Combining cellular immunotherapy was an optional choice for unresectable advanced HCC: a systematic review and meta-analysis

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Abstract

Background The efficacy of cellular immunotherapy in advanced hepatocellular carcinoma (HCC) was controversial. This study was conducted to compare the effectiveness of combining cellular immunotherapy with that of incurable treatment alone. Methods The Ovid Medline, Embase, Cochrane Library and Pubmed were systematically searched due to January 8th 2020. The keywords include “immunotherapy”, “HCC” and study type. Treatment response was evaluated and progression-free survival (PFS) and overall survival (OS) were calculated using hazard ratio (HR) and 95% confidence interval (CI). Results A total of 19 studies with 1,275 patients were included in the meta-analysis. The median complete response rate (CR) was 19% in combining cellular immunotherapy comparing to 9% in the control group (RR=0.55, P=0.003). No significant difference was found in partial response and stable disease (RR=1.06 and 0.78, P>0.05, respectively). The progression disease rate was higher in the non-cellular immunotherapy group (31%) compared to the cellular immunotherapy group (RR=2.20, P=0.002). Patients treating with cellular immunotherapy had a better OS and PFS compared to those without cellular immunotherapy (HR=0.52 and 0.63, P<0.001). In the subgroup analysis, the only CIK infusion therapy and combined DC with CIK perfusion therapy patients had a better OS (HR=0.52 and 0.49, P<0.001 and P=0.002, respectively). Conclusion Our results suggested that combining use of cellular immunotherapy in advanced HCC could increase the complete response rate, and thereafter extend the progression-free and overall survival rate. Subgroup analysis suggested that combining use of CIK and DC or using CIK alone could provide the benefit in survival outcome.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy in the liver, and the prevalence was still increasing in recent years (1, 2). In China, due to the high proportion of hepatitis B patients, HCC is still the fourth common malignancies (3). Currently, surgical resection is still considered to be the most effective approach to treat HCC (4). However, most patients with HCC are already in the advanced stage at the time of diagnosis(5). Previous data show that only about 5% of Western and 40% of Asian non-cirrhotic HCC patients with HCC are suitable for surgical resection (4). In terms of the cancer development process, HCC has its unique biological behavior compared to other malignant tumors, including the background of liver cirrhosis, the presence of multifocal precancerous lesions, the invasion of portal veins and the occurrence of intrahepatic metastases at an early stage (6). Patients with declining reserved liver function have difficulty in undergoing surgery, and given the common pathogenesis in the liver, surgery is difficult to remove the multifocal disease. The above reasons greatly limit the surgical operation in HCC.

Therefore, most guidelines suggested that minimally invasive treatment could be adopted for curing HCC, such as transarterial chemoembolization (TACE) and radiofrequency ablation (RFA) (7–9). Compared with traditional surgery, minimally invasive treatment could maximize the preservation of healthy liver tissue and achieve the purpose of treating tumors. At present, TACE sequential ablation therapy combined with sorafenib targeted therapy has become the basic model for the treatment of liver cancer (10). However, although minimally invasive treatment has made significant progress in the treatment of some early liver cancers, the 5-year survival rate of HCC has not improved significantly. Most patients would experience tumor recurrence and metastasis after undergoing radical treatment such as surgery and RFA (11).

Currently, the application of biological therapy in treating HCC patients has developed for years, and it has become the fourth mode of tumor treatment after surgery, radiotherapy and chemotherapy (12). Significant curative effects have been achieved in the treatment of several tumors, and clinical trials related to the treatment of HCC by biological therapy are now being vigorously carried out (13–15). Adoptive cellular immunotherapy, a category of biological therapy for HCC, refers to the infusion of immune cells with anti-tumor activity or stimulates the human immune response to kill tumor cells. The cells are usually taken from the patient's blood or tumor tissue, grown in large numbers in the laboratory, and then given back to the patient to help the immune system fight cancer (14, 16). Adoptive cellular immunotherapy can be used alone or as a supplement to surgery, radiotherapy and chemotherapy in the clinical treatment, which may improve the efficacy and improve the quality of life of patients (17). The most common cellular immunotherapy in treating HCC was based on the cytokine-induced killer cells (CIK) and dendritic cells (DC). However, the evidence of cellular immunotherapy in advanced HCCs was insufficient and not stratified. Thus, we designed this systematic review and meta-analysis to summarize the efficacy of cellular immunotherapy in treating advanced HCC.

Method

This study was designed based on the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines (18).

Search Strategy

This systematic review and meta-analysis was designed to evaluate the cellular immunotherapy in treating advanced HCC patients, aiming to assess the new treatment for incurable HCC patients. The Ovid Medline, Embase, Cochrane Database of Systematic Reviews and Cochrane Central Register of Controlled Trials and Pubmed were systematically searched due to January 8th 2020. Besides, the grey literature was searched in related website and Google Scholar. The keywords and Mesh were consulted from an experienced librarian. Briefly, the keywords were divided into three part: 1) HCC related keywords, such as “hepatocellular carcinoma”, “liver cancer”, “hepatoma”, “hepatocarcinoma”, and etc; 2) immunotherapy related keywords, such as “immunotherapy”, “immunosuppressive agents”, “immunotherapeutic”, and etc; 3) study type related keywords, “randomized controlled trial”, “cohort study”, “case control”, “review” and etc. The final search strategy was combined with the three parts using “AND” approach. All the studies containing abstracts and titles were imported into Endnote to find duplicate studies and therefore for literature screening.

Inclusion And Exclusion Criteria

All the studies comparing combining cellular immunotherapy and without cellular immunotherapy in treating advanced HCC were involved in our study. The inclusion criteria were: 1) the treatment included the DC, CIK, or natural killer cells (NK) therapy; 2) HCC was defined as intermediate or advanced stage; 3) minimally invasive treatment, such as TACE, RFA, sorafenib and etc., was adopted for combining treatment; 4) study type limited in randomized control trials (RCT), prospective or retrospective cohort studies, and case-control studies. The other meta-analysis, reviews, conference abstracts and comments were reading for the further inclusion of the papers.

The exclusion criteria were: 1) cell or animal experiment; 2) resectable or transplant HCC; 3) Case report or cases less than 10; 4) Without comparison or no immunotherapy were mentioned; 5) no report in survival outcome or treatment response; 6) data could not be fully extracted. Data from the same center in one period will be selected as one for further meta-analysis.

Literature screening and Data extraction

Two investigators (J Wang and L.X Feng) independently screened the titles and abstracts based on the inclusion and exclusion criteria. The full text was further evaluated if the abstracts could not be determined. The third investigator (L.W Zhang) was adapted for discussion if any disagreement existed.

The standard Excel was designed for data extraction and the following information was collected from the original studies. The study characteristics (author, publish year, country, institution, recruitment period, study design and etc.), patient characteristics (treatment, patient sample, gender, median age, tumor number, tumor size, the American Joint Committee on Cancer (AJCC) TNM tumor stage, Barcelona Clinic Liver Cancer (BCLC) tumor stage, Child-Pugh liver function), and outcome assessment (treatment response, toxicity and survival status). The treatment response, including overall response rate (ORR), disease control rate (DCR), complete response (CR), partial response (PR), stable disease (SD), and progression disease (PD) was assessed in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) (19). Progression-free survival (PFS) was defined as the time from randomization to first progression or death. Overall survival (OS) was defined as the time from treatment until death from any cause.

Quality Assessment

Two researchers (J Wang and L.X Feng) independently assess the quality of the including papers. The Jadad scale was used to evaluate the quality of randomized control studies. The scores of low-quality studies ranged from 0 to 3, whereas those of high-quality studies ranged from 4 to 8 (20). For non-randomized studies, the quality was assessed based on the Newcastle-Ottawa Quality Assessment Scale (NCS), with a high quality of 6–9, whereas low quality was scored as 0–5 (21).

Statistical analysis

For assessing the efficacy of cellular immunotherapy in treating advanced HCC, we compared the outcome between cellular immunotherapy group and without cellular immunotherapy group. The treatment response was compared and combined using relative risk (RR) while the survival analysis was combined using hazard ratio (HR). Both were reported with 95% confidence intervals (CI) and P value less than 0.05 was set as a significant difference. If the HR was not described explicitly in the univariate or multivariate analysis, we summarized the time-to-event data through the survival curve based on Tierney's method(22). Fixed effect model was used for calculating the overall pooled HRs, involving the calculation of observed minus expected events and variance of each endpoint in each trial, with the treatment effect expressed as Peto's odds with 95% CI. The I^2 statistic and χ^2 test were used for heterogeneity assessment ($I^2 \geq 50\%$ indicating the presence of heterogeneity). When the heterogeneity not existed, the fix- effects model was used while the random-effect model was used in the opposite way. The statistical analysis was performed by Stata 15.0 software (Stata Corporation, College Station, TX, USA).

Result

Literature selection

A total of 3,594 studies were found based on the search strategy. Figure 1 showed the flowchart of the screening process. After screening the titles and abstracts, 298 studies were scanned in full-text. After excluding the unrelated studies, a total of 19 studies were finally included in the systematic review and meta-analysis (14–17, 23–37).

Characteristics Of Included Studies

The characteristics of the included studies were listed in Table 1. A total of 1,275 patients were involved in our study. The first studies were reported in 2006, and the studies were started in 1997 (31). Sixteen studies were from China, two studies were from Japan and one from Egypt. The recruitment year was from1997 to 2017. Three studies were RCTs, which were evaluated as low-quality, and 16 studies were case-control studies with the NCS ranging from 5 to 7. The male patients occupied 51%-98% in the patient cohort with a median of 57 years-old. Median 72% of patients were Child-Pugh A liver function (range from 36–90%). 58% of patients had the multiple tumors, and 76.7% of patients were staged as TNM III or more.

Table 1
Characteristics of included studies.

Author	Publish year	Country	Recruitment year	Study design	NCS	Treatment	Patient sample	Male (%)	Median age	Multiple number (%)	Median tumor size	Child-pugh A/B	TNM I/II/III/
Zhou et al.	2019	China	2015–2016	Case control	6	Sorafenib + DC + CIK	35	26 (74)	-	-	-	27/8	-
						Sorafenib	36	29 (81)	-	-	-	26/10	-
Yang et al.	2019	China	2015–2016	Case control	7	IRE + NK	18	11 (61)	57	15 (83)	4.8	8/10	0/0/5/
						IRE	22	12 (55)	54	14 (64)	4.7	9/13	0/0/8/
Lin et al.	2017	China	2015–2017	Case control	6	Cryotherapy + NK	35	18 (51)	61	-	-	16/19	0/0/17
						Cryotherapy	26	14 (54)	56	-	-	11/15	0/0/12
Li et al.	2016	China	2010–2013	Case control	7	CIK	37	29 (78)	-	-	-	-	-
						Chemotherapy	37	33 (89)	-	-	-	-	-
Yu et al.	2014	China	2004–2009	Case control	5	BCS + CIK	25	-	-	-	-	-	-
						BCS	25	-	-	-	-	-	-
Niu et al.	2013	China	2004–2011	Case control	5	Cryotherapy + DC + CIK	21	-	-	-	-	-	-
						Cryotherapy	12	-	-	-	-	-	-
El Ansary et al.	2013	Egypt	2009–2010	Case control	6	BSC + DC	15	9 (60)	62	-	-	0/15	-
						BCS	15	11 (73)	60	-	-	0/14	-
Nakamoto et al.	2011	Japan	2004–2006	Case control	7	TAE + DC	13	9 (69)	68	-	3	-	0/4/9/
						TAE	22	13 (59)	70	-	3.2	-	3/8/11
Pan et al.	2010	China	2002–2008	Case control	7	TACE + RFA + CIK	42	37 (88)	53	7 (17)	-	-	-
						TACE + RFA	39	34 (87)	54	6 (15)	-	-	-
Hao et al.	2010	China	2005–2008	Case control	7	TACE + CIK	72	65 (90)	53	-	-	65/7	-
						TACE	74	64 (86)	51	-	-	66/8	-
Weng et al.	2008	China	2002–2004	RCT	-	TACE + RFA + CIK	45	31 (69)	-	-	-	36/9	-
						TACE + RFA	40	29 (73)	-	-	-	33/7	-
Nakamoto et al.	2007	Japan	NG	Case control	6	TACE + DC	10	9 (90)	67	9 (90)	3.7	7/3	0/5/4/
						TACE	11	6 (55)	69	9 (82)	2.7	4/7	1/5/3/
Tong et al.	2013	China	2008–2010	Case control	5	TACE + CIK	20	-	-	-	-	-	-
						TACE	18	-	-	-	-	-	-
Huang et al.	2013	China	1999–2012	Case control	6	TACE + RFA + CIK	85	77 (91)	50	44 (52)	-	76/9	-
						TACE + RFA	89	79 (89)	53	49 (55)	-	74/15	-

Author	Publish year	Country	Recruitment year	Study design	NCS	Treatment	Patient sample	Male (%)	Median age	Multiple number (%)	Median tumor size	Child-pugh A/B	TNM I/II/III/
Deng et al.	2013	China	2008–2009	RCT	-	TACE + RFA + CIK	20	19 (95)	-	13 (65)	-	18/2	-
						TACE + RFA	21	11 (52)	-	12 (57)	-	19/2	-
He et al.	2012	China	2008	RCT	-	TACE + CIK	60	56 (93)	56	-	-	54/6	-
						TACE	58	50 (86)	52	-	-	49/9	-
Zhao et al.	2006	China	2002–2005	Case control	7	TACE + RFA + CIK	33	30 (91)	-	-	-	-	-
						TACE + RFA	31	29 (94)	-	-	-	-	-
Zhang et al.	2006	China	1997–2005	Case control	6	TACE + CIK	16	-	-	-	-	-	-
						TACE	30	-	-	-	-	-	-
Hao et al.	2006	China	2003–2005	Case control	7	TACE + CIK	21	17 (81)	51	-	-	15/4	-
						TACE	46	45 (98)	50	-	-	34/9	-

Abbreviation:
NG not given; RCT randomize control trial; BCS best care support; TACE transarterial chemoembolization; RFA radiofrequency ablation; DC dendritic cells; CIK induced killer cells; NK natural killer cells; IRE irreversible electroporation.

Three studies using DC perfusion as immunotherapy, of which two studies combined with TACE, and 1 study combined with best care support (BCS). Twelve studies using CIK immunotherapy, of which one study compared with chemotherapy, one study combined BCS, five studies combined TACE and rest five studies combine both TACE and RFA. Two studies combined CIK and DC as immunotherapy, of which one combined with sorafenib, and another combined with cryotherapy.

The Treatment Response Of Cellular Immunotherapy

The treatment response was listed in Table 2. Generally, the median CR, PR, SD, and PD rates were 14%, 38%, 25%, and 21%. In combining cellular immunotherapy group, the median CR, PR, SD and PD were 19%, 42%, 29%, and 11%, respectively, compared to 9%, 34%, 22%, and 31%. The comparison between the two groups was calculated in RR, which was shown in Fig. 2. The cellular immunotherapy group was superior in CR (RR = 0.55, 95%CI = 0.38–0.81, P = 0.003). No significant difference was found in PR and SD (RR = 1.06 and 0.78, 95%CI = 0.81–1.39 and 0.53–1.15, P > 0.05, respectively). The occurrence of PD was more common in the non-cellular immunotherapy group than the cellular immunotherapy group (RR = 2.20, 95%CI = 1.34–3.63, P = 0.002).

Table 2
The treatment response and survival outcome data of included studies

Author	Publish year	Treatment	CR (%)	PR (%)	SD (%)	PD (%)	Median OS time	OS 0.5/1/2 year	Median PFS time	PFS 0.5/1/2 year
Zhou et al.	2019	Sorafenib + DC + CIK	4 (11)	14 (40)	13 (37)	4 (11)	18.6	94/78/-	-	-
		Sorafenib	1 (3)	5 (14)	9 (25)	11 (31)	13.8	92/47/-	-	-
Yang et al.	2019	IRE + NK	3 (17)	13 (72)	2 (11)	0 (0)	23.2	100/78/-	-	100/78/-
		IRE	1 (5)	14 (64)	6 (27)	1 (5)	17.9	100/67/-	-	95/43/-
Lin et al.	2017	cryoablation + NK	9 (26)	12 (34)	9 (26)	5 (14)	-	-	9.1	95/-/-
		cryoablation	5 (19)	7 (27)	6 (23)	8 (31)	-	-	7.6	95/-/-
Li et al.	2016	CIK	-	-	-	-	-	90/48/-	-	15/3/-
		chemotherapy	-	-	-	-	-	86/30/-	-	14/3/-
Yu et al.	2014	BCS + CIK	-	-	-	-	13.5	-/-/28	7.4	-/-/20
		BCS	-	-	-	-	5.3	-/-/4	4	-/-/4
Niu et al.	2013	Cryotherapy + DC + CIK	-	-	-	-	-	100/100/20	-	-
		Cryotherapy	-	-	-	-	-	100/95/10	-	-
El Ansary et al.	2013	BSC + DC	0 (0)	2 (13)	9 (60)	4 (27)	7	-	-	-
		BCS	0 (0)	0 (0)	2 (13)	13 (87)	4	-	-	-
Nakamoto et al.	2011	TAE + DC	-	-	-	-	-	-	-	100/81/-
		TAE	-	-	-	-	-	-	-	83/43/-
Pan et al.	2010	TACE + RFA + CIK	-	-	-	-	-	-	10.2	-
		TACE + RFA	-	-	-	-	-	-	6.8	-
Hao et al.	2010	TACE + CIK	-	-	-	-	-	81/73/40	-	62/40/20
		TACE	-	-	-	-	-	54/32/20	-	33/8/2
Weng et al.	2008	TACE + RFA + CIK	-	-	-	-	-	-	-	93/91/-
		TACE + RFA	-	-	-	-	-	-	-	88/73/-
Nakamoto et al.	2007	TACE + DC	-	-	-	-	22.1	100/78/54	9.6	78/60/-
		TACE	-	-	-	-	17.8	80/53/30	7	53/40/-
Tong et al.	2013	TACE + CIK	-	-	-	-	34	89/70/-	12	70/39/-
		TACE	-	-	-	-	13	72/42/-	7	35/12/-
Huang et al.	2013	TACE + RFA + CIK	45 (53)	20 (24)	16 (19)	4 (5)	56	95/83/63	-	82/55/29
		TACE + RFA	22 (25)	49 (55)	8 (9)	10 (11)	31	95/76/40	-	75/35/7
Deng et al.	2013	TACE + RFA + CIK	-	-	-	-	-	85/70/15	-	/50/-
		TACE + RFA	-	-	-	-	-	70/29/5	-	/14/-
He et al.	2012	TACE + CIK	-	-	-	-	10.7	-/86/46	-	-
		TACE	-	-	-	-	4.7	-/52/12	-	-
Zhao et al.	2006	TACE + RFA + CIK	-	-	-	-	-	-	-	90/87/-
		TACE + RFA	-	-	-	-	-	-	-	87/68/-

Author	Publish year	Treatment	CR (%)	PR (%)	SD (%)	PD (%)	Median OS time	OS 0.5/1/2 year	Median PFS time	PFS 0.5/1/2 year
Zhang et al.	2006	TACE + CIK	1 (6)	11 (69)	3 (19)	1 (6)	-	87/50/-	-	-
		TACE	0 (0)	13 (43)	11 (37)	6 (20)	-	60/23/-	-	-
Hao et al.	2006	TACE + CIK	-	-	-	-	22	86/58/-	-	-
		TACE	-	-	-	-	10	69/33/-	-	-

Abbreviation:
BCS best care support; TACE transarterial chemoembolization; RFA radiofrequency ablation; DC dendritic cells; CIK cytokine-induced killer cells; NK natural killer cells; IRE irreversible electroporation; CR complete response; PR partial response; SD stable disease; PD progression disease; OS overall survival; PFS progression-free survival

The Assessment Of Survival Outcome In Cellular Immunotherapy

The survival rate was listed in Table 2. The median OS time was 23 months (range from 7 to 56 months), with median 0.5-, 1-. 3-year OS rate 91.5%, 73.0%, and 38% in cellular immunotherapy group compared to 13 months (range from 4 to 31 months), with median 0.5-, 1-. 3-year OS rate 79.8%, 48.0%, and 17.2% in non-cellular immunotherapy group. Moreover, the median PFS was 6 months (range from 4 to 7 months), with a median 0.5-, 1-. 3-year PFS rate 67%, 33% and 4% in non-cellular immunotherapy group compared to 9 months (range from 7 to 12 months), with median 0.5-, 1-. 3-year PFS rate 78%, 58%, and 23% in cellular immunotherapy group.

The comparison of HRs in OS was shown in Fig. 3. Generally, patients treating with cellular immunotherapy had a better OS compared to those without cellular immunotherapy (HR = 0.52, 95%CI = 0.43–0.63, I^2 = 0%, P < 0.001). In the subgroup analysis, the only CIK infusion therapy and combined DC with CIK perfusion therapy patients had a better OS (HR = 0.52 and 0.49, 95%CI = 0.42–0.66 and 0.32–0.76, I^2 = 0%, P < 0.001 and P = 0.002, respectively). Only one study only used DC perfusion, and no significant difference was found in OS (HR = 0.56, 95%CI = 0.16–1.98, P = 0.368).

The comparison of HRs in PFS was shown in Fig. 4. Similarly, patients treating with cellular immunotherapy had a better PFS compared to those without cellular immunotherapy (HR = 0.63, 95%CI = 0.52–0.78, I^2 = 0%, P < 0.001). The subgroup analysis showed that patients treating with CIK infusion might have a better PFS than patients without CIK therapy (HR = 0.63, 95%CI = 0.51–0.78, I^2 = 0%, P < 0.001). While no significance was found in patients treating with DC therapy (HR = 0.63, 95%CI = 0.21–1.93, I^2 = 0%, P = 0.420).

Discussion

This is the largest-scale systematic review to evaluate the efficacy of cellular immunotherapy in treating advanced HCC patients. And also, it is the first study using the “time to event” method to combine the survival outcome in this area. Our meta-analysis suggested that CIK infusion immunotherapy combined with minimally invasive treatment or BCS could remarkably increase the lifespan in advanced HCC patients while there was still no evidence suggested that using DC or NK infusion immunotherapy could prolong the outcome of advanced HCC patients.

CIK cells are a type of T lymphocytes that are mainly distributed in the liver in humans and account for 25% of lymphocytes in the liver (38). The CIK cells currently used in the clinical treatment are mainly derived from the patient's own peripheral blood, and few patients are collected from the bone marrow. After the CIK cells are collected, the cell need to be cultured and amplified. The immunophenotypes of CD3, CD56, CD4, and CD8 of CIK cells are not different from the CIK cells in normal human blood during the culture process (38). When CIK cells are stimulated by exogenous anti-CD3 monoclonal antibodies or cancer cells, the MHC molecules on the surface of the CIK cells are tightly bound to the stimulus. After the two are combined, the CIK cells are activated, and the activated CIK cells will secrete a cytotoxic particle to the outside. This kind of particles can selectively recognize HCC cells so that the cell membranes are lysed and then to kill cancer cells (39). On the other hand, when CIK cells are activated by monoclonal antibodies and tumor cell surface antigens, perforin, and granzyme are produced, which can also play a role similar to cytotoxic granules (40). In addition, tumor cells can indirectly activate lymphocytes. A related functional antigen (LFA-1) on the surface of lymphocytes binds to the CD3 receptor of CIK cells, causing the release of cytotoxic particles and exerting tumor-killing effects. Several experimental studies have shown that during the in vitro culture of CIK, a large number of Th1 cytokines are secreted, such as interleukin-2, interleukin-12, and interferon -γ (IFN-γ)(39, 41). These cytokines can affect the ability of CIK cells to attack tumor cells by regulating the release of other cytokines and plasma levels, also increase the sensitivity of CIK cells to tumor antigen (41). In addition, CIK cells can also secrete a tumor cell regulatory factor, which can activate the apoptosis genes of tumor cells after entering the tumor cells (42). In addition to the above therapeutic effects, CIK cells can also regulate T lymphocyte activity, activate the immune system, and achieve the effect of T cells killing liver cancer cells (39).

The effectiveness of CIK cellular immunotherapy relies on the quality and quantity of CIK cells obtained for therapeutic administration. Besides, the infusion pathway and the circle of infusions were also the main factors associated with therapeutic efficacy. CIK cell reinfusion should be selected when the proportion of CD3 + CD56 + T cells is the highest. Studies have shown that the optimal time for the number and state of cells is on the 14th day of culture (43). CIK cells have different infusion pathways, and different infusion pathways will affect the treatment effect (44). Animal experiments have shown that the distribution of CIK cells in various organs has a certain relationship with different infusion routes (44, 45). The main routes of CIK infusion in HCC patients are peripheral vein and transarterial. The peripheral venous approach is widely used in clinical practice due to its simplicity and ease of operation. CIK cells are widely distributed to most organs and tissues after infusion via the peripheral vein route. The CIK cells first entered the lungs after intravenous infusion and reached a

peak in 2–6 hours, and then the lung concentration gradually decreased, and then a secondary distribution appeared, and the distribution of the liver, spleen, and kidney reached a peak. Studies have shown that CIK cells have a better transfusion effect via the arterial route because it may be related to the number of CDC cells remaining in the tumor (17).

In our meta-analysis, we summarized several studies combining TACE and RFA in treating advanced HCC patients, it could increase the complete response rate, and thereafter prolong the progression time and survival period. Although TACE and RFA can reduce tumor burden, some studies suggested that the tumor necrosis can cause tumor antigen exposure during minimally invasive treatment (32). Based on this combined application of CIK cellular immunotherapy, CIK cells can be reintroduced into the body after several times expansion in vitro, which can increase the ratio of CD3+/CD4 + T cells and CD4+/CD8 + T cells. With the decreasing of the number of CD8 + T cells, the immune function in vivo is improved, and the immunosuppressive status in HCC patients is reversed. As result, the combination of the three treatments could improve the quality of life of patients, improve the immune function of patients, and extend the progression-free survival and overall survival. Therefore, we believe that TACE combined with RFA and CIK cells provides a new treatment for advanced HCCs.

DCs are the most powerful professional antigen-presenting cells (APC) in the human body. They are the most important roles for initial initiation, regulation, and maintenance of the body's immune response, and have the function of stimulating T lymphocyte proliferation, uptake, processing, and antigen presentation. Its powerful antigen presentation ability can reach hundreds or even a thousand times of macrophages (46). Theoretically, the co-culture of CIK cells and DCs can not only improve the killing effect of cells but also significantly improve the function of DC. Martin et al. co-cultured CIK and DC and found that DCs secreted IL-12 significantly increased, and significantly increased the cytotoxic activity of the cells against tumor cells (47). In addition, the expression of HLA-I and HLA-II molecules and CD40, CD50, and CD56 molecules on the surface of DCs increased, and the proliferation of CIK cells and the expression of CD3, CD4, CD28, and CD49 molecules on the surface increased (48). Several adopted DC alone or combine CIK immunotherapy in treating advanced HCCs (14, 30, 32, 33, 36). In the subgroup analysis, combination of DC and CIK could still improve the overall survival rate comparing to without combination application of DC and CIK. However, current evidence could not suggest that the application of DCs alone could provide benefit in advanced HCC patients.

There were some limitations in our study. Firstly, the studies were mainly from Asian countries, and thus the results need to be evaluated in Western countries. Secondly, even though we had systematically reviewed all the published studies in cellular immunotherapy, the sample of patients was still scarce. Thirdly, due to the different cell dose and circle existed in different studies, the bias from the covariates could not be ignored. However, the combined HRs were around 0.5, which means even considering the impact of covariates in short and long-term outcomes, the benefit of combining cellular immunotherapy would not easily change. More RCTs and large sample clinical trials need to be undertaken for the application of cellular immunotherapy in advanced HCC.

Conclusion

Our results suggested that combining use of cellular immunotherapy in advanced HCC could increase the complete response rate, and thereafter extend the progression-free and overall survival rate. Subgroup analysis suggested that combining use of CIK and DC or using CIK alone could provide the benefit in survival outcome. Further studies need to be undertaken to evaluate the efficacy of DCs application in advanced HCC.

Abbreviations

hepatocellular carcinoma (HCC)

transarterial chemoembolization (TACE)

radiofrequency ablation (RFA)

cytokine-induced killer cells (CIK)

dendritic cells (DC)

preferred reporting items for systematic review and meta-analysis (PRISMA)

randomized control trials (RCT)

natural killer cells (NK)

American Joint Committee on Cancer (AJCC)

Barcelona Clinic Liver Cancer (BCLC)

overall response rate (ORR)

disease control rate (DCR)

complete response (CR)

partial response (PR)

stable disease (SD)

progression disease (PD)

progression-free survival (PFS)

overall survival (OS)

Response Evaluation Criteria in Solid Tumors (RECIST)

Newcastle-Ottawa Quality Assessment Scale (NCS)

relative risk (RR)

hazard ratio (HR)

confidence intervals (CI)

Declarations

Conflict of interest: none

Author's contribution:

Jing, Wang and Ling Xin, Feng contribute equally in this study.

Design of the meta-analysis: Jing, Wang and Ling Xin, Feng

Literature screening: Jing, Wang and Ling Xin, Feng

Quality assessment: Jing, Wang and Ling Xin, Feng

Statistics analysis: Lin Wei, Zhang

Write and revise: Jing, Wang, Ling Xin, Feng, and Lin Wei, Zhang

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Figures

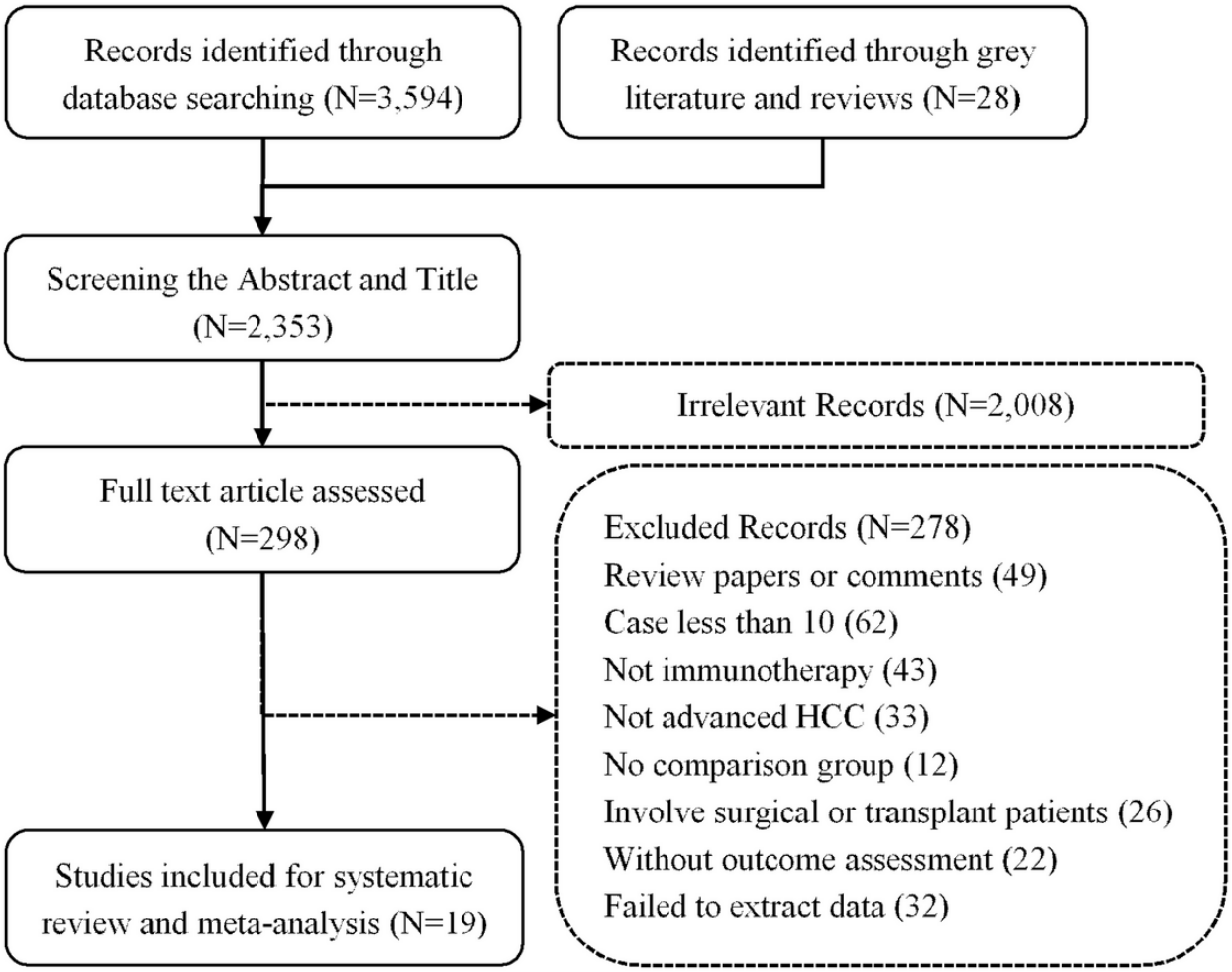

Figure 1

The flowchart of literature screening of the meta-analysis

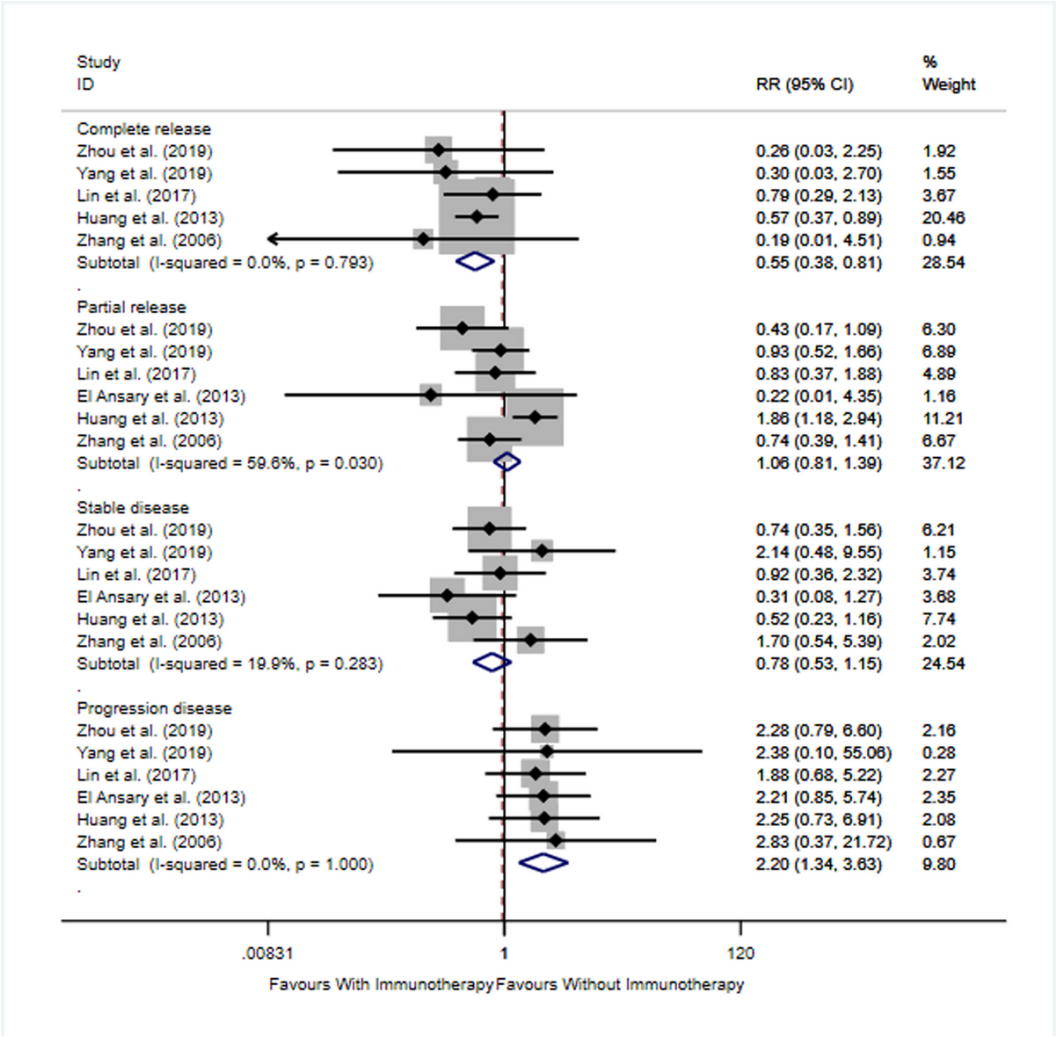

Figure 2

The comparison of complete response, partial response, stable disease and progression disease between the cellular immunotherapy group and non-cellular immunotherapy group

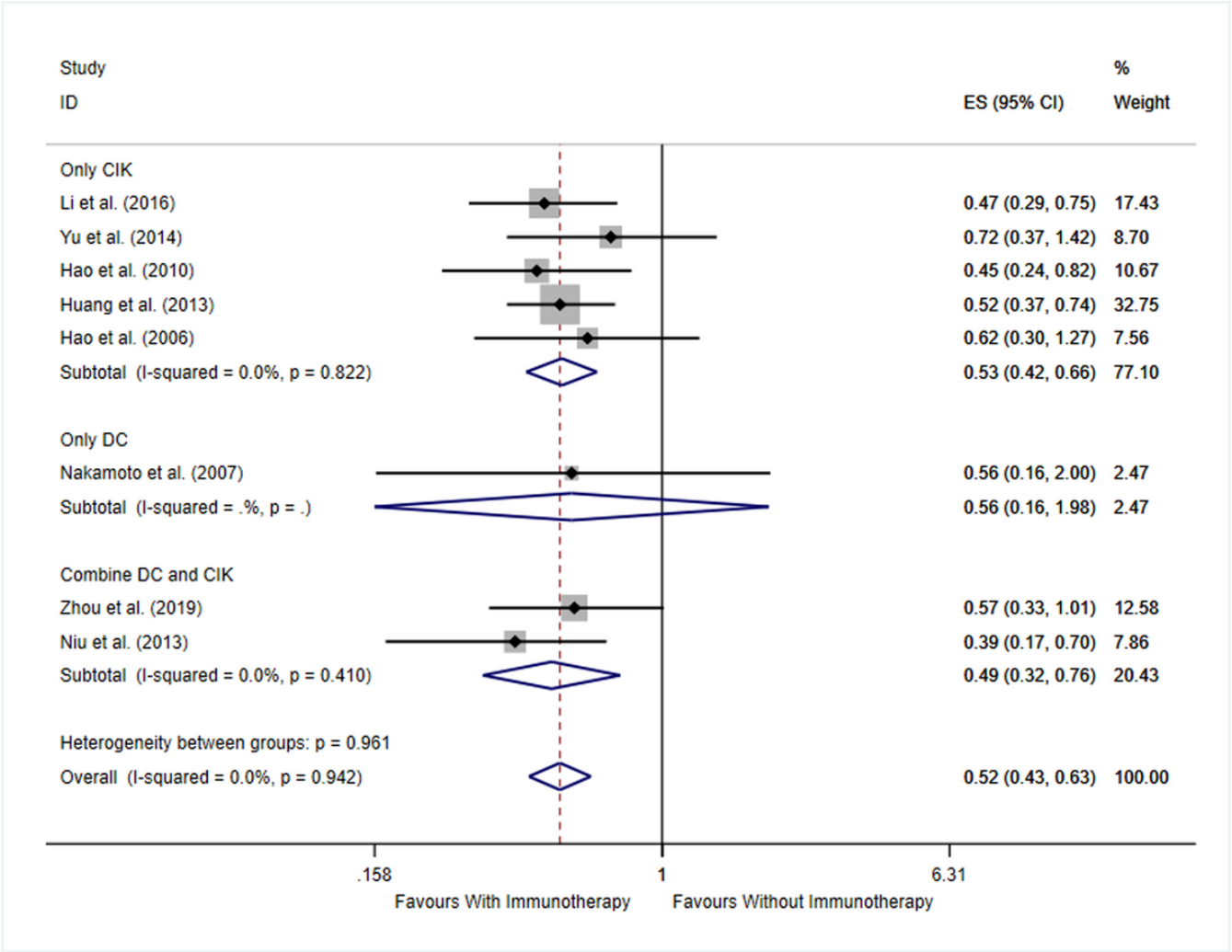

Figure 3

The comparison of HRs in overall survival between the cellular immunotherapy group and non-cellular immunotherapy group.

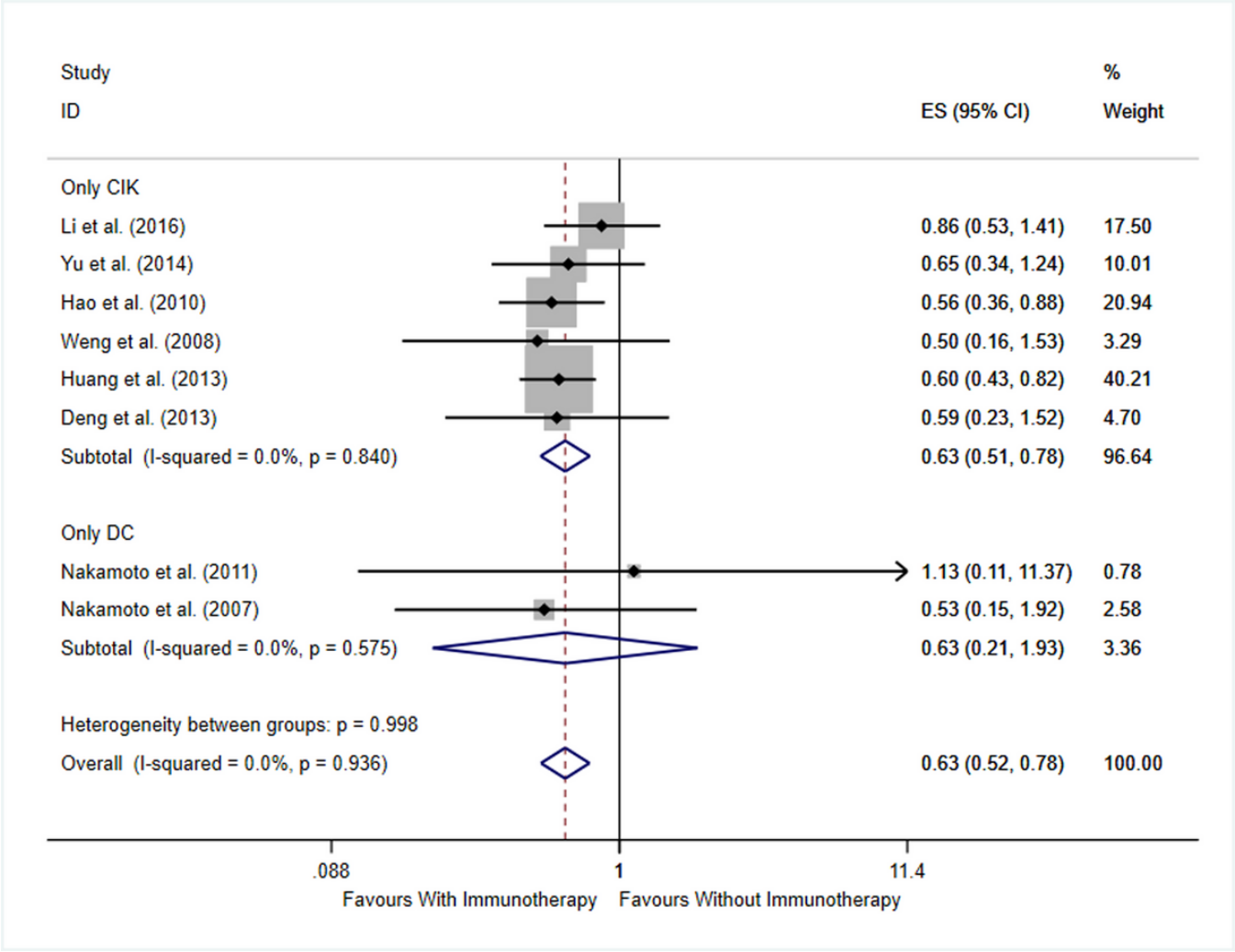

Figure 4

The comparison of HRs in progression-free survival between the cellular immunotherapy group and non-cellular immunotherapy group.