OncoTargets and Therapy

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ORIGINAL RESEARCH

Transcatheter hepatic arterial chemoembolization plus cinobufotalin injection adjuvant therapy for advanced hepatocellular carcinoma: a meta-analysis of 27 trials involving 2,079 patients

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Objective: The aim of this study was to systematically investigate the safety and efficacy of the combination of transcatheter hepatic arterial chemoembolization (TACE) and cinobufotalin injection for advanced hepatocellular carcinoma (HC).

Methods: Clinical trials were searched from Web of Science, Cochrane Library, PubMed, Embase, Chinese Medical Citation Index (CMCI), China National Knowledge Infrastructure (CNKI), Chinese Scientific Journal Database (VIP), and Wanfang database. Outcome measures including therapeutic efficacy, quality of life, liver function, immune function, and adverse events were extracted and evaluated.

Results: After final assessment, 27 studies including 2,079 advanced HC patients were involved in this study. Compared with TACE alone, the combination of TACE with cinobufotalin injection adjuvant therapy significantly prolonged the patients' 1-, 1.5-, 2-, and 3-year overall survival (OS) rate (1-year OS, OR=2.84, 95% CI=2.20–3.67, $P<0.00001$; 1.5-year OS, OR=3.57, 95% CI=1.92–6.66, $P<0.0001$; 2-year OS, OR=3.17, 95% CI=2.36–4.25, $P<0.00001$; 3-year OS, OR=2.88, 95% CI=1.82–4.57, $P<0.00001$). The combined therapy also improved patients' overall response rate (ORR; OR=1.86, 95% CI=1.54–2.24, $P<0.00001$), disease control rate (DCR; OR=2.05, 95% CI=1.59–2.64, $P<0.00001$), and quality of life improved rate (QIR; OR=3.45, 95% CI=2.52–4.72, $P<0.00001$). Moreover, the immune function and liver function of HC patients were all significantly enhanced after the combined therapy of TACE and cinobufotalin injection ($CD3^+$, $P=0.001$; $CD4^+$, $P=0.0006$; $CD4^+/CD8^+$, $P=0.03$; natural killer [NK] cell, $P=0.01$; total bilirubin [TBIL], $P=0.003$; alanine aminotransferase [ALT], $P<0.00001$; aspartate aminotransferase [AST], $P<0.00001$). No serious adverse events occurred during cinobufotalin injection-mediated therapy.

Conclusion: The combination of TACE and cinobufotalin injection adjuvant therapy is safe and more effective for end-stage HC treatment than TACE alone.

Keywords: hepatocellular carcinoma, cinobufotalin injection, transcatheter hepatic arterial chemoembolization, meta-analysis

Introduction

Hepatocellular carcinoma (HC) is a major threat to human health. It is the fifth most common malignancy and caused more than 600,000 deaths every year.[1–3] Over the past 20 years, the number of HC-related deaths has increased by 62%.[4] China is a high-risk area for HC and accounts for more than half of the HC cases worldwide.[4,5] Despite the development of diagnostic methods, early detection of HC is still difficult.[2] In most

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http://dx.doi.org/10.2147/OTT.S182840

patients, HC progressed to the intermediate and advanced stage, and the 5-year survival rate was <17% at this stage.[1] Therefore, only a small proportion of early-stage HC patients are suitable for radical treatment.

Transcatheter hepatic arterial chemoembolization (TACE) is the current standard locoregional treatment for advanced HC.[2,6] Several studies reported that TACE significantly increased the survival time in HC patients compared to supportive treatments.[7,8] However, TACE also has its own limitations, as it can further influence the liver functions and damage the hepatic arterial system of patients.[2,9] In addition, its clinical application was also limited by drug resistance and toxic side effects.[1] In view of these limitations of TACE therapy for HC, complementary and alternative medicine has been increasingly used for the treatment of advanced HC.

In recent years, traditional Chinese medicine has become an important source for novel chemotherapeutic agents and was considered as a powerful method for the cancer treatment.[4,10–12] Cinobufotalin, a cardiotonic steroid or bufadienolide, is extracted from the skin secretions of the traditional Chinese medicinal giant toads.[12–15] Many studies have shown that cinobufotalin has anti-tumor activity and can enhance the treatment effect of chemotherapeutics for malignancies.[12,15,16] It can inhibit the growth of vascular endothelial cells by inhibiting the expression of vascular endothelial growth factor and EGF receptor and then inhibit the growth and metastasis of the tumor.[17] In addition, it can also induce tumor cells apoptosis through decreasing ROS production and by destroying the structure of DNA in cancer cells.[14,16]

Up to now, several clinic trials have been conducted to evaluate the therapeutic effects between TACE and TACE+cinobufotalin injection in advanced HC patients.[18–44] Despite the wide use of cinobufotalin injection in HC treatment for many years, its clinical efficacy was still not well established and recognized. Therefore, we conducted a meta-analysis to investigate the treatment effect and safety of cinobufotalin injection adjuvant therapy combined with TACE in comparison with TACE alone for end-stage HC, to provide scientific reference for the design of future clinical trials.

Materials and methods

Search strategy and selection criteria

Original articles published after 2000 were searched across eight databases, including Web of Science, Cochrane Library, PubMed, Embase, Chinese Medical Citation Index (CMCI), China National Knowledge Infrastructure (CNKI), Chinese Scientific Journal Database (VIP), and Wanfang database, with key terms "huachansu" or "cinobufotalin" "cinobufagin" or "cinobufacini" combined with "hepatocellular carcinoma" or "liver cancer". No language limits were applied. The initial search was performed in May 2018 and updated in July 2018.

Selection criteria of this study are as follows: 1) controlled trials concerning advanced HC patients; 2) literatures comparing the clinical outcomes of TACE plus cinobufotalin injection adjuvant therapy (experimental group) with TACE treatments alone (control group); and 3) articles involving more than 30 HC patients. Exclusion criteria of this study are as follows: 1) non-contrast articles, case studies, and review papers and 2) patients with mixed malignancies.

Data extraction and quality assessment

Data were extracted by two reviewers (Na Guo and Yanyan Miao) independently; disagreements were adjudicated by the third investigator (Mingzhong Sun). The extracted characteristics were summarized as follows: 1) first author's names; 2) years of publication; 3) study locations; 4) tumor stages; 5) number of cases; 6) patient ages; 7) study parameter types; 8) therapeutic regimens; 9) enrollment period and expected survival time of patients; 10) application sequence of cinobufotalin injection; and 11) manufacturer of cinobufotalin injection. The included trial's quality was evaluated according to the Cochrane Handbook.[45]

Outcome definition

Clinical outcomes include therapeutic effect and adverse events. Therapeutic effect was assessed in terms of the overall survival (OS) rates, complete response (CR) rates, partial response (PR) rates, stable disease (SD) rates, progressive disease (PD) rates, overall response rate (ORR; ORR=CR rate+PR rate), disease control rate (DCR; DCR=CR rate+PR rate+SD rate), and quality of life improved rate (QIR). The immune function indicators ($CD3^+$, $CD4^+$, and natural killer (NK) cells percentage and $CD4^+/CD8^+$ ratio) and liver function indexes including total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum albumin (ALB) of HC patients were determined and compared between the two groups. Moreover, adverse events including leukopenia, thrombocytopenia, nausea and vomiting, fever, hepatotoxicity, and myelosuppression were also taken for assessment.

Statistical analyses

The analyses were performed using Review Manager 5.3 and Stata 12.0. Between-study heterogeneity was assessed using the chi-squared statistic and quantified by I^2. $I^2<50\%$ indicated that the studies were homogenous.[46] A fixed effects model was conducted when the heterogeneity did not exist; otherwise, a random effects model was performed. OR was the principal measurement for therapeutic effects and is presented with a 95% CI. We further investigated potential sources of between-study heterogeneity by subgroup analyses based on the some baseline variables (study design and sample sizes). Publication bias was assessed visually by funnel plots and quantified in Egger's test and Begg's regression test.[47,48] When publication bias existed, trim-and-fill method was applied to adjust the pooled estimates of potentially unpublished studies.

Results

Search results

A total of 1,291 articles were identified with the initial retrieve. 717 papers were excluded due to duplication. After title and abstract review, 468 articles were further excluded because they did not include clinical trials (n=344), were reviews or meta-analysis (n=7), were unrelated studies (n=104), or were case reports (n=13), leaving 106 studies as potentially relevant. After detailed assessment of full texts, articles without the control group (n=15), patients not treated by cinobufotalin injection (n=24) or TACE (n=19), studies with insufficient data (n=7), and studies published before 2000 (n=14) were excluded. Finally, 27 trials[19–37] involving 2,079 advanced HC patients were included in this analysis (Figure 1).

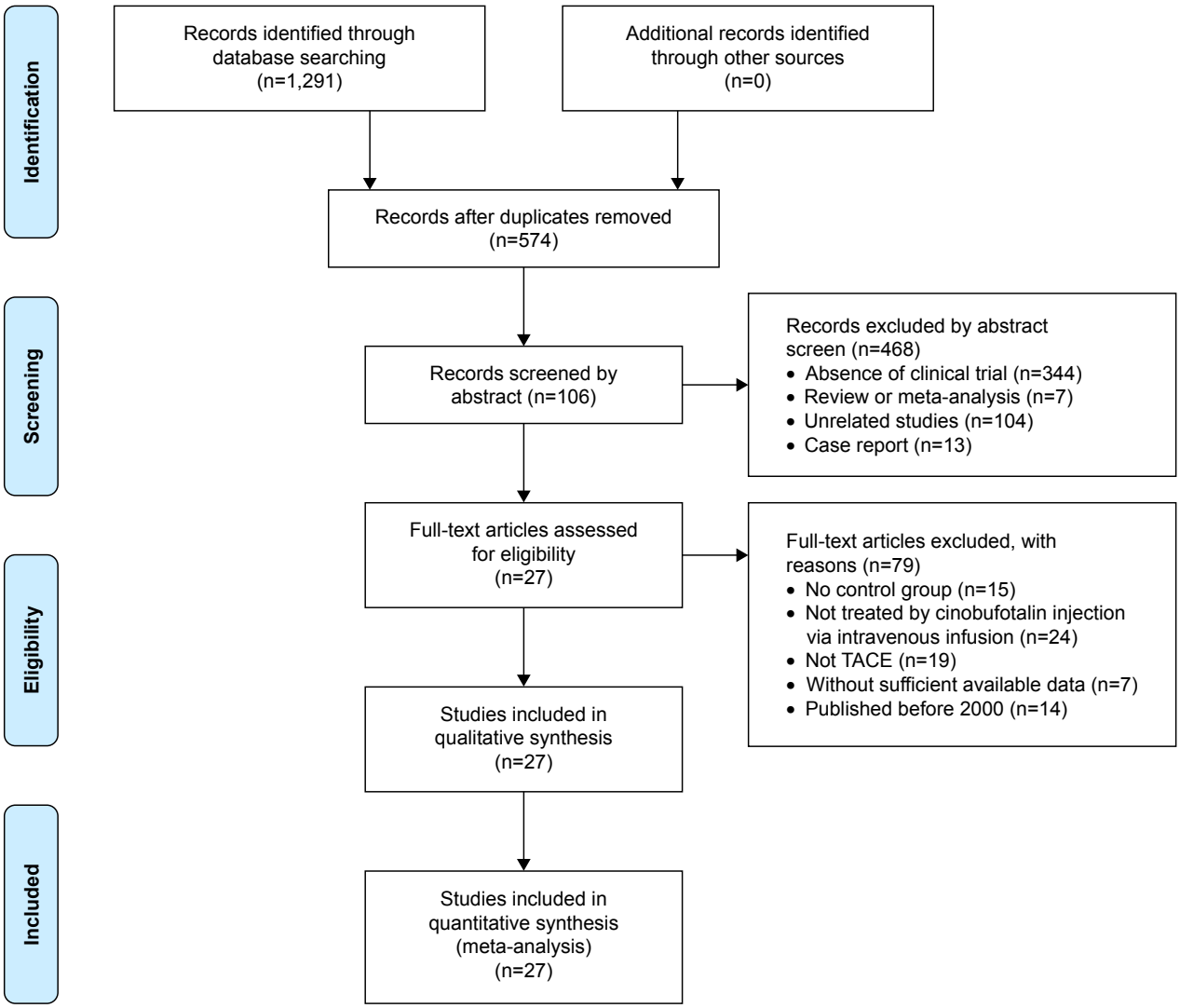


Figure 1 Flow diagram of the selection process.

Patients' characteristics

After selection, all studies were carried out in the hospitals in China since 2000. In total, 1,045 advanced HC patients were treated by TACE in combination with cinobufotalin injection adjuvant therapy, while 1,034 patients were treated by TACE alone. Among all included studies, cinobufotalin injection and TACE were used simultaneously in the 16 trials,[19–21,23,24,26,27,29–31,34,37–40,42] whereas cinobufotalin injection was used after TACE in nine articles[18,22,25,28,32,33,35,36,44] and was used before TACE in two studies.[41,43] Detailed information of the studies involved and HC patients is shown in Tables 1, 2, and S1.

Quality assessment

The evaluation of bias risk is presented in Figure 2. Twenty-five studies had low risk and the other two studies did not have a clear description of randomization process. All included trials did not provide clear description of performance and detection risks. One study was regarded as a high risk due to the absence of follow-up, and 20 trials were considered as unclear risk owing to selective reporting.

Therapeutic efficacy assessments

As shown in Figures 3 and 4 and Table 3, the analysis results showed that patients underwent combined therapy

Table 1 Clinical information from the eligible trials in the meta-analysis

Included studies	Nation	Stage	Patients (Con/exp)	Age (years)		Parameter types
				Con	Exp	
Chen et al (2017)[18]	China	Child-Pugh A–B	36/36	ND	ND	OS, ORR, DCR, QIR
Cui (2008)[19]	China	Child-Pugh A–B	54/61	ND	ND	OS, ORR, DCR, QIR
Deng and Duan (2015)[20]	China	ND	27/26	48.3±16.2 (mean)	48.7±16.1 (mean)	ORR, DCR, QIR, AE
Fu et al (2010)[21]	China	KPS≥60	78/78	56 (median)	58 (median)	ORR, DCR, QIR, AE
He et al (2012)[22]	China	Child-Pugh A–B	25/26	>60 (20)	>60 (19)	ORR, DCR, QIR, AE
Jia (2016)[23]	China	Child-Pugh A–B	46/49	58.1±8.7 (mean)	58.4±8.3 (mean)	ORR, DCR
Ke et al (2011)[24]	China	Child-Pugh A–B	40/38	57.1±11.8 (mean)	58.3±11.6 (mean)	OS, ORR, DCR
Kou and Xu (2011)[25]	China	KPS>60	31/31	41 (mean)	40.5 (mean)	OS, ORR, DCR, QIR
Li et al (2008)[26]	China	Child-Pugh A–B	46/50	ND	ND	OS, ORR, DCR, QIR
Li (2014)[27]	China	ND	25/26	61.7±6.8 (mean)	57.4±6.2 (mean)	ORR, DCR
Liang et al (2008)[28]	China	Child-Pugh A–C	48/48	ND	ND	OS, ORR, DCR, QIR, AE
Liu et al (2009)[29]	China	ND	42/42	ND	ND	OS, ORR, DCR
Liu et al (2010)[30]	China	Child-Pugh A–B	44/38	55.3±11.6 (mean)	54.2±10.3 (mean)	ORR, DCR, AE
Mao (2013)[31]	China	I–III	27/27	48.3±8.9 (mean)	47.6±9.3 (mean)	OS, QIR
Shen (2009)[32]	China	II–III	24/23	ND	ND	AE
Shen and Tan (2015)[33]	China	Child-Pugh A–B	18/18	54.7 (mean)	57.5 (mean)	ORR, DCR
Song (2012)[34]	China	I–II	20/20	49.8±6.4 (mean)	50.3±8.1 (mean)	OS, QIR
Su et al (2013)[35]	China	II–III	30/33	52.7±7.9 (mean)	53.2±8.7 (mean)	ORR, DCR, QIR, AE
Sun et al (2002)[36]	China	ND	118/118	ND	ND	OS, ORR
Wang (2014)[37]	China	III–IV	35/36	ND	ND	ORR, DCR
Xue et al (2010)[38]	China	KPS>60	30/32	45.5±10.7 (mean)	45.8±11.4 (mean)	OS, ORR, DCR, AE
Yan and Bai (2010)[39]	China	II–IV	30/30	63.6 (mean)	65.4 (mean)	ORR, DCR
Yang et al (2014)[40]	China	III–IV	45/45	62.3±7.2 (mean)	61.9±5.4 (mean)	ORR, DCR, AE
Yang et al (2006)[41]	China	ND	40/40	44.3 (mean)	49.6 (mean)	OS, ORR, DCR
Yu (2013)[42]	China	KPS>60	30/30	50.8 (mean)	49.7 (mean)	ORR, DCR
Zeng et al (2009)[43]	China	Child-Pugh A–B	23/23	53.2±3.8 (mean)	52.4±3.7 (mean)	OS, ORR, DCR, QIR
Zhou et al (2006)[44]	China	Child-Pugh A–C	22/21	ND	ND	OS, ORR, DCR

Abbreviations: AE, adverse event; Con, control group (TACE alone group); DCR, disease control rate; Exp, experimental group (TACE plus cinobufotalin injection adjuvant therapy); KPS, Karnofsky performance score; ND, not determined; ORR, overall response rate; OS, overall survival; QIR, quality of life improved rate; TACE, transcatheter hepatic arterial chemoembolization.

Table 2 Information of TACE combined with cinobufotalin injection adjuvant therapy

Included studies	Therapeutic regimen		Enrollment period (year.month)	Expected survival time (months)
	Exp	Con		
Chen et al (2017)[18]	TACE+cinobufotalin injection (IV)	TACE (oxaliplatin, THP)	2014.7–2016.7	>3
Cui (2008)[19]	TACE+cinobufotalin injection (IV)	TACE (5-Fu, ADR, mitomycin, HCPT)	2000.6–2007.6	ND
Deng and Duan (2015)[20]	TACE+cinobufotalin injection (IV)	TACE (DDP, THP)	2011.1–2013.2	ND
Fu et al (2010)[21]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, mitomycin)	2006.6–2009.10	>4
He et al (2012)[22]	TACE+cinobufotalin injection (IV)	TACE (5-Fu, oxaliplatin, THP)	2007.3–2010.8	>3
Jia (2016)[23]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, ADR)	2010.1–2012.6	>3
Ke et al (2011)[24]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, E-ADM)	2006.2–2008.3	>3
Kou and Xu (2011)[25]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, ADR, HCPT)	2003.5–2008.5	>3
Li et al (2008)[26]	TACE+cinobufotalin injection (IV)	TACE (5-Fu, ADR, mitomycin, HCPT)	2001–2005	ND
Li (2014)[27]	TACE+cinobufotalin injection (IV)	TACE (5-Fu, ADR, oxaliplatin, mitomycin)	2012.8–2013.8	ND
Liang et al (2008)[28]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, ADR)	2004.2–2006.2	ND
Liu et al (2009)[29]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, E-ADM)	2002.6–2006.6	ND
Liu et al (2010)[30]	TACE+cinobufotalin injection (IV)	TACE (DDP, THP, mitomycin)	2005.6–2008.1	>3
Mao (2013)[31]	TACE+cinobufotalin injection (IV)	TACE (5-Fu, ADR, mitomycin)	2007.6–2010.6	ND
Shen (2009)[32]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, mitomycin)	2004–2007	>2
Shen and Tan (2015)[33]	TACE+cinobufotalin injection (IV)	TACE (5-Fu, lobaplatin, THP)	2013.3–2014.12	ND
Song (2012)[34]	TACE+cinobufotalin injection (IV)	TACE (5-Fu, ADR, mitomycin)	2007.1–2010.12	>3
Su et al (2013)[35]	TACE+cinobufotalin injection (IV)	TACE (5-Fu, ADR, mitomycin, HCPT)	2008.6–2012.6	>2
Sun et al (2002)[36]	TACE+cinobufotalin injection (IV)	TACE (carboplatin, mitomycin, E-ADM)	1994.6–2000.6	ND
Wang (2014)[37]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, ADR)	ND	ND
Xue et al (2010)[38]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, ADR)	2003.1–2005.10	>3
Yan and Bai (2010)[39]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, ADR, mitomycin, HCPT)	2004.12–2010.1	ND
Yang et al (2014)[40]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, ADMh, mitomycin)	2010.6–2013.6	≥3
Yang et al (2006)[41]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, mitomycin, gemcitabine)	1996.7–2002.3	>3
Yu (2013)[42]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, ADR, mitomycin)	2003.2–2011.5	ND
Zeng et al (2009)[43]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, THP)	2002.2–2006.5	ND
Zhou et al (2006)[44]	TACE+cinobufotalin injection (IV)	TACE (DDP, 5-Fu, mitomycin)	2002.12–2005.12	>2

Abbreviations: ADMh, doxorubicin hydrochloride; ADR, adriamycin; Con, control group (TACE alone group); DDP, cisplatin; E-ADM, pharmorubicin; Exp, experimental group (TACE plus cinobufotalin injection adjuvant therapy); 5-Fu, 5-fluorouracil; HCPT, hydroxycamptothecin; IV, intravenous; ND, not determined; TACE, transcatheter hepatic arterial chemoembolization; THP, pirarubicin.

had significantly improved 1-, 1.5-, 2-, and 3-year OS (1-year OS, OR=2.84, 95% CI=2.20–3.67, $P<0.00001$; 1.5-year OS, OR=3.57, 95% CI=1.92–6.66, $P<0.0001$; 2-year OS, OR=3.17, 95% CI=2.36–4.25, $P<0.00001$; 3-year OS, OR=2.88, 95% CI=1.82–4.57, $P<0.00001$), CR rate (OR=1.73, 95% CI=1.04–2.87, $P=0.03$), PR rate (OR=1.61, 95% CI=1.31–1.97, $P<0.00001$), ORR (OR=1.86, 95% CI=1.54–2.24, $P<0.00001$), and DCR (OR=2.05, 95% CI=1.59–2.64, $P<0.00001$) and significantly decreased PD rate (OR=0.46, 95% CI=0.35–0.59, $P<0.00001$), whereas the 0.5-year OS and SD rate (0.5-year OS, OR=1.40, 95% CI=0.97–2.01, $P=0.07$; SD rate, OR=0.88, 95% CI=0.72–1.09, $P=0.25$) did not show significant difference from patients who received TACE alone.

Quality of life assessment

Thirteen studies[18–22,25,26,28,31,34,35,43,44] assessed the quality of life of advanced HC patients between the TACE+cinobufotalin injection and TACE alone groups. Results showed that quality of life of patients in the combined group was significantly better than that of the control group, indicated by significantly improved QIR (Figure 5; OR=3.45, 95% CI=2.52–4.72, $P<0.00001$).

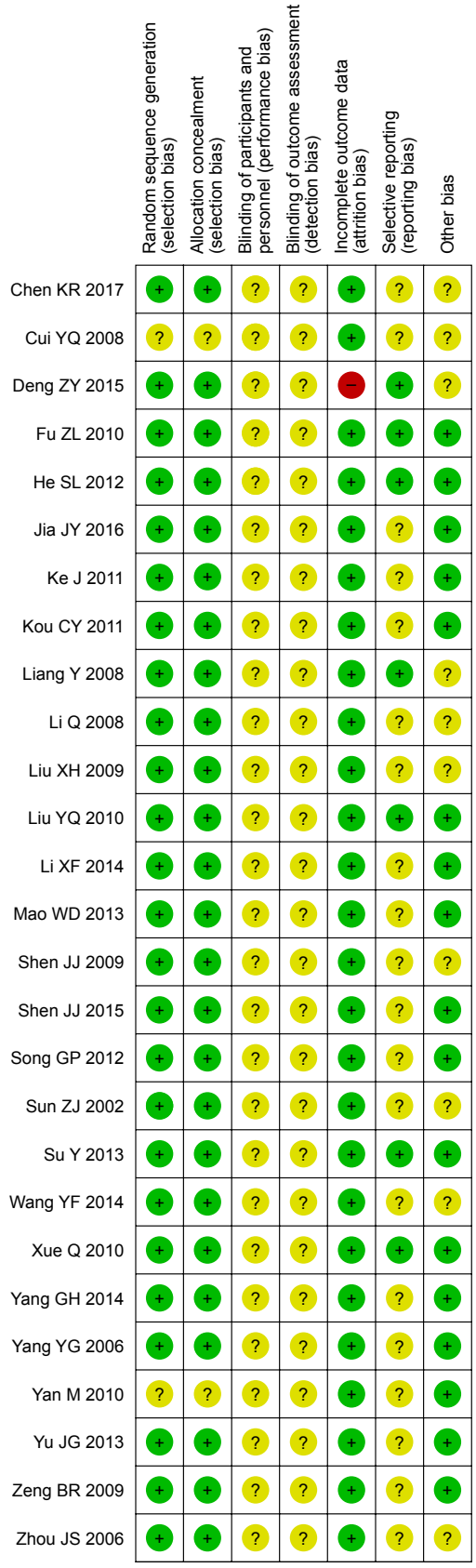


Figure 2 Risk of bias summary: review of authors' judgments about each risk of bias item for included studies.
Notes: Each color represents a different level of bias: red, high risk; green, low risk; yellow, unclear risk.

Liver function evaluation

Five clinical trials[24,26,32,42,44] evaluated the liver function of advanced HC patients between the two groups. As shown in Figure 6, the liver function of HC patients who received combined therapy was significantly improved compared with TACE alone, indicated by obviously reduced TBIL, AST, and ALT (TBIL, OR=−9.21, 95% CI=−15.14 to −3.10, P=0.003; ALT, OR=−30.76, 95% CI=−41.65 to −19.88, $P<0.00001$; AST, OR=−30.66, 95% CI=−42.36 to −18.97, $P<0.00001$; ALB, OR=2.46, 95% CI=−2.75 to 7.67, P=0.35).

Immune function evaluation

The immune status of patients was examined between TACE and TACE+cinobufotalin injection group in five controlled studies.[23,26,29,36,43] Compared with TACE alone, the percentages of $CD3^+$, $CD4^+$, and NK cells, and $CD4^+/CD8^+$ ratio in the combined treatment group were significantly increased (Figure 7; $CD3^+$, OR=9.05, 95% CI=3.62–14.49, P=0.001; $CD4^+$, OR=7.42, 95% CI=3.20–11.63, P=0.0006; NK, OR=10.00, 95% CI=2.08–17.92, P=0.01; $CD4^+/CD8^+$, OR=0.33, 95% CI=0.03–0.62, P=0.03).

Adverse events assessment

Safety of cinobufotalin injection-mediated therapy was evaluated in eight studies.[20–22,28,30,32,35,38] As shown in Figure 8, no serious adverse events were reported during cinobufotalin injection-mediated therapy. The group that received TACE plus cinobufotalin injection had lower rates of myelosuppression (OR=0.29, 95% CI=0.15–0.57, P=0.0003), whereas analysis on other adverse events did not show significant difference (leukopenia, OR=2.74, 95% CI=0.25–30.43, P=0.41; thrombocytopenia, OR=1.08, 95% CI=0.46–2.52, P=0.86; nausea and vomiting, OR=0.57, 95% CI=0.21–1.57, P=0.28; fever, OR=1.23, 95% CI=0.16–9.78, P=0.84; hepatotoxicity, OR=0.83, 95% CI=0.22–3.13, P=0.79).

Publication bias

Publication bias was assessed visually by funnel plots and quantified in Egger's test and Begg's regression test. As shown in Figures 9 and 10 and Table 4, no significant publication bias for OS rate, CR rate, PR rate, SD rate, PD rate, and QIR was observed in these analyses, which confirmed the reliability of our primary conclusions.

Sensitivity analysis

We conducted subgroup analysis to explore the source of heterogeneity in OS rate, ORR, DCR, and QIR with respect to the study design and sample sizes of involved studies.

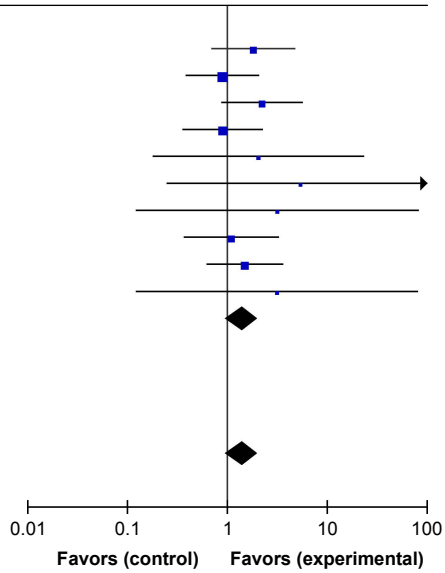
As shown in Table 5, our analysis results showed that no significant difference was found between different study designs and sample sizes of studies in most of the primary indicators except 0.5-year OS.

Discussion

In view of the limitations such as drug resistance and toxic side effects of the current chemotherapy for malignancies, more and more physicians are trying to find more adjunctive or auxiliary therapies to improve patients' survival time or quality of life and to reduce side effects caused by chemotherapy. Traditional Chinese medicine has been utilized as an adjuvant method to treat HC for a long time. Several studies have been reported that the addition of cinobufotalin injection could be beneficial to patients with advanced HC. Even though there were statistical analyses

A

Study or subgroup	Experimental events	Total	Control events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI
0.5-year OS						
Chen KR 2017	25	36	20	36	12.4	1.82 (0.69, 4.78)
Cui YQ 2008	45	61	41	54	23.1	0.89 (0.38, 2.08)
Ke J 2011	27	38	21	40	12.0	2.22 (0.87, 5.66)
Li Q 2008	37	50	35	46	19.2	0.89 (0.35, 2.26)
Liang Y 2008	47	48	46	48	1.9	2.04 (0.18, 23.32)
Mao WD 2013	27	27	25	27	0.9	5.39 (0.25, 117.77)
Song GP 2012	20	20	19	20	0.9	3.15 (0.12, 82.16)
Xue Q 2010	23	32	21	30	12.3	1.10 (0.37, 3.28)
Yang YG 2006	23	40	19	40	16.3	1.50 (0.62, 3.61)
Zeng BR 2009	23	23	22	23	0.9	3.13 (0.12, 81.00)
Subtotal (95% CI)		**375**		**364**	**100**	**1.40 (0.97, 2.01)**
Total events	297		269			
Heterogeneity: χ^2=4.72, *df*=9 (*P*=0.86); I^2=0%						
Test for overall effect: *Z*=1.80 (*P*=0.07)						
Total (95% CI)		**375**		**364**	**100**	**1.40 (0.97, 2.01)**
Total events	297		269			
Heterogeneity: χ^2=4.72, *df*=9 (*P*=0.86); I^2=0%						
Test for overall effect: *Z*=1.80 (*P*=0.07)						
Test for subgroup differences: not applicable						



B

Study or subgroup	Experimental events	Total	Control events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI
1-year OS						
Chen KR 2017	17	36	11	36	8.3	2.03 (0.77, 5.34)
Cui YQ 2008	40	61	22	54	11.5	2.77 (1.30, 5.91)
Ke J 2011	16	38	9	40	7.3	2.51 (0.94, 6.69)
Kou CY 2011	22	31	14	31	5.8	2.97 (1.04, 8.48)
Li Q 2008	32	50	19	46	10.2	2.53 (1.11, 5.76)
Liang Y 2008	40	48	22	48	5.2	5.91 (2.29, 15.25)
Liu XH 2009	33	42	20	42	6.1	4.03 (1.55, 10.47)
Mao WD 2013	24	27	21	27	3.3	2.29 (0.51, 10.29)
Song GP 2012	18	20	16	20	2.3	2.25 (0.36, 13.97)
Sun ZJ 2002	94	118	56	118	16.3	4.34 (2.44, 7.71)
Xue Q 2010	18	32	13	30	8.4	1.68 (0.62, 4.59)
Yang YG 2006	11	40	8	40	8.3	1.52 (0.54, 4.29)
Zeng BR 2009	15	23	14	23	7.0	1.21 (0.36, 4.00)
Subtotal (95% CI)		**566**		**555**	**100**	**2.84 (2.20, 3.67)**
Total events	380		245			
Heterogeneity: χ^2=10.05, *df*=12 (*P*=0.61); I^2=0%						
Test for overall effect: *Z*=7.97 (*P*<0.00001)						
Total (95% CI)		**566**		**555**	**100**	**2.84 (2.20, 3.67)**
Total events	380		245			
Heterogeneity: χ^2=10.05, *df*=12 (*P*=0.61); I^2=0%						
Test for overall effect: *Z*=7.97 (*P*<0.00001)						
Test for subgroup differences: not applicable						

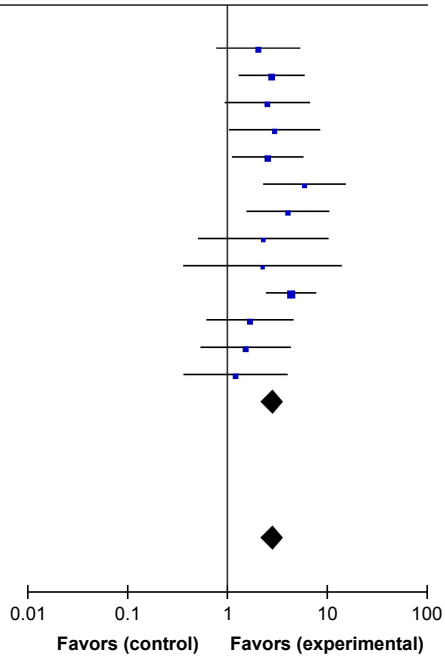
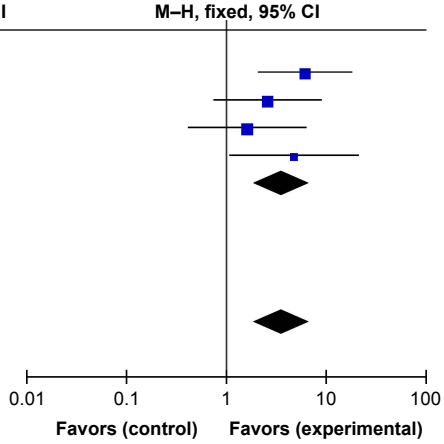


Figure 3 (*Continued*)

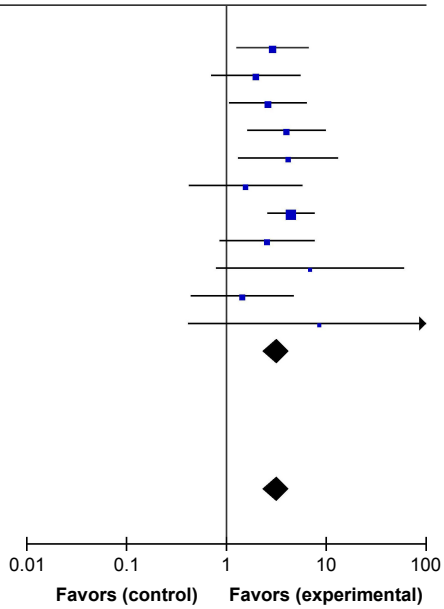
C

Study or subgroup	Experimental events	Total	Control events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI
1.5-year OS						
Liang Y 2008	20	48	5	48	26.5	6.14 (2.07, 18.26)
Mao WD 2013	22	27	17	27	28.6	2.59 (0.74, 9.00)
Song GP 2012	15	20	13	20	29.6	1.62 (0.41, 6.34)
Zhou JS 2006	9	21	3	22	15.2	4.75 (1.07, 21.14)
Subtotal (95% CI)		**116**		**117**	**100**	**3.57 (1.92, 6.66)**
Total events	66		38			
Heterogeneity: χ^2=2.64, *df*=3 (*P*=0.45); I^2=0%						
Test for overall effect: *Z*=4.00 (*P*<0.0001)						
Total (95% CI)		**116**		**117**	**100**	**3.57 (1.92, 6.66)**
Total events	66		38			
Heterogeneity: χ^2=2.64, *df*=3 (*P*=0.45); I^2=0%						
Test for overall effect: *Z*=4.00 (*P*<0.0001)						
Test for subgroup differences: not applicable						



D

Study or subgroup	Experimental events	Total	Control events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI
2-year OS						
Cui YQ 2008	26	61	11	54	13.1	2.90 (1.26, 6.69)
Kou CY 2011	15	31	10	31	10.1	1.97 (0.70, 5.52)
Li Q 2008	21	50	10	46	11.8	2.61 (1.06, 6.40)
Liu XH 2009	28	42	14	42	9.1	4.00 (1.61, 9.91)
Mao WD 2013	20	27	11	27	5.6	4.16 (1.31, 13.17)
Song GP 2012	14	20	12	20	7.0	1.56 (0.42, 5.76)
Sun ZJ 2002	80	118	38	118	23.9	4.43 (2.57, 7.65)
Xue Q 2010	14	32	7	30	8.0	2.56 (0.85, 7.65)
Yang YG 2006	6	40	1	40	1.7	6.88 (0.79, 60.06)
Zeng BR 2009	10	23	8	23	8.8	1.44 (0.44, 4.74)
Zhou JS 2006	3	21	0	22	0.8	8.51 (0.41, 175.57)
Subtotal (95% CI)		**465**		**453**	**100**	**3.17 (2.36, 4.25)**
Total events	237		122			
Heterogeneity: χ^2=6.82, *df*=10 (*P*=0.74); I^2=0%						
Test for overall effect: *Z*=7.70 (*P*<0.00001)						
Total (95% CI)		**465**		**453**	**100**	**3.17 (2.36, 4.25)**
Total events	237		122			
Heterogeneity: χ^2=6.82, *df*=10 (*P*=0.74); I^2=0%						
Test for overall effect: *Z*=7.70 (*P*<0.00001)						
Test for subgroup differences: not applicable						



E

Study or subgroup	Experimental events	Total	Control events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI
3-year OS						
Kou CY 2011	14	31	7	31	17.3	2.82 (0.94, 8.48)
Liu XH 2009	15	42	6	42	17.4	3.33 (1.14, 9.72)
Sun ZJ 2002	42	118	17	118	49.5	3.28 (1.74, 6.21)
Xue Q 2010	5	32	4	30	15.7	1.20 (0.29, 4.98)
Subtotal (95% CI)		**223**		**221**	**100**	**2.88 (1.82, 4.57)**
Total events	76		34			
Heterogeneity: χ^2=1.68, *df*=3 (*P*=0.64); I^2=0%						
Test for overall effect: *Z*=4.51 (*P*<0.00001)						
Total (95% CI)		**223**		**221**	**100**	**2.88 (1.82, 4.57)**
Total events	76		34			
Heterogeneity: χ^2=1.68, *df*=3 (*P*=0.64); I^2=0%						
Test for overall effect: *Z*=4.51 (*P*<0.00001)						
Test for subgroup differences: not applicable						

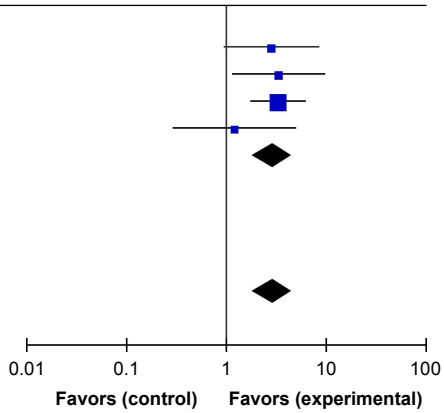
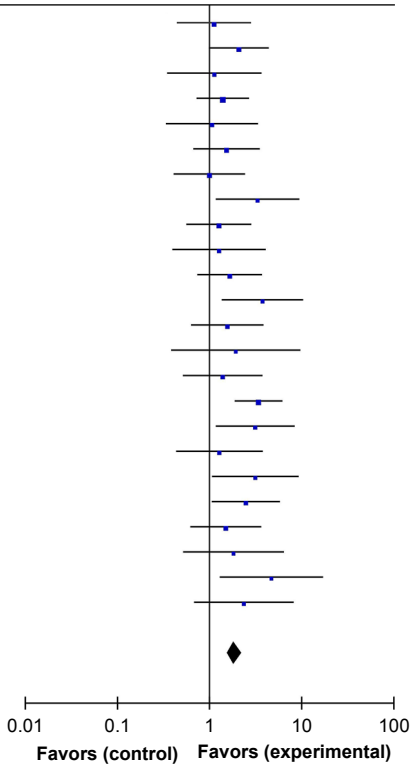


Figure 3 Forest plot of the comparison of 0.5-year (**A**), 1-year (**B**), 1.5-year (**C**), 2-year (**D**), and 3-year (**E**) OS between the experimental and control groups.
Notes: Control group, TACE alone group; experimental group, TACE+cinobufotalin injection combined therapy group. The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.
Abbreviations: OS, overall survival; TACE, transcatheter hepatic arterial chemoembolization.

A

Study or subgroup	Experimental events	Total	Control events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI
Chen KR 2017	18	36	17	36	5.3	1.12 (0.44, 2.82)
Cui YQ 2008	37	61	23	54	5.9	2.08 (0.99, 4.38)
Deng ZY 2015	9	25	8	24	3.2	1.13 (0.35, 3.65)
Fu ZL 2010	32	78	26	78	9.5	1.39 (0.72, 2.67)
He SL 2012	17	26	16	25	3.5	1.06 (0.34, 3.35)
Jia JY 2016	22	49	16	46	5.6	1.53 (0.67, 3.50)
Ke J 2011	18	38	19	40	6.0	0.99 (0.41, 2.42)
Kou CY 2011	21	31	12	31	2.4	3.33 (1.17, 9.44)
Li Q 2008	30	50	25	46	6.4	1.26 (0.56, 2.83)
Li XF 2014	18	26	16	25	3.1	1.27 (0.39, 4.06)
Liang Y 2008	28	48	22	48	5.7	1.65 (0.74, 3.71)
Liu XH 2009	35	42	24	42	2.5	3.75 (1.36, 10.36)
Liu YQ 2010	16	38	14	44	4.6	1.56 (0.63, 3.85)
Shen JJ 2015	5	18	3	18	1.3	1.92 (0.38, 9.65)
Su Y 2013	17	33	13	30	4.1	1.39 (0.51, 3.75)
Sun ZJ 2002	97	118	68	118	7.5	3.40 (1.87, 6.17)
Wang YF 2014	20	36	10	35	2.8	3.13 (1.17, 8.37)
Xue Q 2010	23	32	20	30	3.6	1.28 (0.43, 3.77)
Yan M 2010	22	30	14	30	2.3	3.14 (1.07, 9.27)
Yang GH 2014	29	45	19	45	4.2	2.48 (1.06, 5.80)
Yang YG 2006	24	40	20	40	4.9	1.50 (0.62, 3.64)
Yu JG 2013	8	30	5	30	2.3	1.82 (0.52, 6.38)
Zeng BR 2009	18	23	10	23	1.3	4.68 (1.29, 16.98)
Zhou JS 2006	11	21	7	22	2.0	2.36 (0.68, 8.15)
Total (95% CI)		**974**		**960**	**100**	**1.86 (1.54, 2.24)**
Total events	575		427			

Heterogeneity: χ^2=19.76, *df*=23 (*P*=0.66); I^2=0%
Test for overall effect: *Z*=6.52 (*P*<0.00001)



B

Study or subgroup	Experimental events	Total	Control events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI
Chen KR 2017	30	36	29	36	5.7	1.21 (0.36, 4.02)
Cui YQ 2008	48	61	35	54	9.3	2.00 (0.87, 4.59)
Deng ZY 2015	22	25	19	24	2.7	1.93 (0.41, 9.16)
Fu ZL 2010	70	78	63	78	7.6	2.08 (0.83, 5.24)
He SL 2012	22	26	20	25	3.7	1.38 (0.32, 5.85)
Jia JY 2016	43	49	37	46	5.5	1.74 (0.57, 5.36)
Ke J 2011	32	38	33	40	6.0	1.13 (0.34, 3.73)
Kou CY 2011	26	31	19	31	3.6	3.28 (0.99, 10.90)
Li Q 2008	42	50	41	46	8.0	0.64 (0.19, 2.12)
Li XF 2014	22	26	20	25	3.7	1.38 (0.32, 5.85)
Liang Y 2008	44	48	36	48	3.5	3.67 (1.09, 12.35)
Liu XH 2009	37	42	31	42	4.3	2.63 (0.82, 8.37)
Liu YQ 2010	34	38	38	44	4.4	1.34 (0.35, 5.16)
Shen JJ 2015	13	18	9	18	2.9	2.60 (0.65, 10.38)
Su Y 2013	29	33	23	30	3.4	2.21 (0.57, 8.47)
Wang YF 2014	30	36	26	35	5.2	1.73 (0.54, 5.51)
Xue Q 2010	29	32	26	30	3.0	1.49 (0.30, 7.28)
Yan M 2010	26	30	22	30	3.4	2.36 (0.63, 8.92)
Yang GH 2014	38	45	34	45	6.2	1.76 (0.61, 5.04)
Yang YG 2006	36	40	30	40	3.5	3.00 (0.85, 10.54)
Yu JG 2013	26	30	17	30	2.7	4.97 (1.39, 17.82)
Zeng BR 2009	22	23	17	23	0.9	7.76 (0.85, 70.75)
Zhou JS 2006	20	21	16	22	0.9	7.50 (0.82, 68.83)
Total (95% CI)		**856**		**842**	**100**	**2.05 (1.59, 2.64)**
Total events	741		641			

Heterogeneity: χ^2=13.43, *df*=22 (*P*=0.92); I^2=0%
Test for overall effect: *Z*=5.53 (*P*<0.00001)

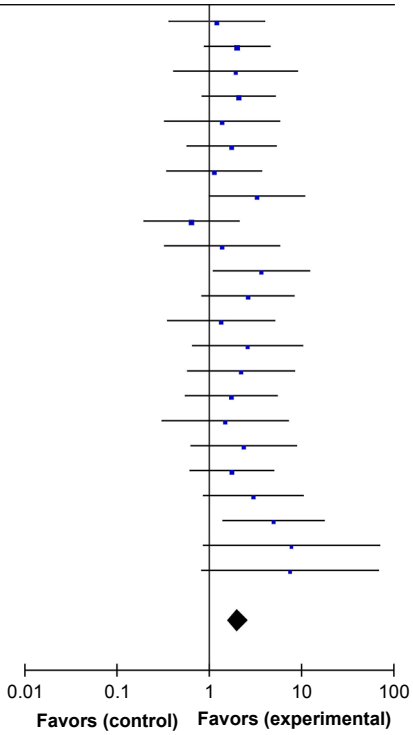


Figure 4 Forest plot of the comparison of ORR (**A**) and DCR (**B**) between the experimental group and the control group.
Notes: Control group, TACE alone group; experimental group, TACE+cinobufotalin injection combined therapy group. The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.
Abbreviations: DCR, disease control rate; ORR, overall response rate; TACE, transcatheter hepatic arterial chemoembolization.

Table 3 Comparison of CR, PR, SD, PD, ORR, and DCR between the TACE and TACE+cinobufotalin injection groups

Parameter	TACE+cinobufotalin injection group (n)	TACE group (n)	Analysis method	Heterogeneity		OR	95% CI	*P*-value
				I^2 (%)	*P*-value			
CR	816	802	Fixed	0	0.93	1.73	1.04–2.87	0.03
PR	816	802	Fixed	0	0.94	1.61	1.31–1.97	<0.00001
SD	856	842	Fixed	0	0.86	0.88	0.72–1.09	0.25
PD	856	842	Fixed	0	0.99	0.46	0.35–0.59	<0.00001
ORR	974	960	Fixed	0	0.66	1.86	1.54–2.24	<0.00001
DCR	856	842	Fixed	0	0.92	2.05	1.59–2.64	<0.00001

Abbreviations: CR, complete response; DCR, disease control rate; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease; TACE, transcatheter hepatic arterial chemoembolization.

of published clinical trials, the exact therapeutic effects were still not systematically evaluated because of small sample sizes and different applied protocols in different studies. In this analysis, we conducted a wide range of online search according to the strict inclusion and exclusion criteria, by which to provide clear and systematical conclusion.

Our meta-analysis revealed that TACE combined with cinobufotalin injection adjuvant therapy is associated with a favorable efficacy compared to HC patients treated by TACE alone. Compared to patients treated by TACE alone, patients treated with combined therapy showed markedly increased 1- to 3-year OS, CR rate, PR rate, ORR, DCR, and QIR ($P<0.05$). Moreover, after TACE and cinobufotalin injection combined treatment, the liver function of HC patients was obviously improved, indicated by increased ALB and decreased TBIL, ALT, and AST, although changes in ALB did not show statistical significance. These results indicated that intravenous infusion of cinobufotalin injection could increase the curative effect of TACE.

The immunosuppressed status of cancer patients has been reported previously.[47] Therefore, immune system reconstruction is one of the critical factors to effectively treat

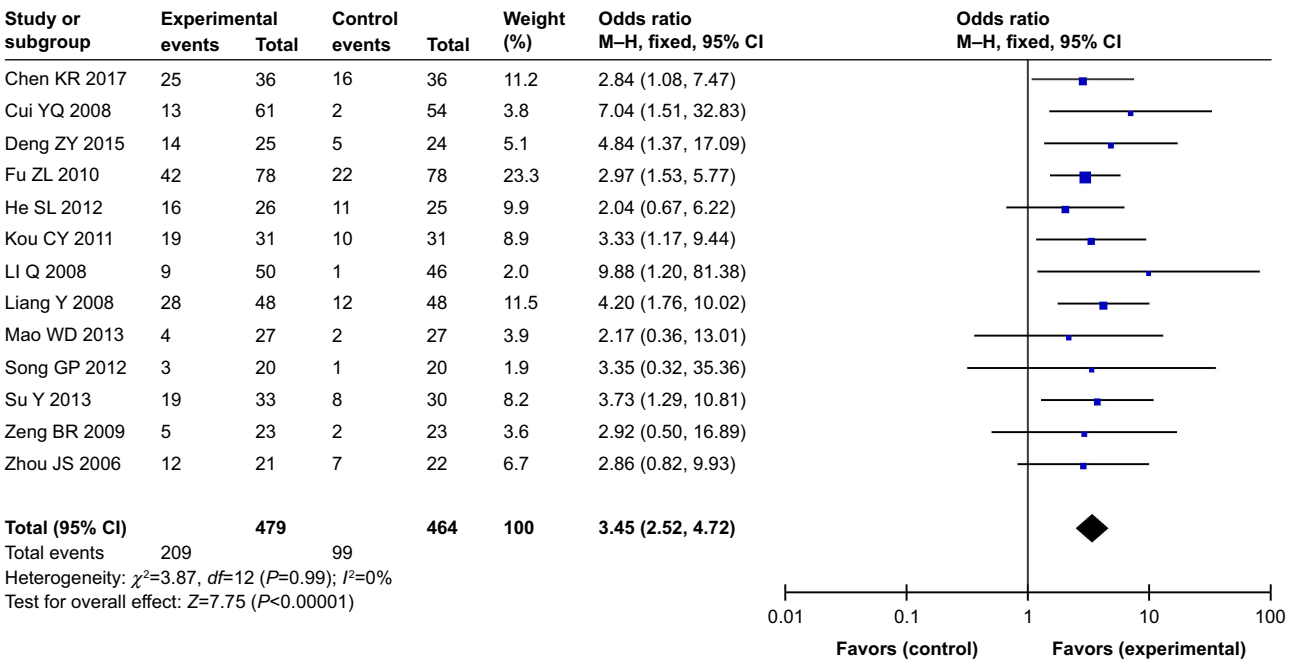


Figure 5 Forest plot of the comparison of QIR between the experimental group and the control group.
Notes: Control group, TACE alone group; experimental group, TACE+cinobufotalin injection combined therapy group. The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.
Abbreviations: M–H, Mantel–Haenszel; QIR, quality of life improved rate; TACE, transcatheter hepatic arterial chemoembolization.

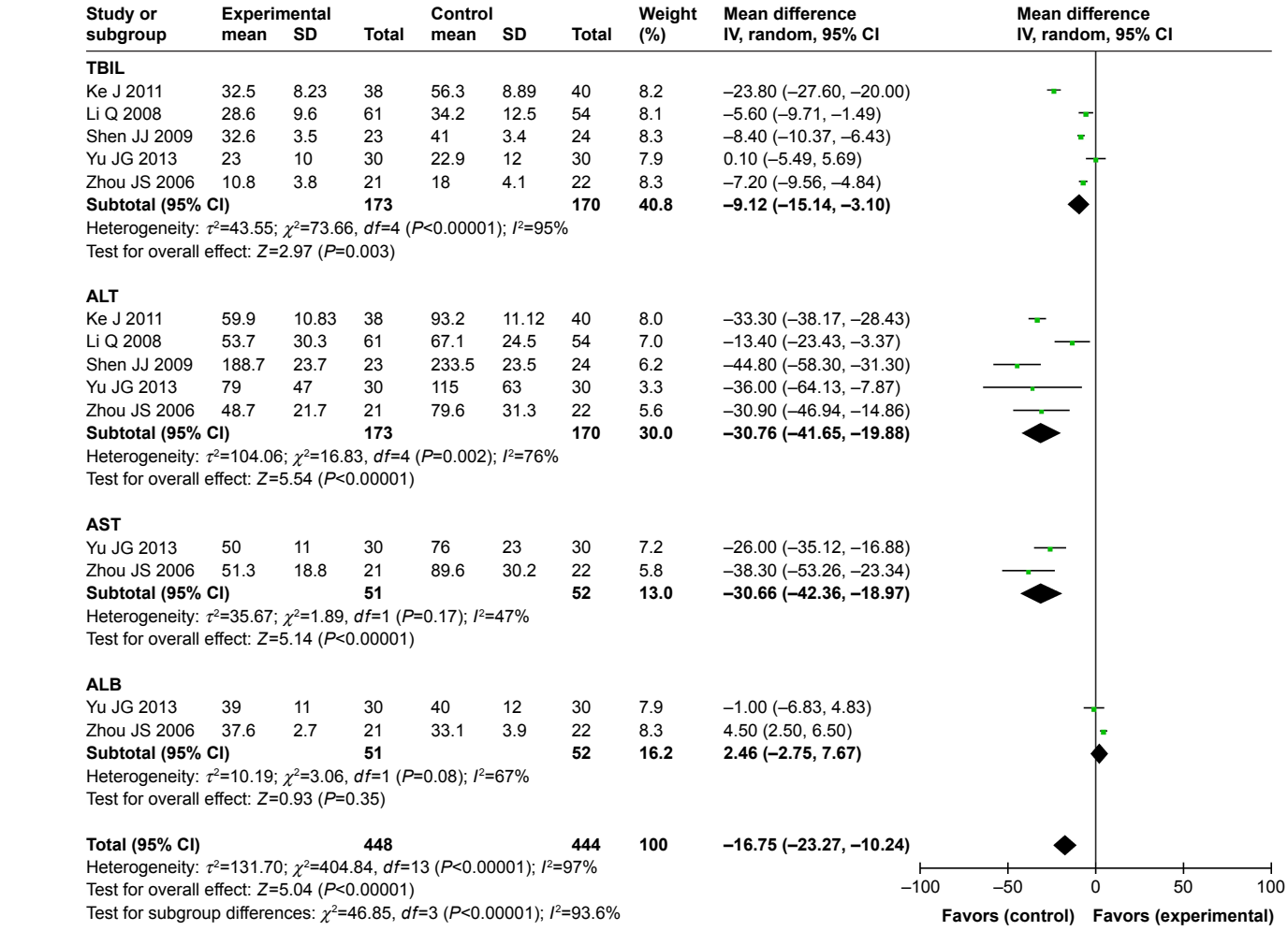


Figure 6 Forest plot of the comparison of liver function indexes including TBIL, ALT, AST, and ALB between the experimental group and the control group.
Notes: Control group, TACE alone group; experimental group, TACE+cinobufotalin injection combined therapy group. The random effects meta-analysis model (inverse variance method) was used.
Abbreviations: ALB, serum albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IV, inverse variance TACE, transcatheter hepatic arterial chemoembolization; TBIL, total bilirubin.

malignancies.[47] Many studies reported that cinobufotalin injection can enhance the ability of body's immunity and resistance to tumors by increasing the IL-2 and interferon (IFN)-γ secretion of T cells and the activities of NK cells and by promoting the maturation of dendritic cells and upregulating the expression of costimulatory molecules in dendritic cells. Our analysis showed significantly increased percentages of $CD3^+$, $CD4^+$, NK, and $CD3^+CD56^+$ T cells and $CD4^+/CD8^+$ ratio, indicating that immune function of HC patients was improved after cinobufotalin injection-mediated therapy.

Safety is the top priority of the clinical treatment, and it is also a key factor for the development of cinobufotalin injection-mediated therapy. Our analysis showed no significant difference in most adverse events between the two groups, while the myelosuppression caused by TACE was obviously alleviated ($P<0.05$), which proves the safety of cinobufotalin injection treatment for advanced HC.

Some factors may have influence on the therapeutic effects of cinobufotalin injection treatment. In our study, subgroup analysis was used for evaluating the impact of study design and sample size on therapeutic effects of cinobufotalin injection-mediated therapy. Our results found no difference between different study designs and sample sizes of studies in most indexes, except 0.5-year OS. However, currently, studies probing the impact of these factors on treatment effects of cinobufotalin injection adjuvant therapy are still insufficient, and these should be further researched and explored.

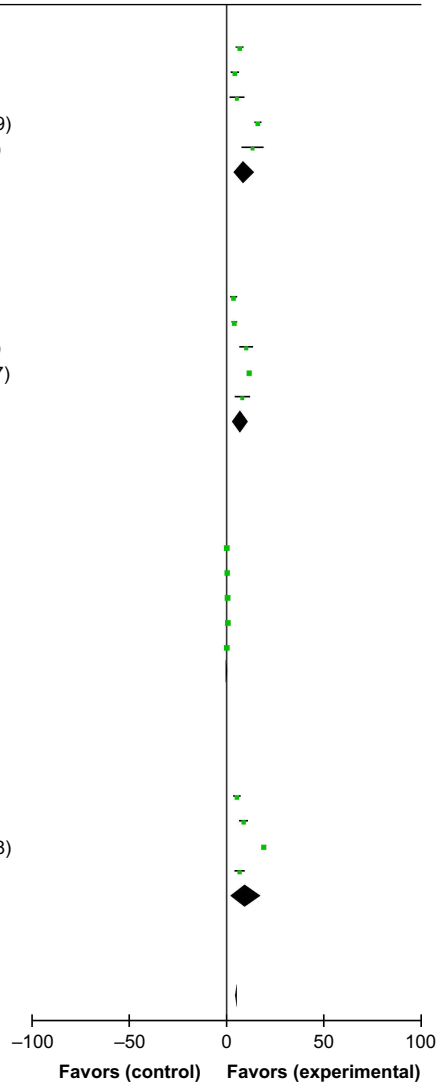


Figure 7 Forest plot of the comparison of immune function indexes including percentage of $CD3^+$, $CD4^+$, and NK cells and $CD4^+/CD8^+$ ratio between the experimental group and the control group.

Notes: Control group, TACE alone group; experimental group, TACE+cinobufotalin injection combined therapy group. The random effects meta-analysis model (inverse variance method) was used.

Abbreviations: NK, natural killer; TACE, transcatheter hepatic arterial chemoembolization.

There are a few limitations in our study. First, all included researches were performed in different medical institutions in China, which may bring in regional bias and influence the clinical application of cinobufotalin injection-mediated therapy worldwide. In addition, different trials evaluated the treatment efficacy with different outcomes, resulting in a reduction in the size of the statistical sample, making it difficult to summarize the results at the same scale. Finally, the therapeutic effects of the combined therapy may be influenced by numerous variables such as chemotherapeutics types, tumor stage, tumor size, and patient's age. Due to the above limitations, future studies and generated data will be valuable to further verify the safety and efficacy of cinobufotalin injection-mediated therapy.

In summary, our study confirmed that TACE combined with cinobufotalin injection adjuvant therapy was an effective treatment for advanced HC patients. Intravenous infusion of cinobufotalin injection markedly enhanced the treatment efficacy of TACE for advanced HC. Moreover, cinobufotalin injection-mediated therapy can effectively improve the quality of life, immune function, and liver function of HC patients. Therefore, cinobufotalin injection-mediated therapy could be recommended as an adjuvant treatment method for end-stage HC.

Study or subgroup	Experimental events	Experimental Total	Control events	Control Total	Weight (%)	Odds ratio M–H, random, 95% CI
Leukopenia						
He SL 2012	23	26	21	25	5.0	1.46 (0.29, 7.30)
Liang Y 2008	43	48	13	48	5.9	23.15 (7.53, 71.23)
Liu YQ 2010	31	38	39	44	5.7	0.57 (0.16, 1.96)
Subtotal (95% CI)		**112**		**117**	**16.6**	**2.74 (0.25, 30.43)**
Total events	97		73			
Heterogeneity: τ^2=4.06; χ^2=20.28, *df*=2 (*P*<0.0001); I^2=90%						
Test for overall effect: *Z*=0.82 (*P*=0.41)						
Thrombocytopenia						
He SL 2012	10	26	9	25	5.9	1.11 (0.36, 3.46)
Liu YQ 2010	33	38	38	44	5.6	1.04 (0.29, 3.73)
Subtotal (95% CI)		**64**		**69**	**11.5**	**1.08 (0.46, 2.52)**
Total events	43		47			
Heterogeneity: τ^2=0.00; χ^2=0.01, *df*=1 (*P*=0.94); I^2=0%						
Test for overall effect: *Z*=0.18 (*P*=0.86)						
Nausea, vomiting						
Deng ZY 2015	3	25	10	24	5.3	0.19 (0.04, 0.82)
Liu YQ 2010	29	38	37	44	5.9	0.61 (0.20, 1.83)
Shen JJ 2009	10	23	9	24	5.8	1.28 (0.40, 4.12)
Subtotal (95% CI)		**86**		**92**	**17.1**	**0.57 (0.21, 1.57)**
Total events	42		56			
Heterogeneity: τ^2=0.40; χ^2=4.01, *df*=2 (*P*=0.13); I^2=50%						
Test for overall effect: *Z*=1.09 (*P*=0.28)						
Fever						
He SL 2012	7	26	19	25	5.7	0.12 (0.03, 0.41)
Liang Y 2008	25	48	2	48	5.2	25.00 (5.44, 114.85)
Liu YQ 2010	33	38	39	44	5.5	0.85 (0.23, 3.18)
Shen JJ 2009	8	23	8	24	5.7	1.07 (0.32, 3.57)
Subtotal (95% CI)		**135**		**141**	**22.1**	**1.23 (0.16, 9.78)**
Total events	73		68			
Heterogeneity: τ^2=4.00; χ^2=29.42, *df*=3 (*P*<0.00001); I^2=90%						
Test for overall effect: *Z*=0.20 (*P*=0.84)						
Hepatotoxicity						
Deng ZY 2015	9	25	16	24	5.8	0.28 (0.09, 0.91)
Liang Y 2008	47	48	45	48	3.9	3.13 (0.31, 31.25)
Liu YQ 2010	33	38	37	44	5.7	1.25 (0.36, 4.31)
Subtotal (95% CI)		**111**		**116**	**15.4**	**0.83 (0.22, 3.13)**
Total events	89		98			
Heterogeneity: τ^2=0.77; χ^2=4.78, *df*=2 (*P*=0.09); I^2=58%						
Test for overall effect: *Z*=0.27 (*P*=0.79)						
Myelosuppression						
Deng ZY 2015	4	25	10	24	5.5	0.27 (0.07, 1.02)
Fu ZL 2010	78	78	78	78		Not estimable
Su Y 2013	14	33	19	30	6.1	0.43 (0.15, 1.18)
Xue Q 2010	5	32	15	30	5.8	0.19 (0.06, 0.61)
Subtotal (95% CI)		**168**		**162**	**17.4**	**0.29 (0.15, 0.57)**
Total events	101		122			
Heterogeneity: τ^2=0.00; χ^2=1.11, *df*=2 (*P*=0.57); I^2=0%						
Test for overall effect: *Z*=3.61 (*P*=0.0003)						
Total (95% CI)		**676**		**697**	**100**	**0.89 (0.45, 1.73)**
Total events	445		464			
Heterogeneity: τ^2=1.67; χ^2=85.31, *df*=17 (*P*<0.00001); I^2=80%						
Test for overall effect: *Z*=0.35 (*P*=0.72)						
Test for subgroup differences: χ^2=8.51, *df*=5 (*P*=0.13); I^2=41.3%						

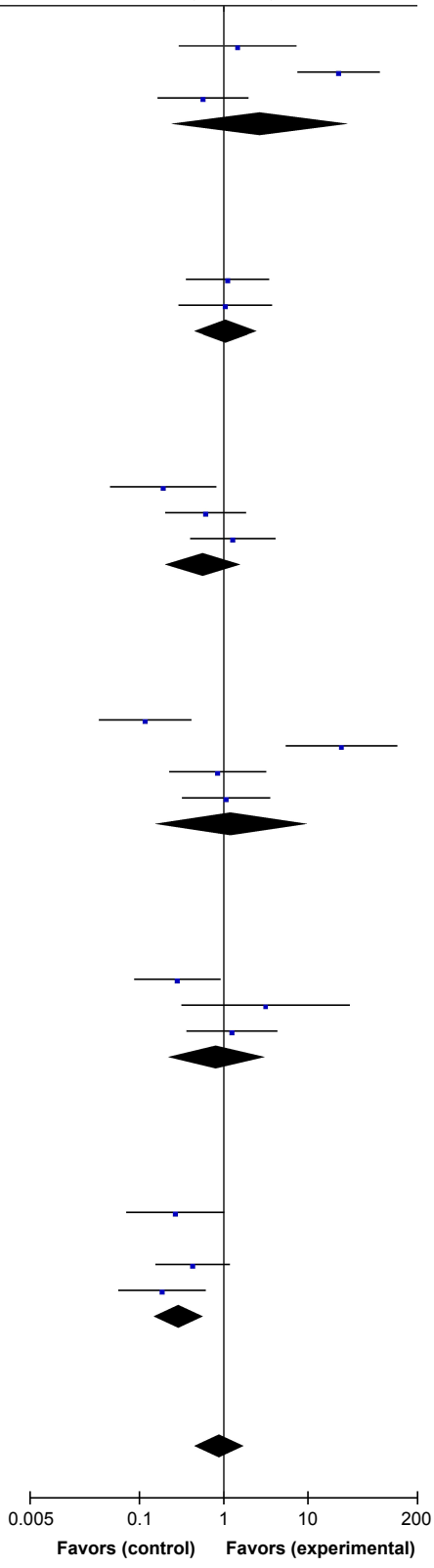


Figure 8 Forest plot of the comparison of adverse effects including leukopenia, thrombocytopenia, diarrhea, nausea and vomiting, fever, hepatotoxicity, and myelosuppression between the experimental group and the control group.

Notes: Control group, TACE alone group; experimental group, TACE+cinobufotalin injection combined therapy group. The random effects meta-analysis model (inverse variance method) was used.

Abbreviation: TACE, transcatheter hepatic arterial chemoembolization.

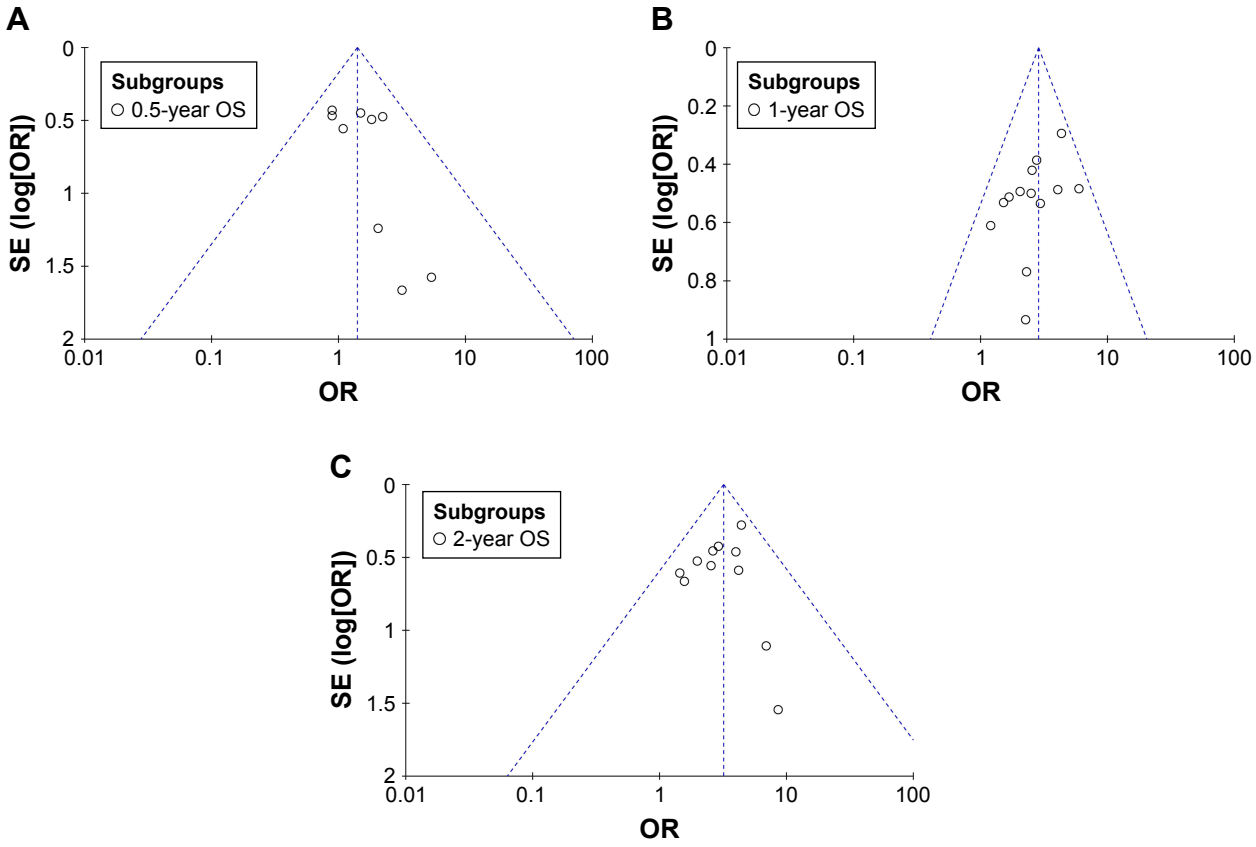


Figure 9 Funnel plot of 0.5-year (**A**), 1-year (**B**), and 2-year (**C**) OS.
Abbreviation: OS, overall survival; SE, standard error.

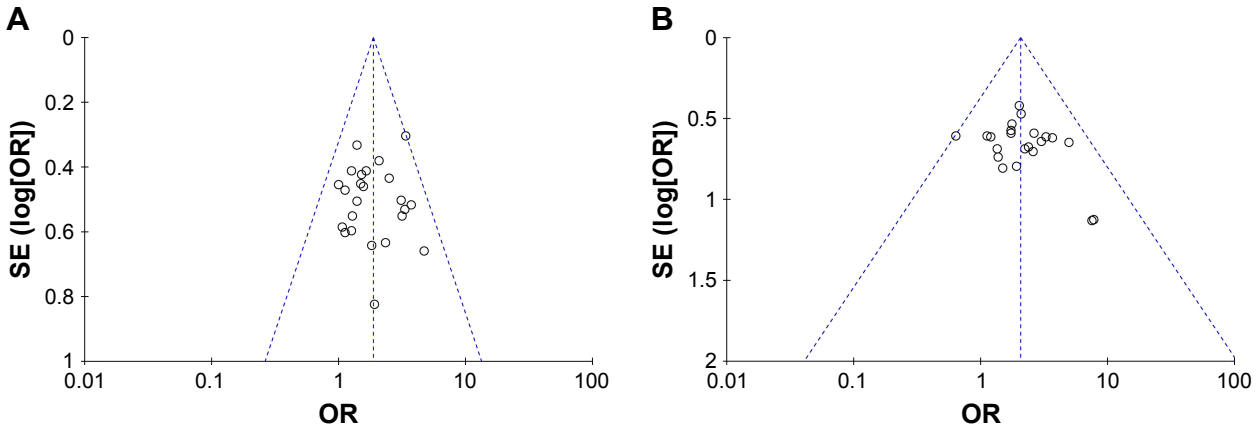


Figure 10 Funnel plot of ORR (**A**) and DCR (**B**).
Abbreviations: DCR, disease control rate; ORR, overall response rate; SE, standard error.

Table 4 Publication bias on OS, CR, PR, SD, PD, ORR, DCR, and QIR

Publication bias	0.5-year OS	1-year OS	2-year OS	CR	PR	SD	PD	ORR	DCR	QIR
Begg	0.152	0.077	0.755	0.436	0.195	0.492	0.413	0.747	0.444	0.300
Egger	0.110	0.070	0.564	0.151	0.191	0.383	0.134	0.821	0.207	0.335

Abbreviations: CR, complete response; DCR, disease control rate; ORR, overall response rate; OS, overall survival; PD, progressive disease; PR, partial response; QIR, quality of life improved rate; SD, stable disease.

Table 5 Subgroup analyses of ORR and DCR between the Exp and Con groups

Parameter	Factors at study level	Exp group (n)	Con group (n)	Analysis method	Heterogeneity		OR	95% CI	*P*-value
					I^2 (%)	*P*-value			
0.5-year OS	**Study sample size**								
	≥80	199	188	Fixed	0	0.77	1.09	0.67–1.79	0.73
	<80	176	176	Fixed	0	0.89	1.86	1.08–3.19	0.02
	Type of control trials								
	RCT	314	310	Fixed	0	0.90	1.55	1.03–2.32	0.03
	Total	375	364	Fixed	0	0.86	1.40	0.97–2.01	0.07
1-year OS	**Study sample size**								
	≥80	359	348	Fixed	4	0.39	3.41	2.47–4.71	<0.00001
	<80	207	207	Fixed	0	0.95	2.07	1.35–3.17	0.0008
	Type of control trials								
	RCT	505	501	Fixed	0	0.53	2.85	2.17–3.75	<0.00001
	Total	566	555	Fixed	0	0.61	2.84	2.20–3.67	<0.00001
2-year OS	**Study sample size**								
	≥80	311	300	Fixed	0	0.80	3.74	2.59–5.38	<0.00001
	<80	154	153	Fixed	0	0.73	2.33	1.42–3.83	0.0009
	Type of control trials								
	RCT	404	399	Fixed	0	0.66	3.21	2.35–4.39	<0.00001
	Total	465	453	Fixed	0	0.74	3.17	2.36–4.25	<0.00001
ORR	**Study sample size**								
	≥80	569	561	Fixed	0	0.49	1.96	1.53–2.50	<0.00001
	<80	405	399	Fixed	0	0.62	1.74	1.30–2.32	0.0002
	Type of control trials								
	RCT	883	876	Fixed	0	0.61	1.82	1.49–2.21	<0.00001
	Total	974	960	Fixed	0	0.66	1.86	1.54–2.24	<0.00001
DCR	**Study sample size**								
	≥80	451	443	Fixed	0	0.71	1.93	1.35–2.75	0.0003
	<80	405	399	Fixed	0	0.85	2.18	1.52–3.14	<0.0001
	Type of control trials								
	RCT	765	758	Fixed	0	0.86	2.04	1.55–2.68	<0.00001
	Total	856	842	Fixed	0	0.92	2.05	1.59–2.64	<0.00001
QIR	**Study sample size**								
	≥80	237	226	Fixed	0	0.57	4.04	2.50–6.52	<0.00001
	<80	242	238	Fixed	0	0.99	3.05	2.02–4.63	<0.00001
	Type of control trials								
	RCT	418	410	Fixed	0	0.99	3.31	2.40–4.56	<0.00001
	Total	479	464	Fixed	0	0.99	3.45	2.52–4.72	<0.00001

Abbreviations: Con, control group (TACE alone group); DCR, disease control rate; Exp, experimental group (TACE plus cinobufotalin injection adjuvant therapy); ORR, overall response rate; OS, overall survival; QIR, quality of life improved rate; RCT, randomized controlled trial.

Author contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Application sequence of and manufacturer of cinobufotalin injection

Included studies	Application sequence of cinobufotalin injection	Manufacturer
Chen et al (2017)[1]	After TACE	No description
Cui (2008)[2]	Used simultaneously	No description
Deng and Duan (2015)[3]	Used simultaneously	No description
Fu et al (2010)[4]	Used simultaneously	Anhui Golden Toad Biochemical Corp, Ltd
He et al (2012)[5]	After TACE	No description
Jia (2016)[6]	Used simultaneously	Anhui Golden Toad Biochemical Corp, Ltd
Ke et al (2011)[7]	Used simultaneously	No description
Kou and Xu (2011)[8]	After TACE	No description
Li et al (2008)[9]	Used simultaneously	No description
Li (2014)[10]	Used simultaneously	No description
Liang et al (2008)[11]	After TACE	No description
Liu et al (2009)[12]	Used simultaneously	No description
Liu et al (2010)[13]	Used simultaneously	No description
Mao (2013)[14]	Used simultaneously	No description
Shen (2009)[15]	After TACE	No description
Shen and Tan (2015)[16]	After TACE	No description
Song (2012)[17]	Used simultaneously	Anhui Golden Toad Biochemical Corp, Ltd
Su et al (2013)[18]	After TACE	Anhui Golden Toad Biochemical Corp, Ltd
Sun et al (2002)[19]	After TACE	No description
Wang (2014)[20]	Used simultaneously	No description
Xue et al (2010)[21]	Used simultaneously	Anhui Golden Toad Biochemical Corp, Ltd
Yan and Bai (2010)[22]	Used simultaneously	No description
Yang et al (2014)[23]	Used simultaneously	Anhui Golden Toad Biochemical Corp, Ltd
Yang et al (2006)[24]	Before TACE	Anhui Golden Toad Biochemical Corp, Ltd
Yu (2013)[25]	Used simultaneously	No description
Zeng et al (2009)[26]	Before TACE	Anhui Golden Toad Biochemical Corp, Ltd
Zhou et al (2006)[27]	After TACE	Anhui Golden Toad Biochemical Corp, Ltd

Abbreviation: TACE, transcatheter hepatic arterial chemoembolization.

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