Is Adjuvant Cellular Immunotherapy Essential after TACE-Predominant Minimally-Invasive Treatment for Hepatocellular Carcinoma? A Systematic Meta-Analysis of Studies Including 1774 Patients

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Abstract

Purpose

Cellular immunotherapy has appeared to be a promising modality for the treatment of malignant tumor. The objective of this study was to evaluate the efficacy of cellular immunotherapy combined with minimally invasive therapy.

Methods

We searched PubMed, Web of Science and The Cochrane Library through March 2016 for relevant studies. Short-term efficacy (the disease control rate, the control rate of quality life and the AFP descent rate) and long-term efficacy (overall survival (OS) and progression-free survival (PFS) rate) were compared as the major outcome measures. The meta-analysis was performed using Review Manager 5.3.

Results

A total of 1174 references in 3 databases were found of which 19 individual studies with 1774 HCC patients enrolled in this meta-analysis. Meta-analysis results showed that cellular immunotherapy combined with minimally-invasive treatment significantly improved the measures of short-term response (the disease control rate (OR = 5.91, P = 0.007), the control rate of quality lift (OR = 3.38, P = 0.003) and the AFP descent rate (OR = 4.48, P = 0.02)). Also higher 6-month PFS (OR = 2.78, P = 0.05), ≥12-month PFS (OR = 3.56, P<0.00001) rate and 6-month OS (OR = 2.81, P = 0.0009), 12-month OS (OR = 3.05, P<0.00001) and 24-month OS (OR = 3.52, P<0.0001) rate were observed in patients undergoing cellular immunotherapy.

Conclusions

This meta-analysis suggested that cellular immunotherapy is a feasible adjuvant treatment that could be beneficial for the improvement of the clinical outcomes for hepatocellular carcinoma (HCC) patients after minimally invasive treatment, including short-term response and long-term survival.

Introduction

Hepatocellular carcinoma (HCC) is the most common type of hepatobiliary cancer, the fifth common malignant cancer and the third most cause of cancer-related deaths worldwide [1]. The growth of annual incidence and mortality of HCC is higher in Asia countries accounting for almost 80%, especially in China [2]. Currently, the recurrence rate of HCC was still high after conventional radical resection therapies. Moreover, not all patients may benefit from hepatectomy because of the high incidence of complications due to chronic liver disease or with intermediate-stage HCC [3,4]. Minimally-invasive treatment has been widely used for patients with unresectable HCC. Transcatheter arterial chemoembolization (TACE) has been found to be an effective method to reduce HCC tumor size and improve overall survival (OS) [5,6]. Also, some studies showed that radiofrequency ablation (RFA) and microwave ablation (MWA) could increase tumor necrosis and prolong survival time for HCC [7,8]. However, there were still some limitations existed in long-term prognosis. The recurrence and metastasis after treatment with the TACE-predominant minimally-invasive treatment were still frequent [9]. The prognosis of HCC remained dismal with a low level of survival (5-year survival rate less than 5%) in patients with advanced HCC at diagnosis [10].

In recent decades, cellular immunotherapy has emerged as a promising strategy for cancer treatment [11,12]. It has been reported that cellular immunotherapy could strengthen the immune state and afford a potential value in enhancing the therapeutic outcome [13], although it has not been considered as a standard therapy for HCC. Several studies and meta-analysis had revealed that the combination of cellular immunotherapy of conventional therapies was more effective [14,15]. However, these analysis were about the combination of specific cellular immunotherapy and specific interventional therapy. There were no studies systematically analyzed whether cellular immunotherapy either CIK or DC-CIK was needed after different interventional therapy. The efficacy of cellular immunotherapy still remains controversial, especially in prolonging progression-free and overall survival [16,17].

Therefore, we summarized the trials of cellular immunotherapy, including CIK and DC-CIK, combined with TACE-predominant minimally-invasive treatment for HCC to systematically evaluate the efficacy of cellular immunotherapy.

Materials and Methods

Search strategy for identification of studies

A comprehensive literature search process was conducted in Pubmed, Web of Science and The Cochrane Library, based on combinations of the following keywords: [hepatocellular carcinoma OR liver neoplasms OR liver cancer] AND [(lymphokine-activated killer OR LAK) OR (cytokine induced killer cell OR CIK) OR (tumor infiltrating lymphocyte OR TIL) OR (cytotoxic T lymphocyte OR CTL) OR (dendritic cell OR DC) OR (natural killer cell OR NK)].

We searched these keywords in Title/Abstract of literatures about human between January 2006 and March 2016. Studies were not limited to language.

Inclusion and exclusion criteria for studies in this meta-analysis

In this systematic analysis, literatures were included that met the following criteria: (i) reported clinical outcomes of cellular immunotherapy for HCC; (ii) the case-control study design; (iii) provided enough information to calculate the odds ratio for short term efficacy, PFS or OS.

The exclusion criteria were as follows: (i) reviews, case reports, in vitro experiments, animal models, other diseases or other treatments; (ii) studies without appropriate control groups or enrolled patients less than 15.

Data extraction and quality assessment

All candidate literatures were reviewed and retrieved by two independent authors (Min Ding and Ying Wang) from these 3 databases, discussed and arrived at a consensus with a third author if disagreement occurred, and extracted data. Articles that could not be categorized according to title or abstract alone were retrieved for full-text review.

The following information was extracted from each included article: authors, year of publication, tumor characteristics, case numbers, regimens, immune cell regimens and culture of immune cells. Two independent authors (Min Ding and Ying Wang) evaluated each study for risk of bias and applicability by using The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool [18]. The QUADAS-2 contained four key domains covering patient selection, index test, reference standard, and flow and timing. The "low risk of bias", "unclear risk of bias" or "high risk of bias" for each article was attributed in Cochrane reviews of interventions. Any disagreement between two authors regarding quality assessment was resolved through discussion with the third author.

Statistical methods

The analysis was carried out using pair-wise comparison between immunotherapy arms and control arms. Review Manager 5.3 [6] was used to conduct meta-analysis and calculated odds ratio (OR) for the disease control rate, the control rate of quality life and the AFP descent rate to reflect short-term efficacy, and 6-, 12-, 24-month PFS and OS to reflect long-term treatment effects across studies. The number of events evaluated in each arm was utilized to calculate the pooled OR with 95% confidence intervals (CI) combined the Mantel-Haenszel statistical method with random effects. The Cochran's Q test (chi-square statistic; x^2) was applied to evaluate the heterogeneity among studies. We considered inconsistency index (I^2) [19] with 25%, 50%, and 75% as representing the evidence of low, moderate, and high heterogeneity, respectively. Publication bias was assessed with Peters test using Meta R package. A p-value of no greater than 0.05 is considered to be statistically significant.

Results

Study characteristics

A search strategy in databases of PubMed, Web of science and The Cochrane Library was applied to find a total of 1174 citations, of which 230 were discarded as they were duplicates. After title and abstract screening, 883 articles were excluded for the following reasons: 68 were reviews, 219 were in vitro experiments, 250 used animal models, 21 were a case report, 200 studied other diseases and 125 used other treatments. Additional 18 literatures were supplemented from meta-analysis studies or reviews. In the third step of the eligibility and detailed
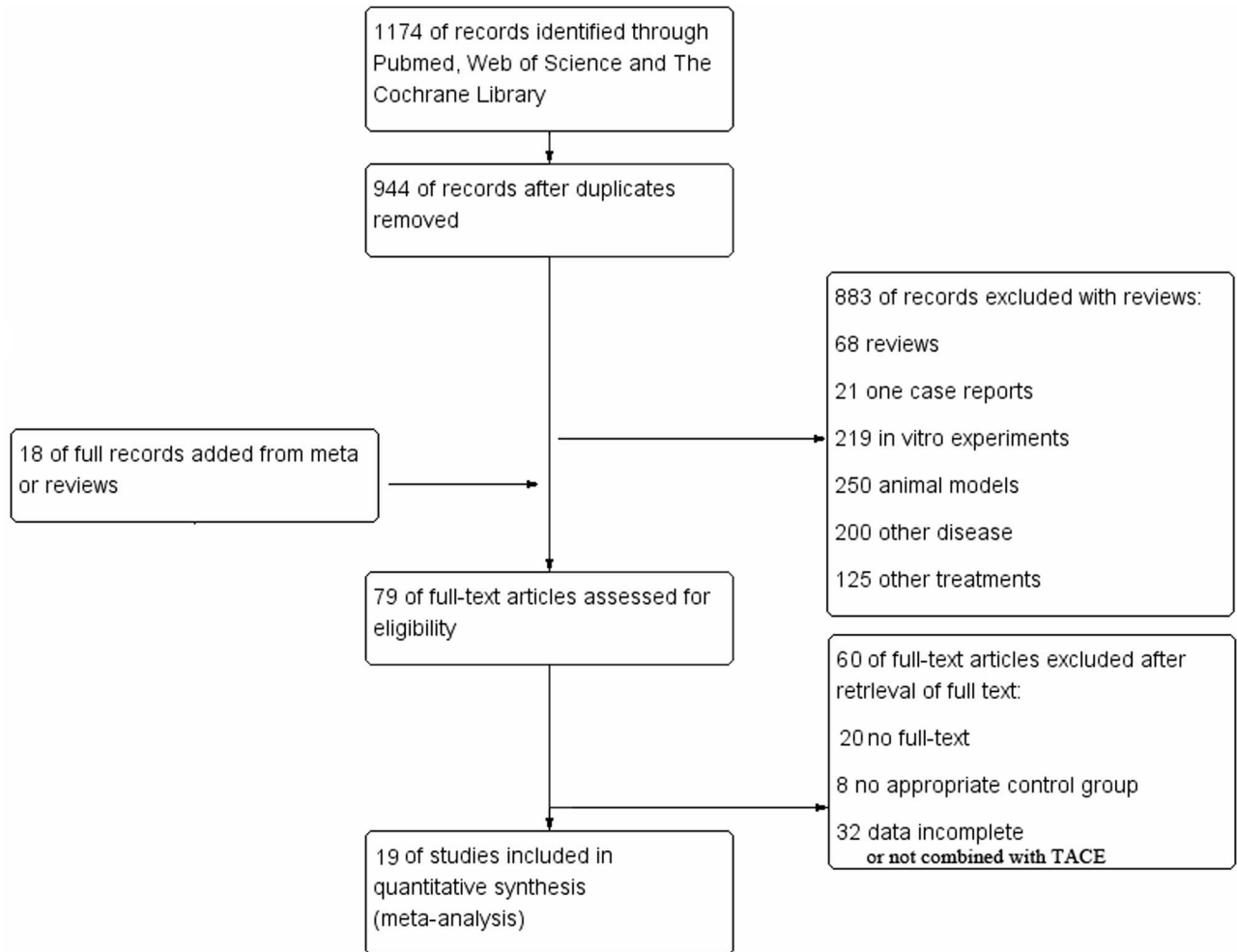
Fig 1. Flow chart of the included studies.

assessment, 79 full-texts were retrieved, and 60 articles without a control group, full-text, complete clinical data or not combined with TACE-predominant minimally-invasive treatment were excluded (Fig 1). Finally, we included 19 studies with 1774 HCC patients in quantitative synthesis for meta-analysis.

The characteristics of the populations presented in these reports were summarized in Table 1. Overall, 13 studies addressed passive immunotherapeutic approaches including cytokine-induced killer cells (CIKs) [20–32]. Other six studies administrated DC-CIK cells [33–38]. Tumor characteristics and regimens were reported in all studies.

Quality assessment

The 19 included papers comprised 11 randomized controlled studies [20–22,26,28,29,32,34,35, 37,38], 1 studies without describing the randomization method [25] and 7 retrospective studies with a matched-pair control group [23,24,27,30,31,33,36]. It contained eleven (57.89%) with all low risk of bias, one (5.26%) with unclear risk and seven (36.84%) with high risk of bias in random sequence generation, allocation concealment, blinding of participants and personnel and blinding of outcome assessment items, as was shown in S1 Fig.

Table 1. Clinical information of the trials for the immunotherapy.

Reference	Tumor characteristics	Patients No (control)	Regimens (per arm)	Immune cell regimens	Culture of immune cell
Zhao 2006	HCC	33(31)	TACE+RFA+CIK, TACE+RFA	$1.1–1.5×10^{10}$/course	CM,CD3MeAb,IL-2,INF-r,IL-1a.
Hao 2006	HCC	21(46)	TACE+CIK,TACE	$1–5×10^{10}$/course	CM, IFN-r, CD3McAb, IL-1a, IL-2.
Zhang 2006	HCC	52(92)	TACE+CIK/TACE +PEI+CIK,TACE/ TACE+PEI	$1.0–1.2×10^{10}$/course	CM, IFN-r, CD3McAb, IL-1a, IL-2.
Shi 2007	HCC	38(214)	TACE+CIK,TACE/ TACE+PEI	$1.0–1.2×10^{10}$/course	CM, IFN-r, CD3McAb, IL-1a, IL-2.
Huang 2007	Primary HCC	55(30)	TACE+RFA+CIK, TACE+RFA		
Weng 2008	HCC	45(40)	TACE+RFA+CIK, TACE+RFA	$1.0–2.0×10^{10}$/course	CM,IFN-r,CD3 McAb,IL-1a,IL-2.
Hao 2010	HCC	74(72)	TACE+CIK,TACE	$1–5×10^{10}$/course	Serum-free culture medium,IFN-r, CD3 McAb,IL-1a,IL-2.
Pan 2010	HCC	42(39)	TACE+RFA+CIK, TACE+RFA	Once every week, at least 4 infusions, more than $1×10^{10}$ cells per course.	
Wu 2012	Primary HCC	32(38)	TACE+DC-CIK,TACE		
Wang 2012	HCC	38(38)	TACE+RFA+CIK, TACE+RFA	Once every twice weeks, at least 3 infusions,$1–1.5×10^{10}$ cells per course.	IFN-r,CD3 McAb, IL-1a,IL-2.
He 2012	Primary liver cancer	60(58)	TACE+CIK,TACE	1 week after TACE	
Huang 2013	HCC	85(89)	TACE+RFA+CIK, TACE+RFA	2 weeks after sequential TACE and RFA, the median successive number of CIK cell infusions was 9 (range, 4–25).	IFN-r, CD3-McAb, IL-2, IL-1a.
Deng 2013	HCC	20(21)	TACE+RFA+CIK, TACE+RFA		
Tong 2013	Primary liver cancer	20(18)	TACE+ CIK,TACE	$1–5×10^{10}$/course	IFN-r, CD3-McAb, IL-2
Xu 2013	Large HCC	40(40)	TACE+PMCT +DC-CIK,TACE +PMCT		
Guo 2014	Primary liver cancer	30(38)	TACE+DC-CIK,TACE	$1.0×10^{10}$/course	AIM-V serum-free culture medium, IFN-r,rhIL-2,CD3 McAb,IL-1, GM-CSF,rhIL-4,TNF-a.
Zhang 2014	liver cancer	41(44)	TACE+RFA+DC-CIK, TACE+RFA	6 times 7 days after TACE and RFA, and the number of DC-CIK cells was above $1.0×10^{10}$	
Liu 2014	Advanced HCC	23(17)	TACE+DC-CIK,TACE		
Cheng 2014	liver cancer	32(28)	TACE+DC-CIK,TACE	4 infusions of DC-CIK cells	IFN-r, CD3-McAb, IL-2, IL-1a, GM-CSF, IL-4, HSP.

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Short-term efficacy

The disease control rate, the control rate of quality life and the AFP descent rate were used to evaluate the short-term efficacy of the cellular immunotherapy. For the disease control rate, the meta-analysis for three CIK studies and one DC-CIK studies showed a significant advantage of the combination therapy (OR = 5.91, 95% CI: 1.62–21.51, P = 0.007). And the heterogeneity was not statistically significant with P value equal to 0.07 (Fig 2A). Four studies, including two CIK studies and two DC-CIK studies were used to evaluate the control rate of quality life in the meta-analysis. The control rate of quality life was significantly higher in patients with cellular immunotherapy compared to without (OR = 3.38, 95% CI: 1.51–7.58, P = 0.003). Also, there was no heterogeneity among the studies (I^2 = 0%, P = 0.97) (Fig 2B).
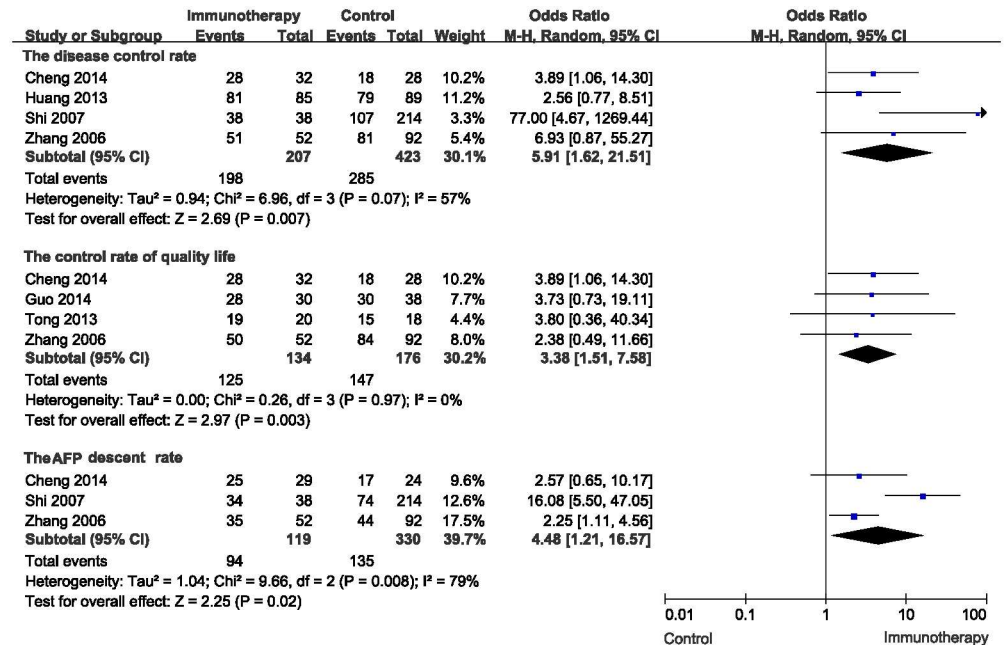
Fig 2. Comparison of short-term efficacy between the patients undergoing cellular immunotherapy or not by using the random effects model (Mantel-Haenszel method).

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For the AFP descent rate, the meta-analysis of three studies (two CIK studies and one DC-CIK studies) showed the combination therapy significantly descent the AFP level (OR = 4.48, 95% CI: 1.21–16.57, P = 0.02) (Fig 2B).

Long-term efficacy

To evaluate the long-term efficacy of the combination of cellular immunotherapy and TACE-predominant minimally-invasive treatment, we performed subgroup analysis to conduct meta-analysis according to 6-month, 12-month and 24-month progression free survival and overall survival.

Progression free survival. For total effect, progression free survival rate was significantly higher in HCC patients undergoing cellular immunotherapy, compared to patients in control groups (OR = 3.46; 95% CI: 2.37–5.06, P<0.00001). All the Cochran's Q-test resulted in a P value of 0.20, and the corresponding I^2 was 24%, indicating that the degree of variability was basically consistent in each result of study.

In subgroup meta-analysis of studies on 6-, > = 12-month progression free survival, patients in cellular immunotherapy treatment group had significantly higher PFS than those in control group, accordingly (6-month: OR = 2.78; 95% CI: 1.02–7.58, P = 0.05; > = 12-month: OR = 3.56; 95% CI: 2.27–5.59, P<0.00001) (Fig 3).

Overall survival. Overall survival rate, including 6-, 12-, and 24-month overall survival rate, was significantly higher in HCC patients undergoing cellular immunotherapy, compared to patients in control groups respectively. For 6-month OS, the meta-analysis for five CIK studies and four DC-CIK studies yielded statistically significant differences (OR = 2.81; 95% CI: 1.53–5.17, P = 0.0009). However, there was no statistically significant differences found in the subgroup of DC-CIK studies (OR = 1.61; 95% CI: 0.64–4.09, P = 0.31). For 12-month OS, the meta analysis for ten CIK studies and five DC-CIK studies showed a higher 12-month rate in the patients with the combination therapy (OR = 3.05; 95% CI: 2.38–3.92, P<0.00001). For

Study or Subgroup	Immunotherapy		Control		Weight	Odds Ratio M-H, Random, 95% CI
	Events	Total	Events	Total		
6-month PFS						
Zhao 2006	31	33	29	31	3.2%	1.07 [0.14, 8.09]
Weng 2008	42	45	36	40	5.1%	1.56 [0.33, 7.42]
Hao 2010	52	72	25	74	16.1%	5.10 [2.52, 10.32]
Subtotal (95% CI)		**150**		**145**	**24.4%**	**2.78 [1.02, 7.58]**
Total events	125		90			
Heterogeneity: Tau² = 0.35; Chi² = 3.42, df = 2 (P = 0.18); I² = 42%						
Test for overall effect: Z = 2.00 (P = 0.05)						
12-month PFS						
Zhao 2006	29	33	23	31	6.7%	2.52 [0.67, 9.43]
Huang 2007	52	55	26	30	5.0%	2.67 [0.56, 12.81]
Weng 2008	41	45	28	40	7.5%	4.39 [1.28, 15.02]
Pan 2010	39	42	30	39	6.2%	3.90 [0.97, 15.67]
Hao 2010	29	72	6	74	10.9%	7.64 [2.93, 19.93]
Wang 2012	30	38	27	38	9.6%	1.53 [0.54, 4.36]
Huang 2013	46	85	31	89	18.7%	2.21 [1.20, 4.06]
Deng 2013	10	20	3	21	5.4%	6.00 [1.33, 27.00]
24-month PFS						
Hao 2010	19	72	2	74	5.4%	12.91 [2.88, 57.81]
Subtotal (95% CI)		**462**		**436**	**75.6%**	**3.56 [2.27, 5.59]**
Total events	295		176			
Heterogeneity: Tau² = 0.13; Chi² = 11.10, df = 8 (P = 0.20); I² = 28%						
Test for overall effect: Z = 5.53 (P < 0.00001)						
Total (95% CI)		**612**		**581**	**100.0%**	**3.46 [2.37, 5.06]**
Total events	420		266			
Heterogeneity: Tau² = 0.10; Chi² = 14.56, df = 11 (P = 0.20); I² = 24%						
Test for overall effect: Z = 6.41 (P < 0.00001)						
Test for subgroup differences: Chi² = 0.20, df = 1 (P = 0.66), I² = 0%						

Fig 3. Comparison of 6, ≥12-month PFS between the patients who received cellular immunotherapy or not by using the random effects model (Mantel-Haenszel method).

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24-month OS, the OR of five CIK studies and three DC-CIK studies was significant (OR = 3.52; 95% CI: 1.90–6.50, P<0.0001), suggesting a higher 24-month OS in HCC patients undergoing cellular immunotherapy. Also, in CIK subgroup and DC-CIK subgroup, the 12-month and 24-month OS were both significantly higher in patients with the combination therapy (P<0.05) (Fig 4).

Assessment of publication bias

In order to guarantee the validity of the conclusions from the meta-analysis, funnel plots and sensitivity analysis were used in subtypes of the short-term efficacy, PFS, and OS (including 6-month, 12-month and 24-month OS). The relative symmetry of all the funnel plots shape suggested the publication bias was not evident. Also, publication bias was also not suggested by Peters test (P > 0.05) (Fig 5).

Discussion

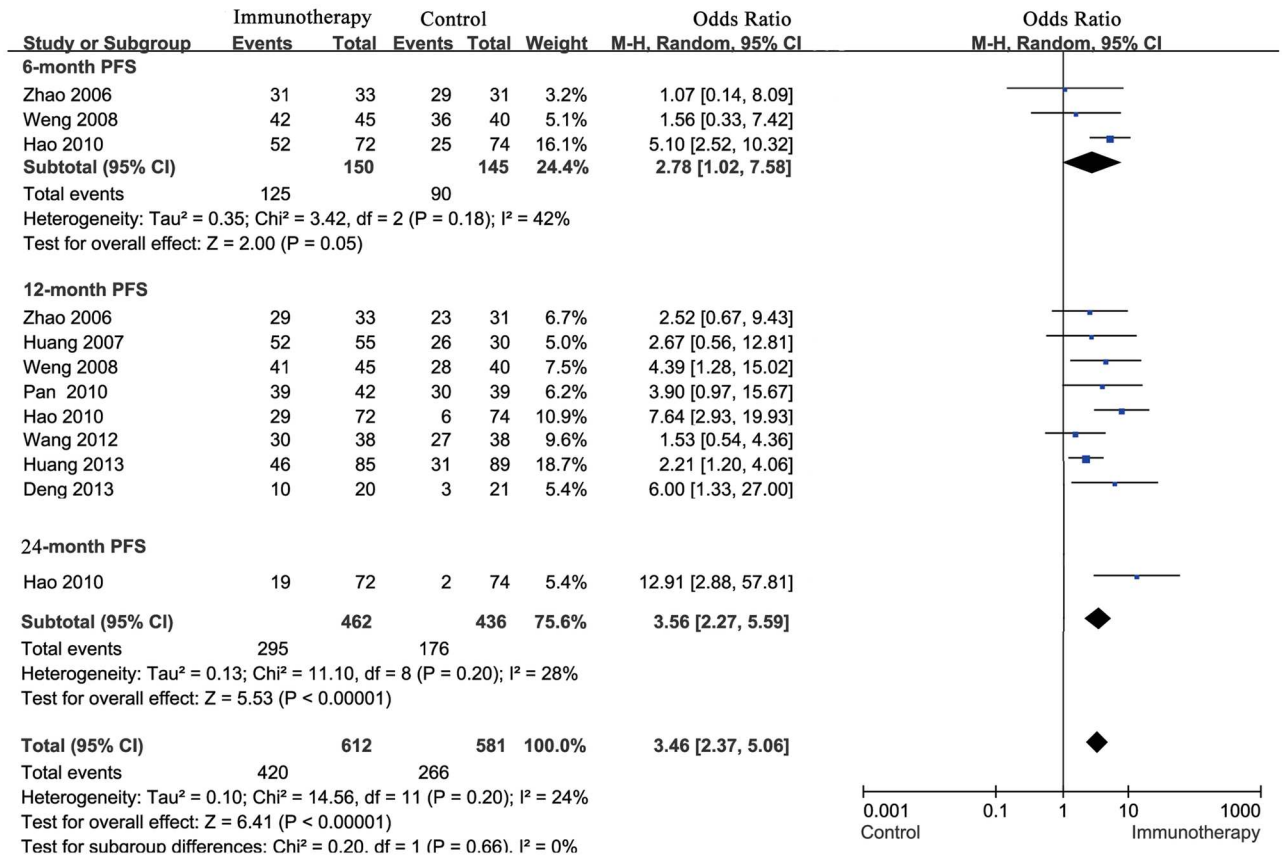Our meta-analysis comprehensively analyzed the outcomes of 19 individual studies with 1744 HCC patients from 3 databases, confirming the efficacy of the combination of cellular immunotherapy and TACE-predominant minimally-invasive Treatment on the prognosis of HCC patients.

A. 6-month OS

Study or Subgroup	Immunotherapy Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
CIK						
Zhang 2006	49	52	73	92	14.3%	4.25 [1.19, 15.14]
Hao 2006	18	21	32	46	12.9%	2.63 [0.66, 10.37]
Shi 2007	38	38	125	214	4.2%	54.91 [3.33, 905.63]
Hao 2010	65	72	55	74	19.9%	3.21 [1.26, 8.20]
Tong 2013	18	20	13	18	8.9%	3.46 [0.58, 20.70]
Subtotal (95% CI)		203		444	60.3%	3.95 [1.93, 8.11]
Total events	188		298			

Heterogeneity: Tau² = 0.15; Chi² = 5.12, df = 4 (P = 0.27); I² = 22%
Test for overall effect: Z = 3.75 (P = 0.0002)

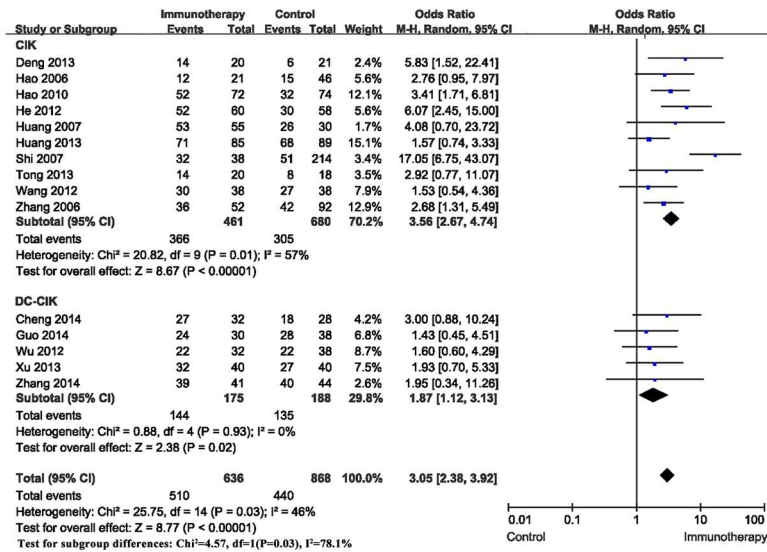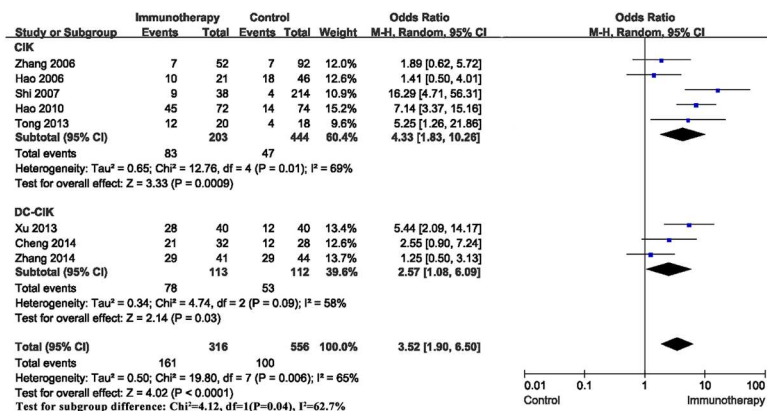|						
DC-CIK						
Wu 2012	28	32	32	38	13.1%	1.31 [0.34, 5.13]
Xu 2013	36	40	38	40	9.1%	0.47 [0.08, 2.75]
Cheng 2014	31	32	26	28	5.3%	2.38 [0.20, 27.81]
Liu 2014	19	23	9	17	12.2%	4.22 [1.00, 17.80]
Subtotal (95% CI)		127		123	39.7%	1.61 [0.64, 4.09]
Total events	114		105			

Heterogeneity: Tau² = 0.19; Chi² = 3.77, df = 3 (P = 0.29); I² = 20%
Test for overall effect: Z = 1.01 (P = 0.31)

Total (95% CI)		330		567	100.0%	2.81 [1.53, 5.17]
Total events	302		403			

Heterogeneity: Tau² = 0.26; Chi² = 11.55, df = 8 (P = 0.17); I² = 31%
Test for overall effect: Z = 3.32 (P = 0.0009)
Test for subgroup differences: Chi²=5.84, df=1(P=0.02), I²=82.9%

B. 12-month OS

Study or Subgroup	Immunotherapy Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
CIK						
Deng 2013	14	20	6	21	2.4%	5.83 [1.52, 22.41]
Hao 2006	12	21	15	46	5.6%	2.76 [0.95, 7.97]
Hao 2010	52	72	32	74	12.1%	3.41 [1.71, 6.81]
He 2012	52	60	30	58	5.6%	6.07 [2.45, 15.00]
Huang 2007	53	55	26	30	1.7%	4.08 [0.70, 23.72]
Huang 2013	71	85	68	89	15.1%	1.57 [0.74, 3.33]
Shi 2007	32	38	51	214	3.4%	17.05 [6.75, 43.07]
Tong 2013	14	20	8	18	3.5%	2.92 [0.77, 11.07]
Wang 2012	30	38	27	38	7.9%	1.53 [0.54, 4.36]
Zhang 2006	36	52	42	92	12.9%	2.68 [1.31, 5.49]
Subtotal (95% CI)		461		680	70.2%	3.56 [2.67, 4.74]
Total events	366		305			

Heterogeneity: Chi² = 20.82, df = 9 (P = 0.01); I² = 57%
Test for overall effect: Z = 8.67 (P < 0.00001)

DC-CIK						
Cheng 2014	27	32	18	28	4.2%	3.00 [0.88, 10.24]
Guo 2014	24	30	28	38	6.8%	1.43 [0.45, 4.51]
Wu 2012	22	32	22	38	8.7%	1.60 [0.60, 4.29]
Xu 2013	32	40	27	40	7.5%	1.93 [0.70, 5.33]
Zhang 2014	39	41	40	44	2.6%	1.95 [0.34, 11.26]
Subtotal (95% CI)		175		188	29.8%	1.87 [1.12, 3.13]
Total events	144		135			

Heterogeneity: Chi² = 0.88, df = 4 (P = 0.93); I² = 0%
Test for overall effect: Z = 2.38 (P = 0.02)

Total (95% CI)		636		868	100.0%	3.05 [2.38, 3.92]
Total events	510		440			

Heterogeneity: Chi² = 25.75, df = 14 (P = 0.03); I² = 46%
Test for overall effect: Z = 8.77 (P < 0.00001)
Test for subgroup differences: Chi²=4.57, df=1(P=0.03), I²=78.1%

C. 24-month OS

Study or Subgroup	Immunotherapy Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
CIK						
Zhang 2006	7	52	7	92	12.0%	1.89 [0.62, 5.72]
Hao 2006	10	21	18	46	12.6%	1.41 [0.50, 4.01]
Shi 2007	9	38	4	214	10.9%	16.29 [4.71, 56.31]
Hao 2010	45	72	14	74	15.2%	7.14 [3.37, 15.16]
Tong 2013	12	20	4	18	9.6%	5.25 [1.26, 21.86]
Subtotal (95% CI)		203		444	60.4%	4.33 [1.83, 10.26]
Total events	83		47			

Heterogeneity: Tau² = 0.65; Chi² = 12.76, df = 4 (P = 0.01); I² = 69%
Test for overall effect: Z = 3.33 (P = 0.0009)

DC-CIK						
Xu 2013	28	40	12	40	13.4%	5.44 [2.09, 14.17]
Cheng 2014	21	32	12	28	12.6%	2.55 [0.90, 7.24]
Zhang 2014	29	41	29	44	13.7%	1.25 [0.50, 3.13]
Subtotal (95% CI)		113		112	39.6%	2.57 [1.08, 6.09]
Total events	78		53			

Heterogeneity: Tau² = 0.34; Chi² = 4.74, df = 2 (P = 0.09); I² = 58%
Test for overall effect: Z = 2.14 (P = 0.03)

Total (95% CI)		316		556	100.0%	3.52 [1.90, 6.50]
Total events	161		100			

Heterogeneity: Tau² = 0.50; Chi² = 19.80, df = 7 (P = 0.006); I² = 65%
Test for overall effect: Z = 4.02 (P < 0.0001)
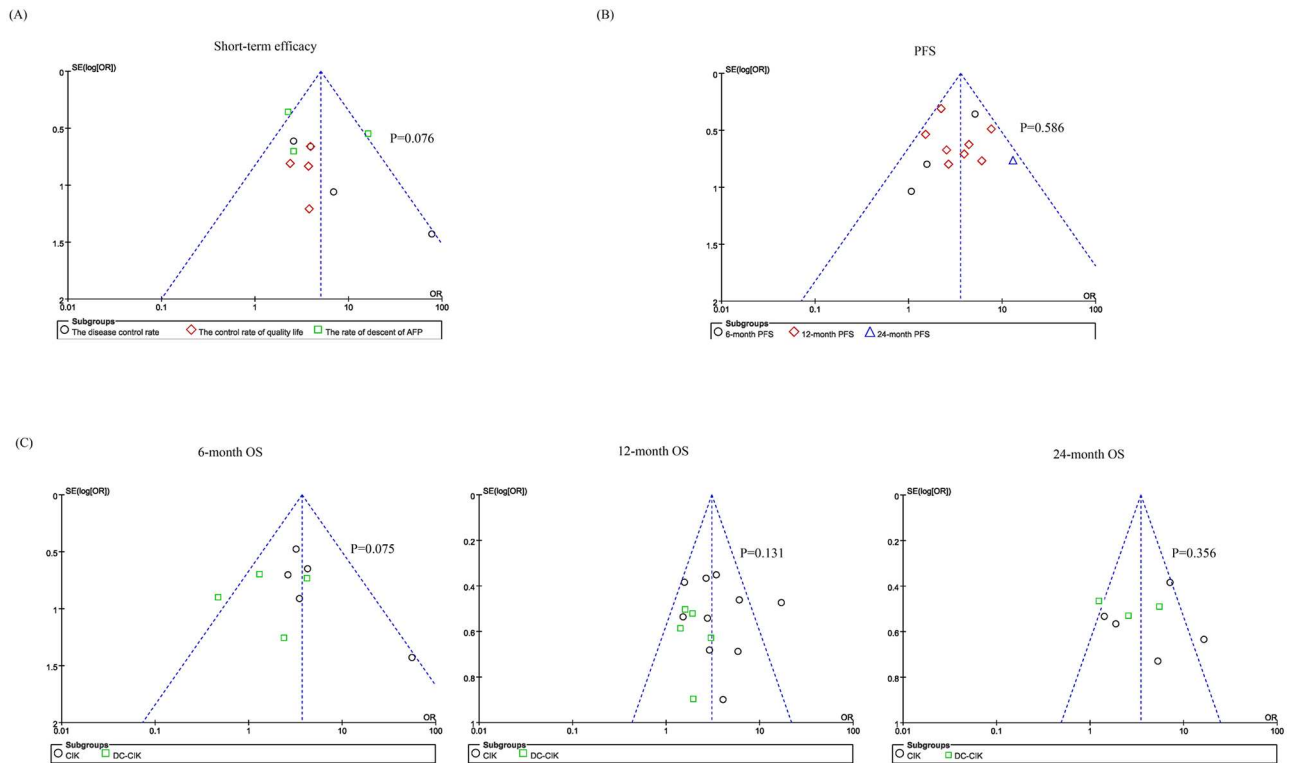Test for subgroup difference: Chi²=4.12, df=1(P=0.04), I²=62.7%

Fig 4. Comparison of 6, 12, 24-month OS between the patients undergoing cellular immunotherapy or not. The random effects model (Mantel-Haenszel method) was used.

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Fig 5. Funnel plots to detect any publication bias. The P value located in this figure indicates the results of Peters test for assessment of publication bias.

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TACE-predominant minimally-invasive Treatment is one of the main treatments for patients with advanced HCC or patients who are not suitable to surgery [6,39,40]. However, TACE-predominant minimally-invasive Treatment might damage the liver function, reduce the resistance and immunity, and lead to tumor recurrence and residual. So, the combination of TACE-predominant minimally-invasive Treatment and immunotherapy might become an effective treatment [41]. From 18 meta-analysis or reviews in this field, 3 out of 18 performed a meta-analysis on HCC. These systematic reviews focused on the efficacy of the combination of CIK/DC-CIK therapeutic treatment and traditional therapy, respectively. Xie et al [42] studied the efficacy of adoptive immunotherapy in postoperative HCC. Li et al [15] demonstrated that CIK cells transfusion therapy could improve the synergistic effect of HCC patients after TACE or TACE+RFA therapy. Su et al [14] evaluated the efficacy and safety of DC-CIK cell therapy in combination with TACE-predominant minimally-invasive treatment for HCC. However, there was no studies systematically analyzed whether the adjuvant cellular immunotherapy after TACE-predominant minimally-invasive treatment is essential. Notably, it is still controversial that whether CIK or DC-CIK cells immunotherapy was efficacy after different interventional therapy, such as TACE, TACE+RFA, TACE+PEI, and TACE+PMCT for HCC. Our meta-analysis comprehensively analyzed the efficacy of the combination of different cell immunotherapy and different strategy of TACE-predominant minimally-invasive treatment. Through short-term and long-term efficacy analysis, we suggested that the group combined with CIK or DC-CIK cell immunotherapy was associated with better prognosis than minimally invasive treatment alone group.

Our analysis demonstrated that the combination therapy could improve the short-term efficacy, including the disease control rate (P = 0.007), the control rate of quality life (P = 0.003),

and the AFP descent rate (P = 0.02). However, the number of included studies for each subgroup was relatively low. And the limitation might lead to bias, even though we found no evidence of publication bias in the meta analysis of the short-term efficacy (P = 0.07) (Fig 5A).

The systematic analysis also showed a significant survival benefit with regard to the PFS and OS when patients undergoing cellular immunotherapy (P≤0.05). There was no heterogeneity across the trails in the meta-analysis for PFS. However, the heterogeneity in 12-month and 24-month OS was statically significant (P<0.05). This demonstrated that the degree of variability was more consistent in each PFS result of study. Notably, for 6-month, 12-month and 24-month OS, the meta analysis was divided into two sub-studies (CIK studies and DC-CIK studies) to reduce the bias caused by different immune cell regimens. For total effect, OS was significantly higher in HCC patients who underwent cellular immunotherapy, compared to patients in control group. However, the heterogeneity in 12-month and 24-month OS in CIK subtypes was statically significant (P<0.05). And the heterogeneity of 6-month, 12-month and 24-month OS between CIK group and DC-CIK group was statically significant (P<0.05) (Fig 4). This result suggested that cellular immunotherapy either CIK or DC-CIK might be needed after different interventional therapy. However, the degree of variability was more consistent in the subtype of DC-CIK studies.

There were some limitations existed in this meta-analysis. First, the number of included studies in each subgroup was relatively low. And the included studies mostly occurred in China lacking multinational larger sample multi-center clinical trials with sufficient statistical power. Second, heterogeneity is a potential issue that may affect the interpretation of the results. The sources of heterogeneity may result from designs and methods, including age distribution, gender, regimens and so on. To address this issue, we employed subgroup analysis and random effects method to perform a meta-analysis on the results of these 19 studies. Third, Publication bias might occurred when the publication of research results depended not just on the quality of the research but on the hypothesis tested, and the significance and direction of effects detected. For meta-analysis, the studies would not be representative when the publication bias presented. In order to guarantee the validity of the conclusions from the meta-analysis, funnel plots and sensitivity analysis (Peters test) were used in the study.

Overall, our systematic analysis demonstrated that the potential clinical value of the combination of cellular immunotherapy with minimally-invasive therapy for prolonging progression free and overall survival. On the basis of the present results, we believe that more advanced and optimized cellular immunotherapy strategies are on their way and may shed a better light on the modality of treatment for HCC.

Supporting Information

S1 Checklist. PRISMA 2009 checklist.
(DOC)

S1 Fig. The quality assessment for included literatures by QUADAS-2.
(TIF)

S2 Fig. PRISMA 2009 flow diagram.
(DOC)

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