The systemic analysis presented herein illustrates the potential value of these approaches especially passive immunotherapies like CIK cell therapies, even though, no firm conclusions can be drawn due to the heterogeneity of the data available. Unlike, non-selective effects of conventional treatments, immunotherapy, theoretically, could selectively target and destroy malignant cells with minimal side effects. The advent of checkpoint inhibitors may help shed a better light on this modality of treatment and its potential value for HCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Study flow diagram. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 2.

Comparison of overall survival between patients in specific immunotherapy group and control group. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 3.

Comparison of recurrence-free survival between patients in specific immunotherapy group and control group. [Color figure can be viewed at wileyonlinelibrary.com]

Table 1.

Table of included studies and their main characteristics

Study (first author, year and place of study)	Controlled clinical trials reporting active immunotherapy	Interventions	Outcomes, results, and relationships
Study patients and main groups			
Kuang et al. 2004 and Peng et al. 2006, China ^{12,13}	Patients with HCC (<i>n</i> = 67) 1. Control group (<i>n</i> = 35) 2. Experimental group (<i>n</i> = 32) • Majority of patients had hepatitis B • Hepatectomy was the front-line therapy	1. Control group: <i>no treatment</i> 2. Experimental group: <i>AFTV</i> AFTV was administered intradermally for three times at two-week intervals. AFTV dose consisted of 40 µL of formalin-fixed autologous HCC fragments, 4000 units of IL-2 and 4000 units of GM-CSF and 50 ng of tuberculin.	<ul style="list-style-type: none"> ➤ Recurrence-free survival in 4.5 years * (HR: 95%CI; <i>P</i> value): 0.54 (0.27–1.08); <i>P</i> = 0.0814 ➤ Toxicity grade 3–4: none was reported ➤ Relationships between Clinicopathologic characteristics and outcome of immunotherapy: AFTV therapy significantly improved recurrence-free and overall survival in patients with tumor size 50mm (<i>n</i> = 39)
Peng et al. 2003, China ¹⁴	Patients with HCC (<i>n</i> = 56) 1. Control group (<i>n</i> = 29) 2. Experimental group (<i>n</i> = 27) • Hepatectomy was the front-line therapy	1. Control group: <i>no treatment</i> 2. Experimental group: <i>AFTV</i> The Dose-1 (–2, or –4) of AFTV was administered intradermally for three times at two-week intervals. The Dose-1 (–2, or –4) of AFTV consisted of 10 (20, or 40, respectively) µL of formalin-fixed autologous HCC fragments with 1000 (2000, or 4000) units of GM-CSF and IL-2, and 0.025 (0.05, or 0.05) µg of tuberculin.	<ul style="list-style-type: none"> ➤ Recurrence-free survival in 2.5 years * (HR: 95%CI; <i>P</i> value): 0.51 (0.21–1.22); <i>P</i> = 0.1303 ➤ Toxicity grade 3–4: none was reported
Peng et al. 2005, China ¹⁵	Patients with HCC (<i>n</i> = 50) 1. Control group (<i>n</i> = 26) 2. Experimental group (<i>n</i> = 24) • Majority of patients had hepatitis B • Hepatectomy was the front-line therapy	1. Control group: <i>no treatment</i> 2. Experimental group: <i>AFTV</i> The Dose-1 and dose-2 of AFTV was administered intradermally for three times at two-week intervals. The Dose-1 consisted of 10 µL of formalin-fixed autologous HCC fragments, 1000 units of GM-CSF, 1000 units of IL-2 and 0.05 µg of tuberculin and dose-2 of AFTV consisted of 10 µL of formalin-fixed autologous HCC fragments, 6000 IU of GM-CSF and 6000 IU of IL-2 and 0.05 µg of tuberculin.	<ul style="list-style-type: none"> ➤ Recurrence-free survival in 2.5 years * (HR: 95%CI; <i>P</i> value): 0.60 (0.25–1.44); <i>P</i> = 0.2528 ➤ Toxicity grade 3–4: none was reported
Gao et al. 2005, China ¹⁶	Patients with HCC (<i>n</i> = 30) 1. Control group (<i>n</i> = 15) 2. Experimental group (<i>n</i> = 15) Hepatectomy was the front-line therapy	1. Control group: <i>chemotherapy with Epirubicin, 5-Fluorouracil and Mitomycin</i> 2. Experimental group: <i>autologous DC vaccine</i> DCs pulsed with hepatoma cell lysates were administered intradermally at three months intervals.	<ul style="list-style-type: none"> ➤ Overall survival in 1.5 years (RR: 95% CI; <i>P</i> value): 0.64 (0.41–0.99); <i>P</i> = 0.0464 ➤ Recurrence-free survival in 1.5 years (RR: 95% CI; <i>P</i> value): 0.25 (0.06–0.99); <i>P</i> = 0.0480 ➤ Toxicity grade 3–4: none was reported
Ansary et al. 2013, Egypt ¹⁷	Patients with advanced HCC (<i>n</i> = 30) 1. Control group (<i>n</i> = 15) 2. Experimental group (<i>n</i> = 15) Hepatectomy and radio-chemotherapy were front-line therapies	1. Control group: <i>no treatment</i> 2. Experimental group: <i>autologous DC vaccine</i> 20 × 10 ⁶ DCs pulsed with HepG2 tumor cell line lysates were administered intradermally.	<ul style="list-style-type: none"> ➤ Overall survival (median difference, <i>P</i> value): 3 months; <i>P</i> = 0.008 ➤ Toxicity grade 3–4: none was reported
Nakamoto et al. 2006, Japan ¹⁸	Patients with HCC stage >1 (<i>n</i> = 21) 1. Control group: TAE 2. Experimental group: TAE + autologous DC vaccine • All of patients had hepatitis B	1. Control group: <i>TAE</i> 2. Experimental group: <i>TAE + autologous DC vaccine</i> 5 × 10 ⁶ immature DCs were administered through an arterial catheter during TAE	<ul style="list-style-type: none"> ➤ Overall survival in 2 years * (HR: 95%CI; <i>P</i> value): 0.53 (0.18–1.57); <i>P</i> = 0.2519 ➤ Recurrence-free survival in 2 years * ➤ Overall survival (HR: 95%CI; <i>P</i> value): 0.67 (0.35–1.28); <i>P</i> = 0.2267 ➤ Toxicity grade 3–4: none was reported

Study (first author, year and place of study)	Controlled clinical trials reporting active immunotherapy	Interventions	Outcomes, results, and relationships
Study patients and main groups			
Controlled clinical trials reporting passive immunotherapy			
Lee et al. 2015, Korea ¹⁹	Patients with HCC stage 1 or 2 ($n = 230$) 1. Control group ($n = 115$) 2. experimental group ($n = 115$) •Majority of patients had hepatitis B •Curative treatment (surgical resection, RFA, or PEI) were front-line therapies	1. Control group: <i>no treatment</i> 2. Experimental group: CIK cell therapy 6.4×10^9 CIK cells were administered intravenously for each cycle of 16 cycles (4 cycles once per week, followed by 4 cycles at 2 weeks intervals, then 4 cycles at 4 weeks intervals, and 4 cycles at 8 weeks intervals).	➤ Overall survival in 4.5 years (HR; 95%CI; P value): 0.21 (0.06–0.75); $P = 0.0163$ ➤ Recurrence-free survival in 4.5 years (HR; 95%CI; P value): 0.63 (0.43–0.94); $P = 0.0236$ ➤ Toxicity grade 3–4 (RR, 95% CI, P value): 1.75 (0.53–5.81); $P = 0.3611$ ➤ Relationships between Clinicopathophysiologic characteristics and outcome of immunotherapy: CIK cell therapy significantly improved recurrence-free survival in patients with HBV ($n = 186$)
Huang et al. 2013, Weng et al. 2007, Zhou et al. 2006, Zhao et al. 2006 and Pan et al. 2010, China ^{20–24}	Patients with HCC ($n = 174$) 1. Control group ($n = 89$) 2. Experimental group ($n = 85$) •Majority of patients had hepatitis B •TACE and RFA were frontline therapies	1. Control group: <i>no treatment</i> 2. Experimental group: CIK cell therapy 10^{10} cells were administered through a hepatic arterial catheter 4 times with two weeks intervals.	➤ Overall survival in 8 years* (HR; 95%CI; P value): 0.69 (0.41–1.15); $P = 0.1545$ ➤ Recurrence-free survival in 5 years* (HR; 95%CI; P value): 0.31 (0.09–1.07); $P = 0.0639$ ➤ Toxicity grade 3–4: none was reported
Hao et al. 2009, China ²⁵	Patients with unresectable HCC ($n = 146$) 1. Control group ($n = 74$) 2. Experimental group ($n = 72$) •Majority of patients had hepatitis B •TACE was the frontline therapy	1. Control group: <i>no treatment</i> 2. Experimental group: CIK cell therapy 1–5 $\times 10^{10}$ CIK cells were administered intravenously in one course. A maximum of 4 courses of treatment was given in one year	➤ Overall survival in 2.5 years* (HR; 95%CI; P value): 0.37 (0.24–0.56); $P < 0.0001$ ➤ Progression-free survival in 1.5 years* (HR; 95%CI; P value): 0.47 (0.36–0.62); $P < 0.0001$ ➤ Toxicity grade 3–4: none was reported ➤ Relationships between Clinicopathophysiologic characteristics and outcome of Immunotherapy: CIK cell therapy
Yu et al. 2014, China ²⁶	Patients with HCC ($n = 132$) 1. Control group ($n = 66$) 2. Experimental group ($n = 66$) •Almost half of the patients had hepatitis B •Hepatectomy or TACE were frontline therapies	1. Control group: BCS 2. Experimental group: CIK cell therapy CIK cells were administered intravenously once a month for up to 36 months	significantly Improved progression-free survival In patients with ECOG performance status of 0–1 ($n = 127$) ➤ Overall survival in 6 years* (HR; 95%CI; P value): 0.52 (0.33–0.81); $P = 0.0038$ ➤ Progression-free survival in 6 years* (HR; 95%CI; P value): 0.61 (0.43–0.87); $P = 0.0056$ ➤ Toxicity grade 3–4: none was reported ➤ Relationships between Clinicopathophysiologic characteristics and outcome of immunotherapy: no significant positive or negative predictive factors were identified by a multivariate analysis
Dong et al. 2008, China ²⁷	Patients with HCC ($n = 127$) 1. control group ($n = 43$) 2. experimental group 1 ($n = 41$) 3. experimental group 2 ($n = 43$) •Majority of patients had hepatitis B •Hepatectomy was the front-line therapy	1. Control group: <i>no treatment</i> 3. Experimental group 1: CIK cell therapy (3 courses) 4. Experimental group 2: CIK cell therapy (6 courses) 1–2 $\times 10^{10}$ cells were administered through a hepatic arterial catheter in each course in two-week Intervals	➤ Overall survival in 5 years for experimental groups vs control* (HR; 95%CI; P value): 0.94 (0.33–2.66); $P = 0.9072$ ➤ Recurrence-free survival in 4.5 years for experimental groups vs control* (HR; 95%CI; P value): 0.29 (0.17–0.49); $P < 0.0001$ ➤ Toxicity grade 3–4: none was reported

Study (first author, year and place of study)	Controlled clinical trials reporting active immunotherapy	Interventions	Outcomes, results, and relationships
Study patients and main groups			
Zhou et al. 2002, China ²⁶	Patients with HCC (<i>n</i> = 121) 1. Control group 1 (<i>n</i> = 30) 2. Control group 2 (<i>n</i> = 27) 3. Experimental group 1 (<i>n</i> = 31) 4. Experimental group 2 (<i>n</i> = 33) •Hepatectomy was the front-line therapy	1. Control group 1: <i>no treatment</i> 2. Control group 2: <i>chemotherapy</i> 3. Experimental group 1: <i>LAK cell therapy</i> 4. Experimental group 2: <i>LAK cell therapy + chemotherapy</i> LAK cells with 10^7 IU of IFN- γ and 10^6 IU of IL-2 were administered intravenously for four times at two-day intervals. Some patients underwent second treatment 6–8 weeks later.	➤ Recurrence-free survival in 3 years* (HR; 95%CI; <i>P</i> value): 0.57 (0.37–0.87); <i>P</i> = 0.0092 ➤ Toxicity grade 3–4: none was reported
Uchino et al. 1993 and Kawata et al. 1995, Japan ^{7,29}	Patients with HCC (<i>n</i> = 24) 1. Control group (<i>n</i> = 12) 2. Experimental group (<i>n</i> = 12) •Hepatectomy, splenectomy and chemotherapy with Adriamycin were front-line therapies	1. Control group: <i>no treatment</i> 2. Experimental group: <i>LAK cell therapy</i> 1.0–18.1 $\times 10^9$ LAK cells were administered intravenously three times once a week	➤ Overall survival in 3 years* (HR; 95%CI; <i>P</i> value): 0.03 (0–not estimated); <i>P</i> = 0.936 ➤ Recurrence-free survival in 3 years* (HR; 95%CI; <i>P</i> value): 0.53 (0.20–1.39); <i>P</i> = 0.1969 ➤ Toxicity grade 3–4: none was reported
Takayama et al. 2000, Japan ²⁸	Patients with HCC (<i>n</i> = 155) 1. Control group (<i>n</i> = 76) 2. Experimental group (<i>n</i> = 79) •Majority of patients had hepatitis C •Hepatectomy was the front-line therapy	1. Control group: <i>no treatment</i> 2. Experimental group: ALT $>3 \times 10^{10}$ autologous lymphocytes were administered intravenously at weeks 2, 3, 4, 12, and 24 after surgery	➤ Overall survival in 6 years* (HR; 95%CI; <i>P</i> value): 0.61 (0.22–1.71); <i>P</i> = 0.3473 ➤ Recurrence-free survival in 6 years* (HR; 95%CI; <i>P</i> value): 0.56 (0.35–0.90); <i>P</i> = 0.0156 ➤ Toxicity grade 3–4: none was reported
Bian et al. 2014, China ³⁰	Patients with HCC stage 1 or 2 (<i>n</i> = 127) 1. Control group (<i>n</i> = 65) 2. Experimental group (<i>n</i> = 62) •Majority of patients had hepatitis B •RFA was the front-line therapy	1. Control group: <i>no treatment</i> 2. Experimental group: <i>Licartin</i> 27.75 MBq/kg of Licartin was administered intravenously within 30 days of the last RFA	➤ Overall survival in 3 years (HR; 95%CI; <i>P</i> value): 0.66 (0.30–1.46); <i>P</i> = 0.3050 ➤ Recurrence-free survival in 3 years (HR; 95%CI; <i>P</i> value): 0.60 (0.38–0.96); <i>P</i> = 0.0332 ➤ Toxicity grade 3–4 (RR, 95% CI, <i>P</i> value): none was reported ➤ Relationships between Clinicopathologic characteristics and outcome of immunotherapy: Licartin therapy significantly improved recurrence-free survival in patients with BCLC stage 0–A (<i>n</i> = 94), tumor size ≤ 30 mm (<i>n</i> = 49) and single lesion (<i>n</i> = 66)
Xu et al. 2007, China ³³	Patients with HCC with stage >2 (<i>n</i> = 60) 1. Control group (<i>n</i> = 30) 2. Experimental group (<i>n</i> = 30) •Majority of patients had hepatitis B •OLT was the frontline therapy	1. Control group: <i>placebo</i> 2. Experimental group: <i>Licartin</i> 15.4 MBq/kg of Licartin was administered intravenously for 3 times at an interval of 28 days beginning from the 4th week after OLT	➤ Overall survival in 2.5 years (HR; 95%CI; <i>P</i> value): 0.26 (0.08–0.81); <i>P</i> = 0.0202 ➤ Recurrence-free survival in 1.5 years (HR; 95%CI; <i>P</i> value): 0.28 (0.12–0.67); <i>P</i> = 0.0042 ➤ Toxicity grade 3–4: none was reported
Order et al. 1990, USA ³²	Patients with unresectable HCC (<i>n</i> = 98) 1. Control group (<i>n</i> = 50) 2. Experimental group (<i>n</i> = 48) •Radiotherapy was the frontline therapy	1. Control group: <i>chemotherapy</i> "EntryTbL _{st2} Experimental group: <i>low dose chemotherapy + ¹³¹I antiferitin antibody</i> 20–30 mCi of ¹³¹ I antiferitin antibody was administered intravenously at day 0, and 10–15 mCi on day 5	➤ Overall survival in 1.5 years (HR; 95%CI; <i>P</i> value): 1.06 (0.73–1.56); <i>P</i> = 0.7637 ➤ Toxicity grade 3–4 (RR, 95%CI; <i>P</i> value): 0.97 (0.7–1.35); <i>P</i> = 0.8668
Zeng et al. 1998, China ³¹	Patients with unresectable HCC (<i>n</i> = 65) 1. Control group (<i>n</i> = 33) 2. Experimental group (<i>n</i> = 32) •Majority of patients had hepatitis B	1. Control group: <i>chemotherapy</i> 2. Experimental group: <i>¹³¹I-Hepama-1 mAb</i> 25 $\pm$ 108 mCi of ¹³¹ I-Hepama-1 mAb was administered through a hepatic artery catheter	➤ Overall survival in 5 years (HR; 95%CI; <i>P</i> value): 0.78 (0.46–1.32); <i>P</i> = 0.3546 ➤ Toxicity grade 3–4: none was reported ➤ Controlled clinical trials reporting passive and active Immunotherapy

Study (first author, year and place of study)	Controlled clinical trials reporting active immunotherapy	Interventions	Outcomes, results, and relationships
Study patients and main groups			
Shimizu et al. 2014, Japan ³⁴	Patients with Invasive HCC (<i>n</i> = 94) 1. Control group (<i>n</i> = 52) 2. Experimental group (<i>n</i> = 42) •Majority of patients had hepatitis C •Hepatectomy was the front-line therapy	1. Control group: <i>no treatment</i> 2. Experimental group: <i>ATVAC</i> DC vaccine incl. autologous tumor lysate-pulsed DCs. A total of 3 DC vaccinations and CAT transfer were administered within 2 months of surgery. Some patients received an additional ATVAC once a month until their tumor lysate was finished.	➤ Overall survival in 10 years* (HR; 95% CI; <i>P</i> value): 0.30 (0.10–0.93); <i>P</i> = 0.0370 ➤ Recurrence-free survival in 10 years* (HR; 95% CI; <i>P</i> value): 0.66 (0.38–1.14); <i>P</i> = 0.1362 ➤ Toxicity grade 3–4: none was reported
Li et al. 2015, China ³⁵	Patients with HCC (<i>n</i> = 60)1. Control group (<i>n</i> = 30) 2. Experimental group (<i>n</i> = 30) •TACE was the front-line therapy	1. Control group: <i>no treatment</i> 2. Experimental group: <i>autologous DC-CIK cell therapy</i> A total of 2 autologous DC-CIK cell infusions were administered once a month.	➤ Overall survival in 1 year* (HR; 95% CI; <i>P</i> value): 0.61 (0.34–1.11); <i>P</i> = 0.1056
Qiu et al. 2011, China ³⁶	Patients with HCC stage III (<i>n</i> = 18) 1. Control group (<i>n</i> = 9) 2. Experimental group (<i>n</i> = 9) •Hepatectomy and radio-chemotherapy was the front-line therapy	1. Control group: <i>no treatment</i> 2. Experimental group: <i>autologous DC-CIK cell therapy</i> DCs pulsed with synthesized α1, 3-galactosyl epitope and CIK cells were administered intravenously every week.	➤ Overall survival in 1.5 years (HR; 95% CI; <i>P</i> value) : 1.34 (0.31–5.81) ; <i>P</i> = 0.6958 ➤ Toxicity grade 3–4: none was reported

Abbreviations: AFTV, autologous formalin-fixed tumor vaccine; ALT, autologous lymphocyte therapy; ATVAC, autologous tumor lysate-pulsed dendritic cells plus activated T cell transfer; BCLC, Barcelona Clinic Liver Cancer staging system; CIK, cytokine induced killer cells; ECOG, the Eastern Cooperative Oncology Group; GM-CSF, Granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; IL-2, interleukin 2; LAK, lymphokine activated killer cells; OLT, orthotopic liver transplantation; PEI, percutaneous ethanol injection; RFA, Radiofrequency ablation; TACE, Transcatheter arterial chemoembolization; TAE, transarterial embolization.

* HR was reproduced from Kaplan Mayer survival curves

Table 2

Risk of bias in included studies

	Random sequence generation (selection bias)	Allocation concealment bias (selection bias)	Blinding of participants and personnel for overall survival	Blinding of participants and personnel for recurrence free survival	Blinding of outcome assessment for overall survival	Blinding of outcome assessment for recurrence free survival	Incomplete outcome data for overall survival	Incomplete outcome data for recurrence free survival	Selective reporting for overall survival	Selective reporting for recurrence free survival
Kuang et al 2004 and Peng et al 2006 (12, 13)			NA		NA		NA			
Peng et al 2003 (14)			NA		NA		NA			
Peng et al 2005 (15)			NA		NA		NA			
Gao et al 2005 (16)										
Ansary et al 2013 (17)				NA		NA		NA		
Nakamoto et al 2006 (18)										
Lee et al 2015 (19)										
Huang et al 2013, Weng et al 2007, Zhou et al 2006, Zhao et al 2006 and Pan et al 2010 (20-24)										
Hao et al 2009 (25)										
Yu et al 2014 (26)										
Dong et al 2008 (27)										
Zhou et al 2002 (26)			NA		NA		NA			
Uchino et al 1993 and Kawata et al 1995 (7, 29)										
Takayama et al 2000 (28)										
Bian et al 2014 (30)										
Xu et al 2007 (33)										
Order et al 1990 (32)				NA		NA		NA		
Zeng et al 1998 (31)				NA		NA		NA		
Shimizu et al 2014 (34)										
Li et al 2015(35)				NA		NA		NA		NA
Qiu et al 2011(36)				NA		NA		NA		

Low risk of bias
 Unclear risk of bias
 High risk of bias