# 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

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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<sup>+</sup>, P=0.001; CD4<sup>+</sup>, P=0.0006; CD4<sup>+</sup>/CD8<sup>+</sup>, 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.<sup>1-3</sup> Over the past 20 years, the number of HC-related deaths has increased by 62%.<sup>4</sup> China is a high-risk area for HC and accounts for more than half of the HC cases worldwide.<sup>4,5</sup> Despite the development of diagnostic methods, early detection of HC is still difficult.<sup>2</sup> In most

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Transcatheter hepatic arterial chemoembolization (TACE) is the current standard locoregional treatment for advanced HC.<sup>2,6</sup> Several studies reported that TACE significantly increased the survival time in HC patients compared to supportive treatments.<sup>7,8</sup> However, TACE also has its own limitations, as it can further influence the liver functions and damage the hepatic arterial system of patients.<sup>2,9</sup> In addition, its clinical application was also limited by drug resistance and toxic side effects.<sup>1</sup> 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.<sup>4,10–12</sup> Cinobufotalin, a cardiotonic steroid or bufadienolide, is extracted from the skin secretions of the traditional Chinese medicinal giant toads.<sup>12–15</sup> Many studies have shown that cinobufotalin has anti-tumor activity and can enhance the treatment effect of chemotherapeutics for malignancies.<sup>12,15,16</sup> 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.<sup>17</sup> In addition, it can also induce tumor cells apoptosis through decreasing ROS production and by destroying the structure of DNA in cancer cells.<sup>14,16</sup>

Up to now, several clinic trials have been conducted to evaluate the therapeutic effects between TACE and TACE+cinobufotalin injection in advanced HC patients.<sup>18-44</sup> 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.<sup>45</sup>

#### 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<sup>+</sup>/CD8<sup>+</sup> 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

# **Results** Search results

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.<sup>46</sup> 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 betweenstudy 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.<sup>47,48</sup> When publication bias existed, trim-and-fill method was applied to adjust the pooled estimates of potentially unpublished studies.

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<sup>19-37</sup> involving 2,079 advanced HC patients were included in this analysis (Figure 1).



Figure I 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,<sup>19–21,23,24,26,27,29–31,34,37–40,42</sup> whereas cinobufotalin injection was used after TACE in nine articles<sup>18,22,25,28,32,33,35,36,44</sup> and was used before TACE in two studies.<sup>41,43</sup> 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. Twentyfive 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

| Included studies                   | Nation | Stage          | Patients  | Age (years)      |                  | Parameter types       |
|------------------------------------|--------|----------------|-----------|------------------|------------------|-----------------------|
|                                    |        |                | (Con/exp) | Con              | Ехр              |                       |
| Chen et al (2017) <sup>18</sup>    | China  | Child-Pugh A–B | 36/36     | ND               | ND               | OS, ORR, DCR, QIR     |
| Cui (2008) <sup>19</sup>           | China  | Child-Pugh A–B | 54/61     | ND               | ND               | OS, ORR, DCR, QIR     |
| Deng and Duan (2015) <sup>20</sup> | China  | ND             | 27/26     | 48.3±16.2 (mean) | 48.7±16.1 (mean) | ORR, DCR, QIR, AE     |
| Fu et al (2010) <sup>21</sup>      | China  | KPS≥60         | 78/78     | 56 (median)      | 58 (median)      | ORR, DCR, QIR, AE     |
| He et al (2012) <sup>22</sup>      | China  | Child-Pugh A–B | 25/26     | >60 (20)         | >60 (19)         | ORR, DCR, QIR, AE     |
| Jia (2016) <sup>23</sup>           | China  | Child-Pugh A–B | 46/49     | 58.1±8.7 (mean)  | 58.4±8.3 (mean)  | ORR, DCR              |
| Ke et al (2011) <sup>24</sup>      | China  | Child-Pugh A–B | 40/38     | 57.1±11.8 (mean) | 58.3±11.6 (mean) | OS, ORR, DCR          |
| Kou and Xu (2011) <sup>25</sup>    | China  | KPS>60         | 31/31     | 41 (mean)        | 40.5 (mean)      | OS, ORR, DCR, QIR     |
| Li et al (2008) <sup>26</sup>      | China  | Child-Pugh A–B | 46/50     | ND               | ND               | OS, ORR, DCR, QIR     |
| Li (2014) <sup>27</sup>            | China  | ND             | 25/26     | 61.7±6.8 (mean)  | 57.4±6.2 (mean)  | ORR, DCR              |
| Liang et al (2008) <sup>28</sup>   | China  | Child-Pugh A–C | 48/48     | ND               | ND               | OS, ORR, DCR, QIR, AE |
| Liu et al (2009) <sup>29</sup>     | China  | ND             | 42/42     | ND               | ND               | OS, ORR, DCR          |
| Liu et al (2010) <sup>30</sup>     | China  | Child-Pugh A–B | 44/38     | 55.3±11.6 (mean) | 54.2±10.3 (mean) | ORR, DCR, AE          |
| Mao (2013) <sup>31</sup>           | China  | 1–111          | 27/27     | 48.3±8.9 (mean)  | 47.6±9.3 (mean)  | OS, QIR               |
| Shen (2009) <sup>32</sup>          | China  | 11–111         | 24/23     | ND               | ND               | AE                    |
| Shen and Tan (2015) <sup>33</sup>  | China  | Child-Pugh A–B | 18/18     | 54.7 (mean)      | 57.5 (mean)      | ORR, DCR              |
| Song (2012) <sup>34</sup>          | China  | I–II           | 20/20     | 49.8±6.4 (mean)  | 50.3±8.1 (mean)  | OS, QIR               |
| Su et al (2013) <sup>35</sup>      | China  | 11–111         | 30/33     | 52.7±7.9 (mean)  | 53.2±8.7 (mean)  | ORR, DCR, QIR, AE     |
| Sun et al (2002) <sup>36</sup>     | China  | ND             | 118/118   | ND               | ND               | OS, ORR               |
| Wang (2014) <sup>37</sup>          | China  | III–IV         | 35/36     | ND               | ND               | ORR, DCR              |
| Xue et al (2010) <sup>38</sup>     | China  | KPS>60         | 30/32     | 45.5±10.7 (mean) | 45.8±11.4 (mean) | OS, ORR, DCR, AE      |
| Yan and Bai (2010) <sup>39</sup>   | 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) <sup>42</sup>            | China  | KPS>60         | 30/30     | 50.8 (mean)      | 49.7 (mean)      | ORR, DCR              |
| Zeng et al (2009) <sup>43</sup>    | 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) <sup>44</sup>    | China  | Child-Pugh A–C | 22/21     | ND               | ND               | OS, ORR, DCR          |

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

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.

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

#### **Table 2** Information of TACE combined with cinobufotalin injection adjuvant therapy

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<sup>18–22,25,26,28,31,34,35,43,44</sup> 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<sup>24,26,32,42,44</sup> 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.<sup>23,26,29,36,43</sup> Compared with TACE alone, the percentages of CD3<sup>+</sup>, CD4<sup>+</sup>, and NK cells, and CD4<sup>+</sup>/CD8<sup>+</sup> ratio in the combined treatment group were significantly increased (Figure 7; CD3<sup>+</sup>, OR=9.05, 95% CI=3.62–14.49, *P*=0.001; CD4<sup>+</sup>, 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<sup>+</sup>/CD8<sup>+</sup>, 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.<sup>20–22,28,30,32,35,38</sup> 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=0.24–2.52, 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,

## Α

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

| Study or                  | Experime              | ental                   | Control |       | Weight | Odds ratio          | Odds             | ratio                 |
|---------------------------|-----------------------|-------------------------|---------|-------|--------|---------------------|------------------|-----------------------|
| subgroup                  | events                | Total                   | events  | Total | (%)    | M–H, fixed, 95% Cl  | M–H, f           | ixed, 95% Cl          |
| 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)   |                  | <b>_</b>              |
| Ke J 2011                 | 27                    | 38                      | 21      | 40    | 12.0   | 2.22 (0.87, 5.66)   |                  | <b>—</b>              |
| Li Q 2008                 | 37                    | 50                      | 35      | 46    | 19.2   | 0.89 (0.35, 2.26)   |                  | <b>_</b>              |
| 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)   |                  | <b>_</b>              |
| Yang YG 2006              | 23                    | 40                      | 19      | 40    | 16.3   | 1.50 (0.62, 3.61)   | -                | + <b>-</b>            |
| 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: $\chi^{2}$ | =4.72, df=9 (         | P=0.86); I <sup>2</sup> | =0%     |       |        |                     |                  |                       |
| Test for overall eff      | fect: Z=1.80 (        | <i>P</i> =0.07)         |         |       |        |                     |                  |                       |
| Total (95% CI)            |                       | 375                     |         | 364   | 100    | 1.40 (0.97, 2.01)   |                  |                       |
| Total events              | 297                   |                         | 269     |       |        |                     |                  | •                     |
| Heterogeneity: $\chi^{2}$ | =4.72, <i>df</i> =9 ( | P=0.86); I <sup>2</sup> | =0%     |       |        | F                   |                  | +                     |
| Test for overall eff      | fect: Z=1.80 (        | <i>P</i> =0.07)         |         |       |        | 0.0                 | 1 0.1            | 1 10 100              |
| Test for subgroup         | differences:          | not applical            | ble     |       |        |                     | Favors (control) | Favors (experimental) |

#### В

| Study or<br>subgroup  | Experime<br>events                             | ental<br>Total                              | Control events    | Total | Weight<br>(%) | Odds ratio<br>M–H, fixed, 95% Cl |           | Odds<br>M–H,     | ratio<br>fixed, 95% Cl |         |
|---|--|---|-------------------|-------|---------------|----------------------------------|-----------|------------------|------------------------|---------|
| 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)               |           |                  | <b>—</b>               |         |
| Sun ZJ 2002   | 94   | 118   | 56                | 118   | 16.3          | 4.34 (2.44, 7.71)                |           |                  | _ <b>_</b>             |         |
| 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)                |           | -                | <b></b>                |         |
| Zeng BR 2009  | 15   | 23  | 14                | 23    | 7.0           | 1.21 (0.36, 4.00)                |           | _                | _ <b>_</b>             |         |
| Subtotal (95% Cl  | I)   | 566   |                   | 555   | 100           | 2.84 (2.20, 3.67)                |           |                  | •                      |         |
| Total events  | 380  |   | 245               |       |               |                                  |           |                  | •                      |         |
| Heterogeneity: $\chi^2$<br>Test for overall eff   | =10.05, <i>df</i> =1<br>fect: <i>Z</i> =7.97 ( | 2 ( <i>P</i> =0.61);<br>( <i>P</i> <0.00001 | /²=0%<br>)        |       |               |                                  |           |                  |                        |         |
| Total (95% CI)  |  | 566   |                   | 555   | 100           | 2.84 (2.20, 3.67)                |           |                  | •                      |         |
| Total events  | 380  |   | 245               |       |               |                                  |           |                  |                        |         |
| Heterogeneity: $\chi^2$ =10.05, <i>df</i> =12 ( <i>P</i> =0.61); <i>l</i> <sup>2</sup> =0%<br>Test for overall effect: <i>Z</i> =7.97 ( <i>P</i> <0.0001) |  |   | /²=0%<br>)<br>ple |       |               | 0                                | ).01<br>- | 0.1              | 1 10                   | 100     |
|   |  | applica                                     |                   |       |               |                                  | F         | -avors (control) | Favors (experi         | mental) |

Figure 3 (Continued)

### С

| Study or<br>subgroup    | Experime<br>events    | ental<br>Total          | Control events | Total | Weight<br>(%) | Odds ratio<br>M–H, fixed, 95% Cl | Odds ratio<br>M–H, fixed, 95% Cl       |
|-------------------------|-----------------------|-------------------------|----------------|-------|---------------|----------------------------------|--|
| 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)                | <b>_</b>                               |
| Zhou JS 2006            | 9                     | 21                      | 3              | 22    | 15.2          | 4.75 (1.07, 21.14)               |  |
| Subtotal (95% Cl        | )                     | 116                     |                | 117   | 100           | 3.57 (1.92, 6.66)                | •                                      |
| Total events            | 66                    |                         | 38             |       |               |                                  |  |
| Heterogeneity: $\chi^2$ | =2.64, df=3 (         | P=0.45); I <sup>2</sup> | =0%            |       |               |                                  |  |
| Test for overall effe   | ect: Z=4.00 (         | <i>P</i> <0.0001)       |                |       |               |                                  |  |
| Total (95% CI)          |                       | 116                     |                | 117   | 100           | 3.57 (1.92, 6.66)                | •                                      |
| Total events            | 66                    |                         | 38             |       |               |                                  | •                                      |
| Heterogeneity: $\chi^2$ | =2.64, <i>df</i> =3 ( | P=0.45); I <sup>2</sup> | =0%            |       |               | ⊢                                |  |
| Test for overall effe   | ect: Z=4.00 (         | <i>P</i> <0.0001)       |                |       |               | 0.01                             | 0.1 1 10 100                           |
| Test for subgroup       | differences:          | not applica             | ble            |       |               |                                  | Favors (control) Favors (experimental) |

#### D

| Study or<br>subgroup   | Experime<br>events   | ntal<br>Total                                    | Control events          | Total | Weight<br>(%) | Odds ratio<br>M–H, fixed, 95% Cl | Odds ratio<br>M–H, fixed, 95% Cl       |
|--|--|--|-------------------------|-------|---------------|----------------------------------|--|
| 2-year OS  |  |  |                         |       |               |                                  |  |
| Cui YQ 2008  | 26   | 61   | 11                      | 54    | 13.1          | 2.90 (1.26, 6.69)                | <b>_</b>                               |
| 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% C  | 1)   | 465  |                         | 453   | 100           | 3.17 (2.36, 4.25)                | •                                      |
| Total events<br>Heterogeneity: $\chi^2$<br>Test for overall ef | 237<br><sup>2</sup> =6.82, <i>df</i> =10<br>fect: <i>Z</i> =7.70 ( | ( <i>P</i> =0.74); <i>I</i><br><i>P</i> <0.00001 | 122<br><sup>2</sup> =0% |       |               |                                  |  |
| Total (95% CI)   |  | 465  |                         | 453   | 100           | 3.17 (2.36, 4.25)                | •                                      |
| Total events<br>Heterogeneity: $\chi^2$                        | 237<br><sup>2</sup> =6.82, <i>df</i> =10                           | ( <i>P</i> =0.74); <i>I</i>                      | 122<br><sup>2</sup> =0% |       |               | H                                |  |
| Test for subgroup  | differences:   | not applicat                                     | )<br>ble                |       |               | 0.0                              |  |
|  |  |  |                         |       |               |                                  | Favors (control) Favors (experimental) |

| Study or<br>subgroup  | Experime<br>events                          | ental<br>Total           | Control events | Total | Weight<br>(%) | Odds ratio<br>M–H, fixed, 95% ( | CI   |     | Odds<br>M–H, f | ratio<br>ixed, 98 | 5% CI       |         |
|-----------------------|---|--------------------------|----------------|-------|---------------|---------------------------------|------|-----|----------------|-------------------|-------------|---------|
| 3-year OS             |   |                          |                |       |               |                                 |      |     |                |                   |             |         |
| Kou CY 2011           | 14  | 31                       | 7              | 31    | 17.3          | 2.82 (0.94, 8.48)               |      |     |                | -                 | <u> </u>    |         |
| 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)               |      |     |                | - <b>I</b>        | -           |         |
| Xue Q 2010            | 5   | 32                       | 4              | 30    | 15.7          | 1.20 (0.29, 4.98)               |      |     |                | -                 |             |         |
| Subtotal (95% C       | CI)   | 223                      |                | 221   | 100           | 2.88 (1.82, 4.57)               |      |     |                |                   |             |         |
| Total events          | 76  |                          | 34             |       |               |                                 |      |     |                |                   |             |         |
| Heterogeneity: $\chi$ | <sup>2</sup> =1.68, <i>df</i> =3 (          | (P=0.64); I <sup>2</sup> | =0%            |       |               |                                 |      |     |                |                   |             |         |
| Test for overall e    | ffect: Z=4.51                               | ( <i>P</i> <0.00001      | )              |       |               |                                 |      |     |                |                   |             |         |
| Total (95% CI)        |   | 223                      |                | 221   | 100           | 2.88 (1.82, 4.57)               |      |     |                |                   |             |         |
| Total events          | 76  |                          | 34             |       |               |                                 |      |     |                |                   |             |         |
| Heterogeneity: $\chi$ | <sup>2</sup> =1.68, <i>df</i> =3 (          | (P=0.64); I <sup>2</sup> | =0%            |       |               |                                 | ⊢    |     |                | _                 |             |         |
| Test for overall e    | Test for overall effect: Z=4.51 (P<0.00001) |                          | )              |       |               |                                 | 0.01 |     | 0.1            | 1                 | 10          | 100     |
| Test for subgroup     | p differences:                              | not applica              | ble            |       |               |                                 |      | Fav | ors (control)  | Favo              | rs (experim | nental) |

Figure 3 Forest plot of the comparison of 0.5-year (A), I-year (B), I.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.

#### Α

| Study or<br>subgroup    | Experime<br>events    | ental<br>Total | Control<br>events | Total | Weight<br>(%) | Odds ratio<br>M–H, fixed, 95% C |      | Odd:<br>M–H   | s ratio<br>, fixed, 95% Cl |           |
|-------------------------|-----------------------|----------------|-------------------|-------|---------------|---------------------------------|------|---------------|----------------------------|-----------|
| 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)               |      |               | <b></b>                    |           |
| He SL 2012              | 17                    | 26             | 16                | 25    | 3.5           | 1.06 (0.34, 3.35)               |      | -             | <b>_</b>                   |           |
| Jia JY 2016             | 22                    | 49             | 16                | 46    | 5.6           | 1.53 (0.67, 3.50)               |      |               | <b></b>                    |           |
| Ke J 2011               | 18                    | 38             | 19                | 40    | 6.0           | 0.99 (0.41, 2.42)               |      | -             | <b>_</b>                   |           |
| 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)               |      |               | - <b>-</b>                 |           |
| 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)               |      |               | <b></b>                    |           |
| 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)               |      |               | _ <b>_</b>                 |           |
| 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)               |      |               | <b>—</b>                   |           |
| 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: $\chi^2$ | =19.76, <i>df</i> =23 | 3 (P=0.66);    | /²=0%             |       |               |                                 | H    |               |                            | —         |
| Test for overall eff    | ect: Z=6.52 (         | P<0.00001)     |                   |       |               |                                 | 0.01 | 0.1           | 1 10                       | 100       |
|                         |                       |                |                   |       |               |                                 | Favo | ors (control) | Favors (expe               | rimental) |

#### В

| Study or<br>subgroup    | Experim<br>events | nental<br>Total | Control events | Total | Weight<br>(%) | Odds ratio<br>M–H, fixed, 95% C | :1   | Odds<br>M–H,  | ratio<br>fixed, 95% Cl |           |
|-------------------------|-------------------|-----------------|----------------|-------|---------------|---------------------------------|------|---------------|------------------------|-----------|
| 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)               |      |               | <b></b>                |           |
| 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)               |      |               | <b>—</b>               |           |
| Ke J 2011               | 32                | 38              | 33             | 40    | 6.0           | 1.13 (0.34, 3.73)               |      | _             | <b>-</b>               |           |
| 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)               |      |               | <b></b>                |           |
| 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)               |      |               | <b>—</b>               |           |
| 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)              |      |               | <b>—</b>               |           |
| Su Y 2013               | 29                | 33              | 23             | 30    | 3.4           | 2.21 (0.57, 8.47)               |      |               | <b>—</b>               |           |
| 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)               |      |               | <b>—</b>               |           |
| Yang GH 2014            | 38                | 45              | 34             | 45    | 6.2           | 1.76 (0.61, 5.04)               |      |               | <b>—</b>               |           |
| Yang YG 2006            | 36                | 40              | 30             | 40    | 3.5           | 3.00 (0.85, 10.54)              |      |               | <b>—</b>               |           |
| 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: $\chi^2$ | =13.43, df=2      | 22 (P=0.92);    | /²=0%          |       |               |                                 | H    |               |                        |           |
| Test for overall eff    | ect: Z=5.53       | (P<0.00001)     |                |       |               |                                 | 0.01 | 0.1           | 1 10                   | 100       |
|                         |                   |                 |                |       |               |                                 | Favo | ors (control) | Favors (expe           | rimental) |

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.

| Parameter | TACE+cinobufotalin  | TACE      | Analysis | Heterogen | eity    | OR   | 95% CI    | P-value  |
|-----------|---------------------|-----------|----------|-----------|---------|------|-----------|----------|
|           | injection group (n) | group (n) | method   | l² (%)    | 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 |

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

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.<sup>47</sup> Therefore, immune system reconstruction is one of the critical factors to effectively treat

| Study or<br>subgroup  | Experim<br>events | ental<br>Total     | Control<br>events | Total | Weight<br>(%) | Odds ratio<br>M–H, fixed, 95% Cl |      | Oddsra<br>M–H, fix | tio<br>ed, 95% Cl     |     |
|-----------------------|-------------------|--------------------|-------------------|-------|---------------|----------------------------------|------|--------------------|-----------------------|-----|
| Chen KR 2017          | 25                | 36                 | 16                | 36    | 11.2          | 2.84 (1.08, 7.47)                |      |                    |                       |     |
| Cui YQ 2008           | 13                | 61                 | 2                 | 54    | 3.8           | 7.04 (1.51, 32.83)               |      |                    |                       |     |
| Deng ZY 2015          | 14                | 25                 | 5                 | 24    | 5.1           | 4.84 (1.37, 17.09)               |      |                    |                       |     |
| Fu ZL 2010            | 42                | 78                 | 22                | 78    | 23.3          | 2.97 (1.53, 5.77)                |      |                    | _ <b></b>             |     |
| He SL 2012            | 16                | 26                 | 11                | 25    | 9.9           | 2.04 (0.67, 6.22)                |      | _                  |                       |     |
| Kou CY 2011           | 19                | 31                 | 10                | 31    | 8.9           | 3.33 (1.17, 9.44)                |      |                    |                       |     |
| LI Q 2008             | 9                 | 50                 | 1                 | 46    | 2.0           | 9.88 (1.20, 81.38)               |      |                    |                       | _   |
| Liang Y 2008          | 28                | 48                 | 12                | 48    | 11.5          | 4.20 (1.76, 10.02)               |      |                    |                       |     |
| Mao WD 2013           | 4                 | 27                 | 2                 | 27    | 3.9           | 2.17 (0.36, 13.01)               |      |                    |                       |     |
| Song GP 2012          | 3                 | 20                 | 1                 | 20    | 1.9           | 3.35 (0.32, 35.36)               |      |                    | · · · · · ·           |     |
| Su Y 2013             | 19                | 33                 | 8                 | 30    | 8.2           | 3.73 (1.29, 10.81)               |      |                    |                       |     |
| Zeng BR 2009          | 5                 | 23                 | 2                 | 23    | 3.6           | 2.92 (0.50, 16.89)               |      |                    | • • •                 |     |
| Zhou JS 2006          | 12                | 21                 | 7                 | 22    | 6.7           | 2.86 (0.82, 9.93)                |      | -                  |                       |     |
| Total (95% CI)        |                   | 479                |                   | 464   | 100           | 3.45 (2.52, 4.72)                |      |                    | •                     |     |
| Total events          | 209               |                    | 99                |       |               |                                  |      |                    | •                     |     |
| Heterogeneity: $\chi$ | 2=3.87, df=       | 12 ( <i>P</i> =0.9 | 9); /²=0%         |       |               |                                  | L    | 1                  |                       |     |
| Test for overall e    | effect: Z=7.7     | 5 ( <i>P</i> <0.00 | 0001)             |       |               | C                                | ).01 | 0.1                | 1 10                  | 100 |
|                       |                   |                    |                   |       |               |                                  | Favo | s (control)        | Favors (experimental) |     |

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.

| Study or<br>subgroup | Experi<br>mean         | mental<br>SD       | Total            | Contro<br>mean           | SD                            | Total               | Weight<br>(%) | Mean difference<br>IV, random, 95% Cl |      | Mean dif<br>IV, rando | fference<br>om, 95% | СІ       |        |
|----------------------|------------------------|--------------------|------------------|--------------------------|-------------------------------|---------------------|---------------|---------------------------------------|------|-----------------------|---------------------|----------|--------|
| TBIL                 |                        |                    |                  |                          |                               |                     |               |                                       |      |                       |                     |          |        |
| Ke J 2011            | 32.5                   | 8.23               | 38               | 56.3                     | 8.89                          | 40                  | 8.2           | -23.80 (-27.60, -20.00                | )    | -                     |                     |          |        |
| Li Q 2008            | 28.6                   | 9.6                | 61               | 34.2                     | 12.5                          | 54                  | 8.1           | -5.60 (-9.71, -1.49)                  | ,    | -                     | -                   |          |        |
| Shen JJ 2009         | 32.6                   | 3.5                | 23               | 41                       | 3.4                           | 24                  | 8.3           | -8.40 (-10.37, -6.43)                 |      | -                     |                     |          |        |
| Yu JG 2013           | 23                     | 10                 | 30               | 22.9                     | 12                            | 30                  | 7.9           | 0.10 (-5.49, 5.69)                    |      |                       | +                   |          |        |
| Zhou JS 2006         | 10.8                   | 3.8                | 21               | 18                       | 4.1                           | 22                  | 8.3           | -7.20 (-9.56, -4.84)                  |      |                       |                     |          |        |
| Subtotal (95%        | CI)                    |                    | 173              |                          |                               | 170                 | 40.8          | -9.12 (-15.14, -3.10)                 |      | •                     | •                   |          |        |
| Heterogeneity:       | $\tau^2 = 43.55;$      | $\chi^2 = 73.66$   | 5, df=4 (        | P<0.0000                 | 1); /2=95                     | %                   |               |                                       |      | -                     |                     |          |        |
| Test for overall     | effect: Z=             | 2.97 ( <i>P</i> =0 | 0.003)           |                          | ,,                            |                     |               |                                       |      |                       |                     |          |        |
| ALT                  |                        |                    |                  |                          |                               |                     |               |                                       |      |                       |                     |          |        |
| Ke J 2011            | 59.9                   | 10.83              | 38               | 93.2                     | 11.12                         | 40                  | 8.0           | -33.30 (-38.17, -28.43                | )    | -                     |                     |          |        |
| Li Q 2008            | 53.7                   | 30.3               | 61               | 67.1                     | 24.5                          | 54                  | 7.0           | -13.40 (-23.43, -3.37)                | ,    |                       | -                   |          |        |
| Shen JJ 2009         | 188.7                  | 23.7               | 23               | 233.5                    | 23.5                          | 24                  | 6.2           | -44.80 (-58.30, -31.30                | )    |                       |                     |          |        |
| Yu JG 2013           | 79                     | 47                 | 30               | 115                      | 63                            | 30                  | 3.3           | -36.00 (-64.13, -7.87)                | ,    |                       |                     |          |        |
| Zhou JS 2006         | 48.7                   | 21.7               | 21               | 79.6                     | 31.3                          | 22                  | 5.6           | -30.90 (-46.94, -14.86                | )    |                       |                     |          |        |
| Subtotal (95%        | CI)                    |                    | 173              |                          |                               | 170                 | 30.0          | -30.76 (-41.65, -19.88                | )    | •                     |                     |          |        |
| Heterogeneity:       | $\tau^2 = 104.06$      | $S; \chi^2 = 16.8$ | 33, <i>df</i> =4 | ( <i>P</i> =0.002        | ); <i>I</i> <sup>2</sup> =76% | Ď                   |               |                                       |      | •                     |                     |          |        |
| Test for overall     | effect: Z=             | 5.54 (P<           | 0.00001)         |                          |                               |                     |               |                                       |      |                       |                     |          |        |
| AST                  |                        |                    |                  |                          |                               |                     |               |                                       |      |                       |                     |          |        |
| Yu JG 2013           | 50                     | 11                 | 30               | 76                       | 23                            | 30                  | 7.2           | -26.00 (-35.12, -16.88                | )    |                       |                     |          |        |
| Zhou JS 2006         | 51.3                   | 18.8               | 21               | 89.6                     | 30.2                          | 22                  | 5.8           | -38.30 (-53.26, -23.34                | )    |                       |                     |          |        |
| Subtotal (95%        | CI)                    |                    | 51               |                          |                               | 52                  | 13.0          | -30.66 (-42.36, -18.97                | )    | •                     |                     |          |        |
| Heterogeneity:       | $\tau^2 = 35.67;$      | $\chi^2 = 1.89$ ,  | df=1 (P          | =0.17); <i>I</i> 2:      | =47%                          |                     |               |                                       |      | •                     |                     |          |        |
| Test for overall     | effect: Z=             | 5.14 ( <i>P</i> <  | 0.00001)         |                          |                               |                     |               |                                       |      |                       |                     |          |        |
| ALB                  |                        |                    |                  |                          |                               |                     |               |                                       |      |                       |                     |          |        |
| Yu JG 2013           | 39                     | 11                 | 30               | 40                       | 12                            | 30                  | 7.9           | -1.00 (-6.83, 4.83)                   |      |                       | +                   |          |        |
| Zhou JS 2006         | 37.6                   | 2.7                | 21               | 33.1                     | 3.9                           | 22                  | 8.3           | 4.50 (2.50, 6.50)                     |      |                       | -                   |          |        |
| Subtotal (95%        | CI)                    |                    | 51               |                          |                               | 52                  | 16.2          | 2.46 (-2.75, 7.67)                    |      |                       | •                   |          |        |
| Heterogeneity:       | $\tau^2 = 10.19;$      | $\chi^2 = 3.06$ ,  | df=1 (P          | =0.08); I <sup>2</sup> : | =67%                          |                     |               | ,                                     |      |                       | ľ                   |          |        |
| Test for overall     | effect: Z=             | 0.93 ( <i>P</i> =0 | 0.35)            |                          |                               |                     |               |                                       |      |                       |                     |          |        |
| Total (95% CI)       |                        |                    | 448              |                          |                               | 444                 | 100           | –16.75 (–23.27, –10.24                | )    | ٠                     |                     |          |        |
| Heterogeneity:       | τ <sup>2</sup> =131.70 | ); χ²=404          | .84, <i>df=</i>  | 13 ( <i>P</i> <0.0       | 0001); <i>I</i> 2             | =97%                |               |                                       | H    | -+                    |                     |          |        |
| Test for overall     | effect: Z=             | 5.04 (P<           | 0.00001)         |                          |                               |                     |               | -                                     | -100 | -50                   | 0                   | 50       | 100    |
| Test for subgrou     | up differei            | nces: $\chi^2 =$   | 46.85, di        | f=3 (P<0.                | 00001); <i>I</i>              | <sup>2</sup> =93.6% |               |                                       | Fav  | ors (control)         | Favors              | (experim | ental) |

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.<sup>47</sup> 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)- $\gamma$  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<sup>+</sup>, CD4<sup>+</sup>, NK, and CD3<sup>+</sup>CD56<sup>+</sup> T cells and CD4<sup>+</sup>/CD8<sup>+</sup> ratio, indicating that immune function of HC patients was improved after cinobufotalin injectionmediated 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.

| Study or<br>subgroup                                 | Experi<br>mean          | imental<br>SD                    | Total              | Contro<br>mean    | SD                  | Total | Weight<br>(%) | Mean difference<br>IV, random, 95% C |        | Mean o<br>IV, rand | lifference<br>Iom, 95% | ,<br>CI    |     |
|--|-------------------------|----------------------------------|--------------------|-------------------|---------------------|-------|---------------|--------------------------------------|--------|--------------------|------------------------|------------|-----|
| CD3  |                         |                                  |                    |                   |                     |       |               |                                      |        |                    |                        |            |     |
| Jia JY 2016  | 61.33                   | 5.02                             | 49                 | 54.61             | 4.91                | 46    | 4.2           | 6.72 (4.72, 8.72)                    |        |                    | -                      |            |     |
| Li Q 2008  | 44.65                   | 5.4                              | 50                 | 40.41             | 4.8                 | 46    | 4.1           | 4.24 (2.20, 6.28)                    |        |                    | ÷                      |            |     |
| Liu XH 2009  | 43.1                    | 7.2                              | 42                 | 37.8              | 10.1                | 42    | 1.8           | 5.30 (1.55, 9.05)                    |        |                    |                        |            |     |
| Sun ZJ 2002  | 52.17                   | 6.65                             | 118                | 36.09             | 6.73                | 118   | 4.9           | 16.08 (14.37, 17.79                  | )      |                    |                        |            |     |
| Zena BR 2009   | 43.57                   | 10.67                            | 23                 | 30.25             | 8.76                | 23    | 0.9           | 13.32 (7.68, 18.96)                  | ,<br>, |                    |                        |            |     |
| Subtotal (95% CI)                                    |                         |                                  | 282                |                   |                     | 275   | 15.9          | 9.05 (3.62, 14.49)                   |        |                    |                        |            |     |
| Heterogeneity: $\tau^2=3$                            | 5.66; χ <sup>2</sup> =  | =96.56, d                        | f=4 (P<0           | .00001); /        | <sup>2</sup> =96%   |       |               |                                      |        |                    | 1                      |            |     |
| Test for overall effect                              | et: Z=3.27              | 7 ( <i>P</i> =0.00               | )1) `              | ,,                |                     |       |               |                                      |        |                    |                        |            |     |
| CD4  |                         |                                  |                    |                   |                     |       |               |                                      |        |                    |                        |            |     |
| Jia JY 2016  | 34.7                    | 4.33                             | 49                 | 31.11             | 4.16                | 46    | 4.9           | 3.59 (1.88, 5.30)                    |        |                    |                        |            |     |
| Li Q 2008  | 33.19                   | 3.6                              | 50                 | 29.22             | 3.5                 | 46    | 5.7           | 3.97 (2.55, 5.39)                    |        |                    |                        |            |     |
| Liu XH 2009  | 28.8                    | 8.8                              | 42                 | 18.7              | 7.2                 | 42    | 2.1           | 10.10 (6.66, 13.54)                  |        |                    | -                      |            |     |
| Sun ZJ 2002  | 35.02                   | 3.72                             | 118                | 23.44             | 3.21                | 118   | 7.3           | 11.58 (10.69, 12.47)                 | )      |                    |                        |            |     |
| Zeng BR 2009   | 35.61                   | 6.42                             | 23                 | 27.49             | 7.04                | 23    | 1.7           | 8.12 (4.23, 12.01)                   |        |                    | -                      |            |     |
| Subtotal (95% CI)                                    |                         |                                  | 282                |                   |                     | 275   | 21.6          | 7.42 (3.20, 11.63)                   |        |                    | •                      |            |     |
| Heterogeneity: r <sup>2</sup> =2                     | 1.52; χ <sup>2</sup> =  | =118.04, (                       | df=4 (P<           | 0.00001);         | I <sup>2</sup> =97% |       |               |                                      |        |                    |                        |            |     |
| Test for overall effect                              | t: Z=3.45               | 5 ( <i>P</i> =0.00               | 06)                |                   |                     |       |               |                                      |        |                    |                        |            |     |
| CD4/CD8  |                         |                                  |                    |                   |                     |       |               |                                      |        |                    |                        |            |     |
| Jia JY 2016  | 1.49                    | 0.16                             | 49                 | 1.42              | 0.15                | 46    | 8.9           | 0.07 (0.01, 0.13)                    |        |                    | - t                    |            |     |
| Li Q 2008  | 1.47                    | 0.29                             | 50                 | 1.17              | 0.25                | 46    | 8.9           | 0.30 (0.19, 0.41)                    |        |                    | - t                    |            |     |
| Liu XH 2009  | 1.27                    | 0.28                             | 42                 | 0.82              | 0.23                | 42    | 8.9           | 0.45 (0.34, 0.56)                    |        |                    | - t                    |            |     |
| Sun ZJ 2002  | 1.72                    | 0.14                             | 118                | 1.05              | 0.12                | 118   | 8.9           | 0.67 (0.64, 0.70)                    |        |                    | - t                    |            |     |
| Zeng BR 2009   | 1.58                    | 0.18                             | 23                 | 1.44              | 0.21                | 23    | 8.9           | 0.14 (0.03, 0.25)                    |        |                    | - t                    |            |     |
| Subtotal (95% CI)                                    |                         |                                  | 282                |                   |                     | 275   | 44.5          | 0.33 (0.03, 0.62)                    |        |                    |                        |            |     |
| Heterogeneity: $\tau^2=0$                            | .11; χ²=3<br>           | 335.89, di<br>3 ( <i>P</i> =0.03 | f=4 ( <i>P</i> <0) | .00001); <i>I</i> | ² <b>=99%</b>       |       |               |                                      |        |                    |                        |            |     |
|  |                         | 5 (1 0.00                        | <i>'</i> )         |                   |                     |       |               |                                      |        |                    |                        |            |     |
| NK   |                         |                                  |                    |                   |                     |       |               |                                      |        |                    |                        |            |     |
| Li Q 2008  | 32.8                    | 4.2                              | 50                 | 27.5              | 5.2                 | 46    | 4.4           | 5.30 (3.40, 7.20)                    |        |                    |                        |            |     |
| Liu XH 2009  | 27.9                    | 7.2                              | 42                 | 19.2              | 1.8                 | 42    | 3.7           | 8.70 (6.46, 10.94)                   |        |                    |                        |            |     |
| Sun ZJ 2002  | 32.06                   | 5.16                             | 118                | 12.91             | 2.46                | 118   | 6.9           | 19.15 (18.12, 20.18                  | )      |                    |                        |            |     |
| Zeng BR 2009   | 31.5                    | 4.8                              | 23                 | 24.8              | 4.1                 | 23    | 3.1           | 6.70 (4.12, 9.28)                    |        |                    | 1                      |            |     |
| Subtotal (95% CI)                                    |                         |                                  | 233                |                   |                     | 229   | 18.1          | 10.00 (2.08, 17.92)                  |        |                    |                        |            |     |
| Heterogeneity: $\tau^2=6$<br>Test for overall effect | 4.25; χ²=<br>ct: Z=2.47 | =228.22,<br>7 ( <i>P</i> =0.01   | df=3 (P<<br>)      | 0.00001);         | 12=99%              |       |               |                                      |        |                    |                        |            |     |
|  |                         |                                  | 1 070              |                   |                     | 1 054 | 100           | E 07 / A 74 E 00)                    |        |                    |                        |            |     |
| Hotorogonoity: -2-0                                  | 02: 22-0                | 761 74                           | 1,0/9              |                   | 1). 12-00           | 1,054 | 100           | 5.27 (4.71, 5.63)                    |        |                    | 11                     |            |     |
| Tost for overall office                              | .υΖ, χ-=2<br>+ 7-10 °   | 2,101.11,<br>26 (D-0.0           | 0001)              | ~0.0000           | ), <b>1</b> 99      | /0    |               |                                      | _100   | -50                | 0                      | 50         | 10  |
| Test for subgroup di                                 | fforences               | $v^2 = 26'$                      | 27 df=3            | (P<0 000)         | 11) <i>I</i> ²=8    | 8.6%  |               |                                      | Fo     | vora (aantral)     | Favor                  | - (avnarin | .o. |

Figure 7 Forest plot of the comparison of immune function indexes including percentage of CD3<sup>+</sup>, CD4<sup>+</sup>, and NK cells and CD4<sup>+</sup>/CD8<sup>+</sup> 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<br>subgroup   | Experime<br>events                              | ental<br>Total                     | Control<br>events                  | Total               | Weight<br>(%) | Odds ratio<br>M–H, random, 95% ( | CI    | Odds ra<br>M–H, ra | atio<br>andom, 95% Cl                   |         |
|--|---|------------------------------------|------------------------------------|---------------------|---------------|----------------------------------|-------|--------------------|---|---------|
| 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)                |       |                    | <u> </u>                                |         |
| Subtotal (95% CI)  |   | 112                                |                                    | 117                 | 16.6          | 2.74 (0.25, 30.43)               |       |                    |   |         |
| Total events   | 97  |                                    | 73                                 |                     |               |                                  |       |                    |   |         |
| Heterogeneity: $\tau^2$ =4.06;<br>Test for overall effect: Z                     | ; χ²=20.28,<br>2=0.82 (P=                       | , <i>df</i> =2 (P<<br>0.41)        | :0.0001); <i>I</i> ²=              | 90%                 |               |                                  |       |                    |   |         |
| Thrombocytopenia   |   |                                    |                                    |                     |               |                                  |       |                    |   |         |
| He SL 2012   | 10  | 26                                 | 9                                  | 25                  | 5.9           | 1.11 (0.36, 3.46)                |       |                    | <b>-</b>                                |         |
| Liu YQ 2010  | 33  | 38                                 | 38                                 | 44                  | 5.6           | 1.04 (0.29, 3.73)                |       |                    | <u>+</u>                                |         |
| Subtotal (95% CI)  |   | 64                                 |                                    | 69                  | 11.5          | 1.08 (0.46, 2.52)                |       |                    | $\blacktriangleright$                   |         |
| Total events   | 43  |                                    | 47                                 |                     |               |                                  |       |                    |   |         |
| Heterogeneity: $\tau^2$ =0.00;<br>Test for overall effect: Z                     | ; χ²=0.01, α<br>2=0.18 (P=                      | df=1 (P=0<br>0.86)                 | .94); /²=0%                        |                     |               |                                  |       |                    |   |         |
| 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<br>Heterogeneity: $\tau^2$ =0.40;<br>Test for overall effect: Z     | 42<br>; χ²=4.01, α<br>?=1.09 (P=                | df=2 (P=0<br>0.28)                 | 56<br>.13); /²=50%                 | )                   |               |                                  |       |                    |   |         |
| Fovor  |   |                                    |                                    |                     |               |                                  |       |                    |   |         |
| He SI 2012   | 7   | 26                                 | 19                                 | 25                  | 57            | 0 12 (0 03 0 41)                 |       |                    |   |         |
| Liang Y 2008   | 25  | 48                                 | 2                                  | 48                  | 5.2           | 25.00 (5.44, 114, 85)            |       |                    | l                                       |         |
| Liu YO 2010  | 33  | 38                                 | 39                                 | 44                  | 5.5           | 0.85 (0.23, 3.18)                |       |                    |   |         |
| Shen 11 2009   | 8   | 23                                 | 8                                  | 24                  | 5.7           | 1 07 (0 32 3 57)                 |       |                    |   |         |
| Subtotal (95% CI)  | 0   | 135                                | 0                                  | 1/1                 | 22.1          | 1 23 (0 16 9 78)                 |       |                    |   |         |
| Total events   | 73  | 155                                | 68                                 | 141                 | 22.1          | 1.25 (0.10, 5.70)                |       |                    |   |         |
| Heterogeneity: $\tau^2$ =4.00;<br>Test for overall effect: Z                     | ; χ <sup>2</sup> =29.42,<br>2=0.20 ( <i>P</i> = | <i>df</i> =3 ( <i>P</i> <<br>0.84) | :0.00001); <i>I</i> <sup>2</sup> ; | =90%                |               |                                  |       |                    |   |         |
| 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: <i>τ</i> <sup>2</sup> =0.77;<br>Test for overall effect: <i>Z</i> | ; χ²=4.78, α<br>=0.27 (P=                       | df=2 (P=0<br>0.79)                 | .09); /²=58%                       | )                   |               |                                  |       |                    |   |         |
| Myelosuppression   |   |                                    |                                    |                     |               |                                  |       |                    |   |         |
| Deng ZY 2015   | 4   | 25                                 | 10                                 | 24                  | 5.5           | 0.27 (0.07, 1.02)                |       |                    | 1                                       |         |
| 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<br>Heterogeneity: $\tau^2$ =0.00;<br>Test for overall effect: Z     | 101<br>; χ²=1.11, α<br>=3.61 (P=                | df=2 (P=0<br>0.0003)               | 122<br>.57); /²=0%                 |                     |               |                                  |       |                    |   |         |
| Total (95% CI)   |   | 676                                |                                    | 697                 | 100           | 0.89 (0.45, 1.73)                |       |                    |   |         |
| Total events   | 445   |                                    | 464                                |                     |               |                                  |       |                    | 1                                       |         |
| Heterogeneity: $\tau^2=1.67$ ;   | ; χ²=85.31,                                     | df=17 (F                           | <0.00001); <i>I</i>                | ²=80%               |               |                                  | I     |                    | <b>↓</b>                                |         |
| Test for overall effect: Z   | =0.35 (P=                                       | 0.72)                              |                                    |                     |               |                                  | 0.005 | 0.1                | 1 10                                    | 200     |
| Test for subgroup different  | ences: χ²=                                      | 8.51, <i>df=</i>                   | 5 ( <b>P=</b> 0.13); I             | <sup>2</sup> =41.3% |               |                                  | Fave  | ors (control)      | Favors (experi                          | mental) |

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), I-year (B), and 2-year (C) OS. Abbreviation: OS, overall survival; SE, standard error.

![](_page_13_Figure_4.jpeg)

Figure 10 Funnel plot of ORR  $(\mathbf{A})$  and DCR  $(\mathbf{B})$ .

Abbreviations: DCR, disease control rate; ORR, overall response rate; SE, standard error.

| Table 4  | Dublication | hine o | - 05  | CD  | DD  | <sup>c</sup> D |     |      |      |  |
|----------|-------------|--------|-------|-----|-----|----------------|-----|------|------|--|
| T able 4 | Fublication | Dias O | n Os, | Сκ, | гπ, | <i>зD</i> ,    | гυ, | URR, | DCR, |  |

| Publication bias | 0.5-year OS | I-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.

| Parameter   | Factors at study level | Exp group | Con group | Analysis | Heterogeneity      |         | OR   | 95% CI    | P-value  |  |  |  |
|-------------|------------------------|-----------|-----------|----------|--------------------|---------|------|-----------|----------|--|--|--|
|             |                        | (n)       | (n)       | method   | l <sup>2</sup> (%) | P-value | 1    |           |          |  |  |  |
| 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     |  |  |  |
| I-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      | - 1       | 1         |          |                    |         |      |           |          |  |  |  |
|             | ≥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 |  |  |  |

| Table 5 Subgroup analyses | of ORR and DCR betwe | een the Exp and Con groups |
|---------------------------|----------------------|----------------------------|
|---------------------------|----------------------|----------------------------|

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

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

Table SI Application sequence of and manufacturer of cinobufotalin injection

Abbreviation: TACE, transcatheter hepatic arterial chemoembolization.

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