

# The Clinical Efficacy and Adverse Effects of Entecavir plus Thymosin alpha-1 Combination Therapy versus Entecavir Monotherapy in HBV-related Cirrhosis: A Systematic Review and Meta-analysis

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## Research article

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## Abstract

**Background** Previous studies have demonstrated the benefits of thymosin alpha-1 (Ta1) in anti-virus, immunological enhancement and anti-inflammation. However, it is controversial about the efficacy and safety of entecavir (ETV) plus Ta1 combination therapy versus ETV monotherapy in cirrhosis patients with hepatitis B virus (HBV) infection.

**Objective** The systematic review and meta-analysis of randomized clinical trials (RCTs) were performed to evaluate the efficacy and safety of ETV plus Ta1 compared with ETV monotherapy in HBV-related cirrhosis. **Methods** A systematic search was performed via PubMed, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), Embase, China National Knowledge Infrastructure (CNKI), Chinese Science and Technology Journals Database (VIP), and Chinese Biological Medicine database (CBM). Relative risk (RR) and standardized mean difference (SMD) with a fixed- or random- effect model were calculated. Heterogeneity were assessed with the Cochrane Q-test and I<sup>2</sup> values.

**Results** Seven RCTs involving 1144 subjects were included in the systematic review and meta-analysis. Compared with ETV monotherapy, ETV plus Ta1 combination therapy led to a higher complete response (RR = 1.18; 95% CI, 1.07 - 1.30). The HBV DNA undetectable rate and HBeAg loss rate of ETV plus Ta1 therapy for 24 weeks was higher than the ETV alone (RR = 1.91; 95% CI, 1.56 - 2.35; RR = 2.05; 95% CI, 1.62 - 2.60). However, after treatment for 48 and 52 weeks, there was no significantly different between the combination therapy and ETV monotherapy (RR = 1.07; 95% CI, 0.96 - 1.18; RR = 1.17; 95% CI, 0.89 - 1.55). In comparison with ETV alone, ETV plus Ta1 improved part of biochemical parameters and liver fibrosis. There was significant heterogeneity. In addition, The ETV plus Ta1 significantly reduced adverse events compared to ETV monotherapy (RR = 0.48; 95% CI, 0.24 - 0.95).

**Conclusions** ETV plus Ta1 combination therapy might have a higher clinical response and a lower comprehensive adverse reaction rate in HBV-related cirrhosis patients compared with ETV monotherapy. Meanwhile, the whole patients included in this meta-analysis were from chinese mainland, so that more worldwide RCTs with a large sample size are needed to verify the current findings.

## Introduction

Liver cirrhosis is an end-stage organic disease characterized as the irreversible fibrosis, necrosis of liver cells and multifaceted immune dysfunction [1, 2]. Chronic HBV (CHB) infection is considered as an independent risk factor of the occurrence and progression to cirrhosis. Annually, 2.1 - 6.0 % of HBV-related patients can be diagnosed as cirrhosis [3, 4]. With an estimated yearly more than 200,000 cirrhosis related deaths in CHB patients, the disease ranks as the 13th leading cause of total years of life lost globally [4, 5].

The main goal of therapy for HBV-related cirrhosis is to achieve the long-term suppression of HBV replication, decrease viral load, avoid liver transplantation, prevent progression to hepatocellular carcinoma (HCC) and death, thereby improving the quality of life, prolonging life expectancy and reducing risk of transmission to others. The anti-viral treatment to HBV is critical for clinical outcome of cirrhosis patients. Meanwhile, cirrhosis is also accompanied with serious immunodysfunction, which parallelly presents chronic systemic inflammation and immune inhibition [6]. The present mainstay of HBV treatment is the repression of HBV replication by nucleos(t)ide analogs (NAs), such as entecavir (ETV), lamivudine (LAM) and tenofovir disoproxil fumarate (TDF), and supplementing with the immune-mediated agents such as α-Interferon and Ta1 [7, 8]. Theoretically, the strong immune response is conducive to viral suppression and clearance.

However, practice guidelines have merely recommended NAs in alone or combination as anti-viral therapy drugs for people with cirrhosis [2]. Interferon-alpha (IFNa) or pegylated interferon-alpha (PegIFNa) is cautiously or not recommended for therapy in cirrhosis patients in order to prevent acute severe hepatitis. These guidelines have rarely mentioned Ta1 or Ta1 plus NAs, which has naturally led to the question of whether Ta1 plus NAs is more favourable than NAs monotherapy.

ETV belongs to be one of nucleoside analogs and approved for listing by FDA in 2005, which is preferentially recommended for HBV-related cirrhosis individuals owing to its active inhibition of HBV and seldom viral resistance. The previous clinical trials suggested that ETV could be well tolerated for patients with HBV-related compensated or decompensated cirrhosis and efficacious in improvement of virological, biochemical and histological parameters [9-12]. The incidence of drug resistance with ETV is reported only to be 1.2% at 5 years [13].

Ta1 is a synthetic polypeptide consisting of 28 amino acids, which can not only reduce hepatic inflammation, but also trigger the maturation in lymphocytes and promote T-helper 1 (Th1) response [14, 15]. Ta1 has reflected good therapeutic activities and little side effect with viral hepatitis [16-19]. Meanwhile, the clinical trials comparing ETV alone and Ta1 plus ETV combination therapy have been performed in the past ten years, whereas the results were inconsistent. For example, Jia P and colleagues found that people with ETV plus Ta1 combination treatment seemed to be higher in the level of undetectable HBV DNA than ETV monotherapy (sample size 130; RR = 1.82; 95% CI, 1.34 - 2.48) [20]. However, Xu YQ and colleagues had the opposite results for undetectable HBV DNA (sample size 60; RR = 0.96; 95% CI, 0.73 - 1.25) [21].

To figure out this cause of inconsistency between ETV monotherapy and ETV plus Ta1 combination therapy, the systematic comparisons with the 2 treatment approaches was needed. Therefore, we conducted a systematic review and meta-analysis of existing trials to compare the efficacy and safety between ETV alone and ETV plus Ta1 for HBV-related cirrhosis patients.

## Methods

### Literature search

The electronical search were performed from English-language and Chinese-language databases, including PubMed, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), Embase, China National Knowledge Infrastructure (CNKI), Chinese Science and Technology Journals Database (VIP) and Chinese Biological Medicine database (CBM). The following items were applied to search for relevant publications: "Thymosin" OR "Thymosin α1" OR "Thymosin alpha 1", "Entecavir", "Hepatitis B" OR "HBV", "Cirrhosis" OR "Hepatocirrhosis" OR "Posthepatitic cirrhosis". Furthermore, a manual search of reference lists was conducted to screen out potential eligible clinical trials. The retrieved studies only in abstract form were not systematically evaluated for inadequate data.

### Study selection

The eligible publications comparing the efficacy and adverse effects of ETV combined with Ta1 to ETV monotherapy in HBV-related cirrhosis were included in our meta-analysis and systematic review. The inclusion criteria included: (I) HBV-related cirrhosis patients; (II) randomized controlled trials (RCTs) with a duration of at least 20 weeks and the number of subjects in each group > 10; (III) ETV plus Ta1 as combination therapy group; (IV) ETV monotherapy as the control group. (V) The outcome indexes including at least the following 2 items: Response of subjects including effective response and no response; Virological blood detection such as rates of undetectable HBV DNA or /and the rates of HBeAg loss; Biochemical and clinical variables reflecting liver function such as the levels of ALT, ALB, A/G, TBIL and AST; and adverse effects including nausea, vomit and dizzy.

Exclusion criteria were as follows: (I) duplicate literature; (II) irrelevant topics; (III) reviews; (IV) non-RCT design; (V) unable to extract the data of HBV-related cirrhosis patients; (VI) any publication with incomplete data was not available.

### Data extraction

The information was independently extracted by two review authors (D Peng and H.Y Xing) and imported into Review Manager (RevMan, Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Any disagreements about data were resolved through discussing with the corresponding author (J.H Chen). For the included studies, we extracted characteristics of studies, baseline characteristics of subjects, response of HBV-related cirrhosis

patients, HBV virological response, change of biochemical variables and adverse effects of subjects. The characteristics of studies included first author, region, study design, enrollment period, type of disease, number of patients included, diagnostic criteria of HBC, number of patients in ETV plus Ta1 or ETV monotherapy group, and intervention method. The baseline characteristics of subjects were as follows: age, groups, gender, ALT, ALB, TBIL, AST and A/G. The HBV virological response included the undetectable rate of HBV DNA levels and the negative conversion proportion of HBeAG in serum after treatment. The improvement of hepatic fibrosis were reflected through the serum variables regarding HA, PC- $\square$ , LN and C- $\square$ .

## Risk of Bias

The Cochrane Collaboration's tool was applied independently by two reviewers to summarize the risk of bias across all included studies. The bias items for each included study were as follows: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias. Disagreement between two reviewers was settled by consensus or consultation with a third reviewer (C Li).

## Statistical analysis

The meta-analyses were conducted using the Review Manager (version 5.3) software. Dichotomous data were pooled with fixed- or random- effect model and presented as the odds risk (OR) with 95% confidence interval (CI). For the continuous data, mean  $\pm$  standard deviation (SD) and number of participants were extracted in each group. Fixed- or random-effect model and standardized mean difference (SMD) with 95% CI were employed for statistics of continuous results. Statistical heterogeneity were assessed by the Cochrane Q-test and  $I^2$  values.  $P < 0.1$  or  $I^2 > 50\%$  were represented for significant heterogeneity among the included trials. Sensitivity and subgroup analysis were performed for investigating the source of heterogeneity in each study. The publication bias was evaluated with funnel plots. Besides from Cochran's Q-test,  $P < 0.05$  were expressed as significant difference among the analyzed studies.

## Results

### Characteristics of studies

We totally identified 416 publications through database searching. Seven studies were lastly included for our meta-analysis [20–26] (Fig. 1). The type of disease included HBV-related cirrhosis (HBC), compensated HBV-related cirrhosis (DHBC) and decompensated HBV-related cirrhosis (DHBC). Only two studies included patients with unclassified type of HBC. One studies pointed out patients with DHBC, and other included studies reminded of patients with CHBC. Three studies were based on Guidelines for Prevention and Treatment of Chronic Hepatitis B in 2010 or 2015, and only one study abided by Diagnostic Criteria of HBV in 2000. The diagnostic criteria were not mentioned in 3 other studies. The detailed characteristics of studies were summarized in Table 1.

Table 1  
Characteristics of the included studies

Author [year]	Region	Study design	Enrollment period	Type of disease	Number of patients included	Diagnostic criteria of HBV	Groups	Number of patients in control/ETV plus Ta1 (n)	Intervention method
Shuai TM [2013]	Gansu Province, Jiayuguan	RCT	2009–2012	HBC	30	Diagnostic Criteria of HBV revised by Xian National Viral Hepatitis Conference in 2000	CG VS. EG	15 15	Entecavir 0.5 mg, once per day, treated for 48 weeks. Entecavir 0.5 mg, once per day, combined with Thymosin α1 1.6 mg, twice per week, treated for 24 weeks, and then single Entecavir 0.5 mg, once per day, treated until 48 weeks.
Diao YH [2017]	Henan Province, Nanyang	RCT	2013.4-2014.4	CHBC	80	Guidelines for prevention and treatment of chronic hepatitis B in 2010	CG VS. EG	40 40	Entecavir 0.5 mg, once per day, treated for 24 weeks. Entecavir 0.5 mg, once per day, combined with Thymosin α1 1.6 mg, twice per week, treated for 24 weeks.

Author [year]	Region	Study design	Enrollment period	Type of disease	Number of patients included	Diagnostic criteria of HBV	Groups	Number of patients in control/ ETV plus Ta1 (n)	Intervention method
Wu XN [2018]	China	RCT	2013.01- 2015.09	CHBC	690	NA	CG VS. EG	339 351	After 26 weeks of entecavir treatment (0.5 mg per day), patients were randomly assigned to receive entacavir (0.5 mg per day) or combination with Thymosin $\alpha$ 1 (1.6 mg twice per week ) for 52 weeks.
Wang XR [2018]	Hei longjiang Province, Jiamusi	RCT	2013.4- 2016.4	HBC	50	NA	CG VS. EG	25 25	Entecavir 0.5 mg, once per day, treated for 12 weeks. Entecavir 0.5 mg, once per day, combined with Thymosin $\alpha$ 1 1.6 mg, twice per week, treated for 12 weeks.

Table 1  
(continued)

Author [year]	Region	Study design	Enrollment period	Type of disease	Number of patients included	Diagnostic criteria of HBV	Groups	Number of patients in ETV/ETV plus Ta1 (n)	Intervention method
Xu YQ [2017]	Sichuan Province, Leshan	RCT	2014.1-2016.12	DHBC	60	NA	CG VS. EG	30 30	ETV 0.5 mg, once per day, treated for 48 weeks. ETV 0.5 mg, once per day, combined with Ta1 1.6 mg, twice per week, treated for 48 weeks.
Zhang XX [2018]	Liaoning Province, Shenyang	RCT	2014.5-2017.5	CHBC	104	Guidelines for prevention and treatment of chronic hepatitis B in 2010	CG VS. EG	52 52	ETV 0.5 mg, once per day, treated for 24 weeks. ETV 0.5 mg, once per day, combined with Ta1 1.6 mg, twice per week, treated for 24 weeks.
Jia P [2018]	Heilongjiang Province, Jiamusi	RCT	2016.5-2017.5	CHBC	130	Guidelines for prevention and treatment of chronic hepatitis B in 2015	CG VS. EG	65 65	ETV 0.5 mg, once per day, treated for 6 months. ETV 0.5 mg, once per day, combined with Ta1 1.6 mg, twice per week, treated for 6 months.

Author [year]	Region	Study design	Enrollment period	Type of disease	Number of patients included	Diagnostic criteria of HBV	Groups	Number of patients in ETV/ ETV plus Ta1 (n)	Intervention method
CG (control group), the group with ETV monotherapy; EG (experimental group), the group with ETV plus Ta1 combination therapy; RCT, randomized controlled trials; HBC, HBV-related cirrhosis; CHBC, compensated HBV-related cirrhosis; DHBC, decompensated HBV-related cirrhosis; NA, not available.									

## Characteristics of patients

Five of 7 studies included the mean age of subjects, which ranged from 32 to 69 years old. Gender was provided in 6 studies, and the percentage of males ranged from 43.3–76.0% in ETV alone group, and that is from 50.0–80.0% in ETV plus Ta1 group. At least three baseline measures of ALT, TBIL, AST, ALB and A/G were mentioned in each study. The characteristics of patients were summarized in Additional file 5: Table S1.

## Complete and no response

Three studies with 270 subjects were included in the meta-analysis with regarding complete and no response. The higher complete response was observed in the combination therapy group ( $RR = 1.18$ ; 95% CI, 1.07–1.30,  $P = 0.001$ ). No significant heterogeneity were found between the two treatment groups ( $P = 0.49$ ,  $I^2 = 0\%$ ) (Fig. 2A). Furthermore, the rate of no response in the ETV plus Ta1 group was significantly lower than the ETV alone group ( $RR = 0.32$ ; 95% CI, 0.16–0.66,  $P = 0.002$ ). No significant heterogeneity were observed in the two therapies ( $P = 0.59$ ,  $I^2 = 0\%$ ) (Fig. 2B).

## The HBV DNA undetectable rate

In the meta-analysis, six studies reported the virological response of 1090 patients after treatment of 24 weeks, 48 weeks and 52 weeks. We divided the eligible six studies into two subgroups. Subgroup 1 contained four trials that included 310 patients (155 in ETV plus Ta1 group and 155 in ETV alone group) with treatment of 24 weeks. Subgroup 2 contained three studies that included 780 patients (396 in ETV plus Ta1 group and 384 in ETV alone group) with treatment of more than 24 weeks.

The sub-analysis by treatment duration suggested that The HBV DNA undetectable rate of the combination therapy for 24 weeks was higher than the ETV alone group ( $RR = 1.91$ ; 95% CI, 1.56–2.35,  $P < 0.00001$ ) (Fig. 3A and 3B). There was no significant heterogeneity in subgroup 1 ( $P = 0.40$ ,  $I^2 = 0\%$ ). However, after treatment of 48 and 52 weeks, the HBV DNA undetectable rate in ETV plus Ta1 group was no significantly different with the ETV monotherapy group ( $RR = 1.07$ ; 95% CI, 0.96–1.18,  $P = 0.22$ ) (Fig. 3 and Additional file 2: Fig S2). There was no significant heterogeneity in subgroup 2 ( $P = 0.35$ ,  $I^2 = 0\%$ ).

## The HBeAg Loss Rate

723 subjects were involved in the six studies, which reported the HBeAg loss rate. The heterogeneity of overall tests was significant so that random-effect model was used for analyzing the overall effects ( $P = 0.005$ ,  $I^2 = 70\%$ ). The HBeAg loss rate of the combination therapy group was higher than the monotherapy group among those studies ( $RR = 1.52$ ; 95% CI, 1.16–2.01,  $P = 0.003$ ) (Fig. 4 and Additional file 3: Fig S3).

In the subgroup analyses by treatment duration, which included 24 weeks, 48 weeks and 52 weeks. The results of Subgroup 1, which contained three studies and involved 314 patients with treatment for 24 weeks, reported that the HBeAg loss rate

was greater in ETV plus Ta1 group than in ETV alone group ( $RR = 2.05$ ; 95% CI, 1.62–2.60,  $P < 0.00001$ ). There was no significant heterogeneity ( $P = 0.12$ ,  $I^2 = 53\%$ ). Another three studies and 409 patients were included in the Subgroup 2. The results showed that the HBeAg loss rate of patients treated with ETV plus Ta1 was similar with that of patients treated with ETV alone ( $RR = 1.17$ ; 95% CI, 0.89–1.55,  $P = 0.26$ ). There was a significant heterogeneity ( $P = 0.88$ ,  $I^2 = 0\%$ ).

results about the HBeAG loss rate in ETV plus Ta1 group and ETV alone group. Subgroup analysis for the HBeAG loss rate. The subgroup about treatment duration includes less than or equal to 24 weeks and more than 24 weeks. "Events" represents the number of subjects who experienced the HBeAG loss. "Total" represents the number of subjects in that group. "Test for overall effect" refers to pooled estimate of risk ratio about inducing HBeAG loss after comprehensive analysis of all studies. Blue boxes indicates the dichotomous data in the forest plots. CI, confidence interval; M-H, Mantel-Haensel.

## Biomedical and clinical variables

The biomedical and clinical variables, including ALB, AST, ALT, TBIL and A/G, were extracted from six eligible studies involved 454 participants, including ALB, AST, ALT, TBIL and A/G. The results of meta-analyses were summarized in Table 2. The serum levels of ALB, AST, ALT, TBIL and A/G were significantly enhanced by the treatment with ETV plus Ta1 or ETV alone. Compared to ETV alone, ETV plus Ta1 significantly increased the AST and ALT levels, while there was no obvious difference on the serum levels of ALB, TBIL and A/G. The significant heterogeneity was existed in the most meta-analysis regarding biomedical and clinical variables.

Table 2  
Summary of pooled results and sensitivity analyses including biochemical variables

Variable	Studies included (n)	Patients included (n)	SMD	95%CI	Significance, P	Heterogeneity	
						P	$\chi^2$
<b>ALB</b>							
After treatment, EG vs. CG	2	90	-0.38	-2.12, 1.37	0.67	0.0001	93%
EG, before vs. after	2	90	-1.62	-2.87, -0.36	0.01	0.02	81%
CG, before vs. after	2	90	-1.37	-2.05, -0.68	< 0.0001	0.15	51%
<b>AST</b>							
After treatment, EG vs. CG	4	364	-0.93	-1.68, -0.17	0.02	< 0.00001	91%
EG, before vs. after	4	364	8.89	3.45, 14.32	0.001	< 0.00001	99%
CG, before vs. after	4	364	7.56	2.47, 12.64	0.004	< 0.00001	99%
<b>ALT</b>							
After treatment, EG vs. CG	6	454	-1.12	-1.70, -0.55	0.0001	< 0.00001	87%
EG, before vs. after	6	454	10.45	5.83, 15.08	< 0.00001	< 0.00001	99%
CG, before vs. after	6	454	11.61	6.69, 16.53	< 0.00001	< 0.00001	99%
<b>TBIL</b>							
After treatment, EG vs. CG	6	454	-0.35	1.22, 0.53	0.44	< 0.00001	95%
EG, before vs. after	6	454	3.31	1.28, 5.34	0.001	< 0.00001	98%
CG, before vs. after	6	454	3.26	1.54, 4.99	0.0002	< 0.00001	97%
<b>A/G</b>							
After treatment, EG vs. CG	3	314	0.47	-0.88, 1.82	0.49	< 0.00001	97%
EG, before vs. after	3	314	-1.26	-2.19, -0.33	0.008	< 0.00001	93%
CG, before vs. after	3	314	-1.06	-1.30, -0.83	< 0.00001	0.61	0%

CG (control group), the group with ETV monotherapy; EG (experimental group), the group with ETV plus Ta1 combination therapy; SMD, standardized mean difference; CI, confidence interval; ALT, alanine aminotransferase; ALB, albumin; TBIL, total bilirubin; AST, aspartate aminotransferase; A/G, the albumin globulin ratio.

## Serum variables about hepatic fibrosis

Liver fibrosis is a prominent pathological feature in cirrhosis patients. The serum levels of HA, PC- $\alpha$ , LN and C- $\alpha$  reflected the synthesis and degradation about collagen, proteoglycan and glycoprotein in liver extracellular matrix. In this meta-analysis, only one trials involving 120 subjects reported the serum variables about hepatic fibrosis regarding HA, PC- $\alpha$ , LN and C- $\alpha$ . Compared with ETV alone group, the serum levels of HA, PC- $\alpha$  and C- $\alpha$  were significantly improved in ETV plus Ta1 group, while the serum LN level was significantly changed, which indicated that ETV plus Ta1 combination therapy could significantly decrease the severity of hepatic fibrosis compared to ETV monotherapy (Table 3). However, In most meta-analysis about the serum variables of hepatic fibrosis, there was significant heterogeneity.

Table 3

Summary of pooled results and sensitivity analyses regarding serum variables about hepatic fibrosis

Variable	Studies included (n)	Patients included (n)	SMD	95%CI	Significance, P	Heterogeneity	
						P	$I^2$
<b>HA</b>							
After treatment, EG vs. CG	1	114	-2.38	-2.87, -1.89	< 0.00001	NA	NA
<b>PC-<math>\alpha</math></b>							
After treatment, EG vs. CG	1	114	-2.92	-4.42, -1.43	< 0.00001	NA	NA
<b>LN</b>							
After treatment, EG vs. CG	1	114	-1.99	-4.50, 0.52	0.06	NA	NA
<b>C-<math>\alpha</math></b>							
After treatment, EG vs. CG	1	114	-2.60	-3.86, -1.33	< 0.0001	NA	NA
CG (control group), the group with ETV monotherapy; EG (experimental group), the group with ETV plus Ta1 combination therapy; SMD, standardized mean difference; CI, confidence interval; HA, hyaluronic acid; PC- $\alpha$ , procollagen III; LN, laminin; C- $\alpha$ , type $\alpha$ collagen; NA, not applicable.							

## Adverse events

Three eligible studies involving 270 subjects reported that 35 patients experienced the adverse events including nausea, vomit, allergic and dizzy. The meta-analysis results showed a significant decrease in adverse events after therapy with ETV plus Ta1 compared with ETV monotherapy ( $RR = 0.48$ ; 95% CI, 0.24–0.95,  $P = 0.03$ ) (Fig. 5 and Additional file 4: Fig S4). No significant heterogeneity was found ( $P = 0.98$ ,  $I^2 = 0\%$ ). However, no significant difference was observed between the two groups in nausea, vomit, allergic and dizzy.

## Risk of bias

In the assessment of random sequence generation, 3 included studies had a low risk. The allocation concealment, performance bias and detection bias remained unclear. All bias item of incomplete outcome data and 85% (6/7) bias item of selective reporting had a low risk. Other risk of bias was not estimated for inefficient information in each included study.

## Discussion

CHB patients commonly experience liver fibrosis, cirrhosis, HCC and death as the progression of disease [27, 28]. Although the current expert consensus showed that the effective anti-viral treatment to HBV is vital for improving prognosis and preventing complications in cirrhotic patients, the treatment to adverse reactions, immunosuppression and chronic systemic inflammation should not be ignored [29, 30]. In this meta-analysis, we assessed the efficacy and safety of ETV combined with Ta1 versus ETV monotherapy for treating HBV-related cirrhosis. Seven studies accorded with the inclusion criteria. Our results showed that ETV combined with Ta1 could increase the complete response and contribute to the reduction of adverse reactions, in comparison with ETV monotherapy. The subgroup analyses to type of adverse reactions indicated that ETV combined with Ta1 has no significance with ETV monotherapy in nause, vomit, dizzy and allergy, respectively.

Rehermann B and colleagues supported that the suppress and elimination of HBV depend mainly on the potent and diverse T cell immune response in host [31]. In vitro experiments showed that Ta1 induced the maturation of thymocytes, stimulated differentiation into active T cells and functional recovery of T cells [32]. In vivo experiments verified that Ta1 was associated with the up-regulating activity of natural killer cell in CHB patients [8]. Our results demonstrated that ETV plus Ta1 combination therapy could provide additional benefits of the virological and serological response over ETV monotherapy, which is accorded with previous studies. Substantial heterogeneity was observed in undetectable HBV DNA and HBeAg negative conversion rate ( $I^2 > 50\%$ ), and the subgroup and sensitivity analyses showed that duration of treatment is an important factor for the primary results.

The present meta-analysis showed that the biochemical variables, including ALT, ALB, AST, TBIL and A/G, which were significantly improved by combination therapy during Ta1 add-on, compared with ETV monotherapy, thus indicating that the combination treatment have a better effect on improving the function of hepatocytes and remission of hepatic damage. Yang XL [15] found that Ta1 could protect liver of rat against damage via down-regulating TNF- $\alpha$  and up-regulating IL-10, resulting in the relief to hepatic inflammation and hepatocyte apoptosis, which was also in line with our findings.

HBV-related cirrhosis is an important stage of progressive liver injury or fibrosis [33]. Recovery of cirrhosis was related with degradation of fibrous septa, regeneration of hepatocytes to replace fibrotic tissue and restoration of a lobular architecture [34, 35]. Our current meta-analysis indicated the serum variables about hepatic fibrosis including HA, PC $\square$ , LN and C $\square$  in ETV plus Ta1 group were significantly reduced, reminded that the main components of liver fibrosis remains more degradation and less deposition in combination treatment during Ta1 add-on. However, the included trials was short and small sample size, which limited the ability to objectively analyze potential differences in clinical outcomes. More large, long-term and high-quality studies were still being executed.

In addition, HCC and the complications of cirrhosis included variceal hemorrhage, ascites, spontaneous bacterial peritonitis and hepatic encephalopathy, which have an important impact on the expected life of the human being [27]. Although these endpoint outcomes were not analyzed, the study included from this meta-analysis showed that ETV plus Ta1 combination group has a lower incidence of HCC after treatment of 51 weeks, and yet has no benefits in terms of ascites, hepatic encephalopathy, variceal hemorrhage and liver stiffness, in comparison with ETV alone [26]. The results were consistent with the study of Liang YR which reported that Ta1 therapy improves liver function, and obviously extend recurrence-free and overall survival in HBV-related HCC [36]. Unfortunately, the participants of RCTs were from Chinese mainland. With a view to the diversity among people of different races and regions, more global multicenter randomized double-blind trials will need to be performed.

There were other possible limitations in this meta-analysis. First, the diagnostic criteria of HBV-related cirrhosis was inconsistent among the included studies, and the severity of cirrhosis patients was not exactly same. Second, the characteristic of included subjects were incomplete in some studies, the statistical sample size was sometimes too small to compare the treatment effects between monotherapy and combination therapy. Third, the heterogeneity was remarkable in biochemical and virological variables. Despite the subgroup analysis and sensitivity analysis were detected in this meta-analysis, the resource of heterogeneity was not well clarified. Fourth, the included individuals in RCTs were all from China, the results haven't apply for people in other countries at present.

# Conclusions

This meta-analysis firstly indicated that ETV plus Ta1 combination therapy is more safe and effective than ETV monotherapy in HBV-related cirrhosis patients. However, our observations are better for Chinese, and unable to be generalizable in global countries, so the international, large and well-designed multicenter RCTs need to be performed.

# Abbreviations

HBV: Hepatitis B Virus; RCTs: Randomized Clinical Trials; RR: Relative Risk; SMD: Standardized Mean Difference; CHB: Chronic Hepatitis B Virus; HCC: Hepatocellular Carcinoma; NAs: Nucleos(t)ide Analogs; CI: Confidence Interval; ALT: Alanine Aminotransferase; ALB: Albumin; TBIL: Total Bilirubin; AST: Aspartate Aminotransferase; A/G: the Albumin Globulin Ratio; HA: Hyaluronic Acid; PC- $\alpha$ : Precollagen III; LN: Laminin; C- $\alpha$ : Type  $\alpha$  Collagen.

# Declarations

## Availability of data and materials

All data and materials were presented within the manuscript and additional supporting files.

## Authors' contributions

D Peng designed the study. D Peng and H.Y Xing collected and analyzed the data. X.F Wang and B Li gave statistical support. C Li carried out the quality assessment. D Peng wrote the manuscript. J.H Chen and M Hou reviewed and revised the manuscript. All authors have read and approved the manuscript.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors have no conflicts of interest to declare.

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## Additional Files

**Additional file 1: Fig S1.** Risk of bias assessment. (PDF)

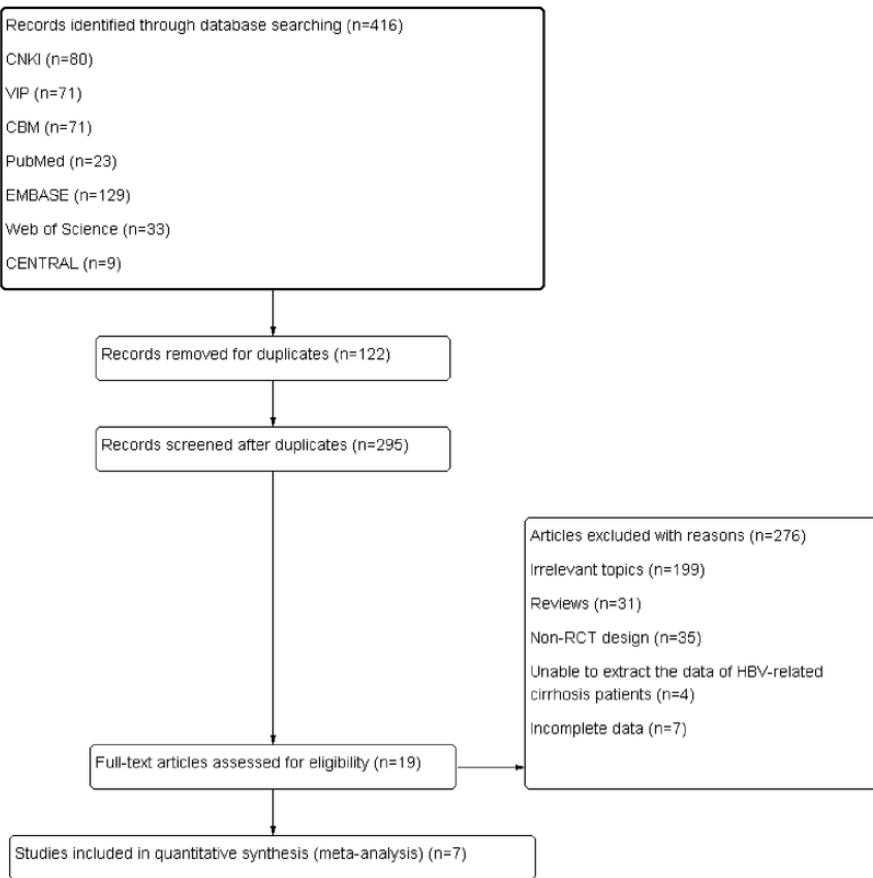
**Additional file 2: Fig S2.** The funnel plot of treatment duration in evaluation for the HBV DNA undetectable rate in ETV plus Ta1 group and ETV alone group. (PDF)

**Additional file 3: Fig S3.** The funnel plot of treatment duration in evaluation for the HBeAG loss rate in ETV plus Ta1 group and ETV alone group. (PDF)

**Additional file 4: Fig S4.** The funnel plot of effect estimate against its standard error to adverse advents. (PDF)

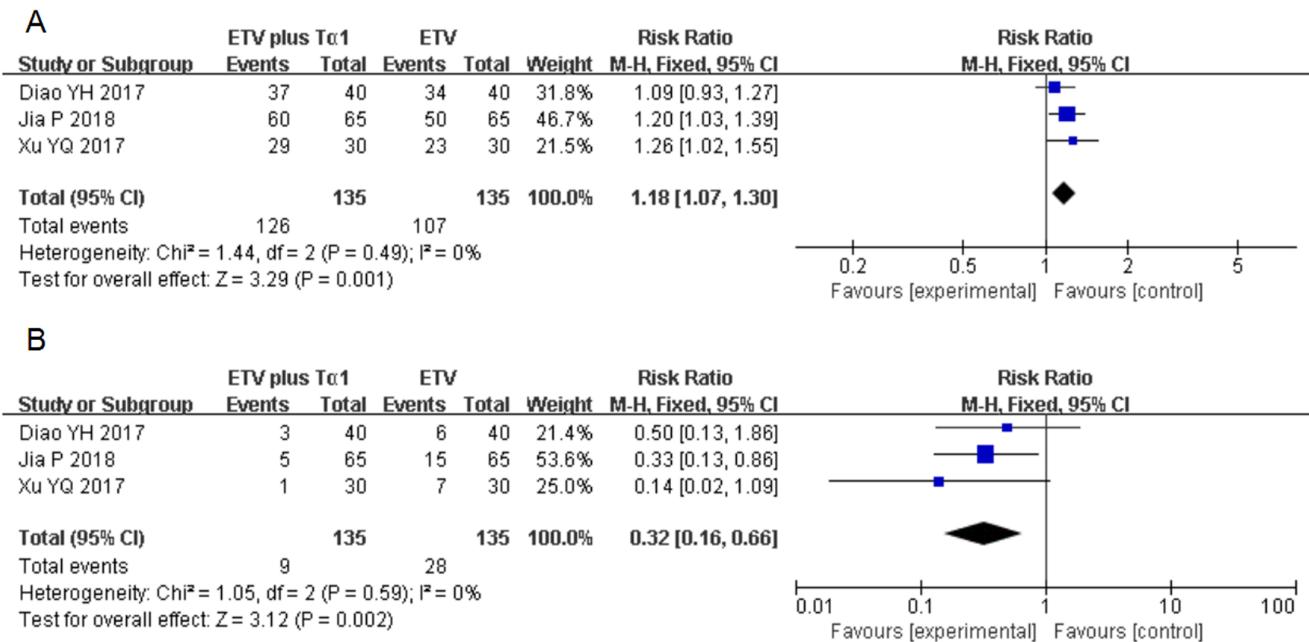
**Additional file 5: Table S1.** Characteristic of the included patients. (PDF)

## Figures



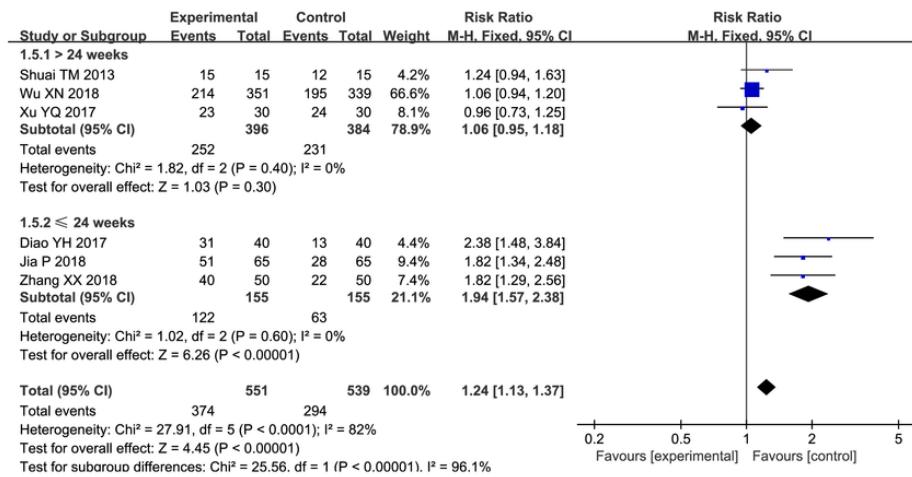
**Figure 1**

Flow chart for selection of relevant publications. The figures represent the number of included articles per stage.



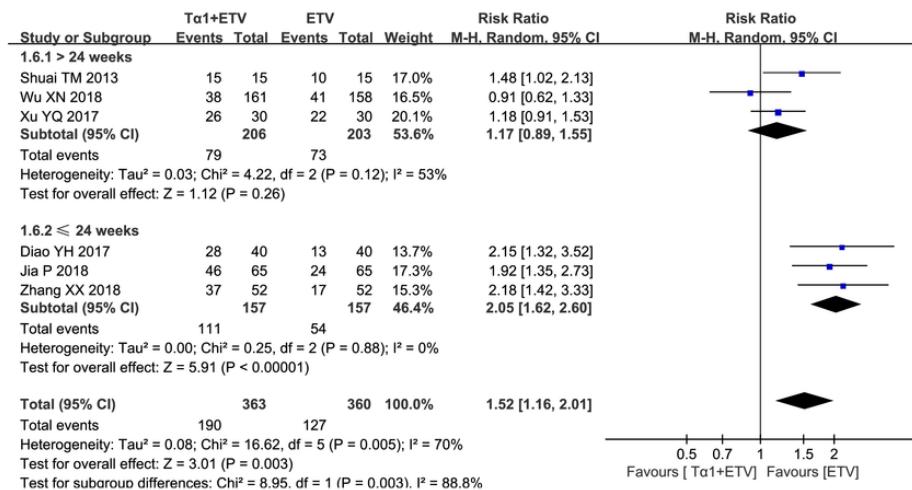
**Figure 2**

Relative risk of the efficacy to HBV-related Cirrhosis patients in ETV plus Ta1 group and ETV alone group. (A) meta-analysis for the effective response; (B) meta-analysis for no response. Blue boxes indicates the dichotomous data in the forest plots. CI, confidence interval; M-H, Mantel-Haensel.



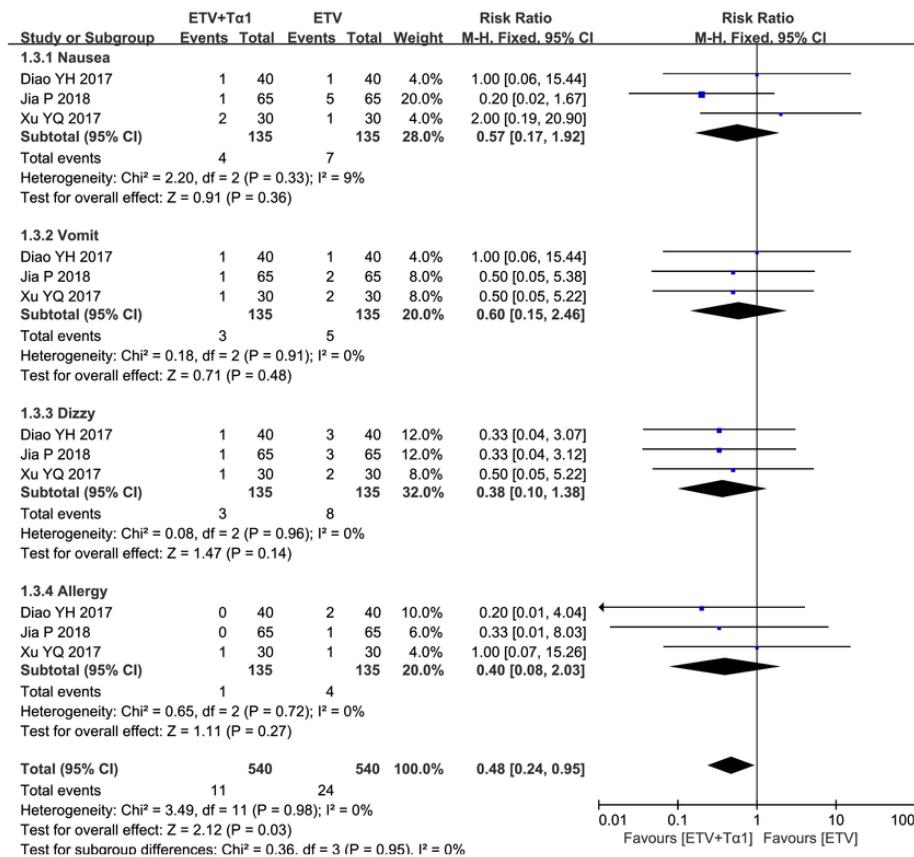
**Figure 3**

Summary of pooled results about the HBV DNA undetectable rate in ETV plus Ta1 group and ETV alone group. Subgroup analysis for the HBV DNA undetectable rate. The subgroup about treatment duration includes less than or equal to 24 weeks and more than 24 weeks. “Events” represents the number of subjects who were undetected with HBV DNA. “Total” represents the number of subjects in that group. “Test for overall effect” represents pooled estimate of risk ratio after comprehensive analysis of all studies. Blue boxes indicates the dichotomous data in the forest plots. CI, confidence interval; M-H, Mantel-Haensel.



**Figure 4**

Summary of pooled results about the HBeAG loss rate in ETV plus Ta1 group and ETV alone group. Subgroup analysis for the HBeAG loss rate. The subgroup about treatment duration includes less than or equal to 24 weeks and more than 24 weeks. “Events” represents the number of subjects who experienced the HBeAG loss. “Total” represents the number of subjects in that group. “Test for overall effect” refers to pooled estimate of risk ratio about inducing HBeAG loss after comprehensive analysis of all studies. Blue boxes indicates the dichotomous data in the forest plots. CI, confidence interval; M-H, Mantel-Haenszel.



**Figure 5**

Summary of pooled results and the funnel plot including adverse advents in ETV plus Ta1 group and ETV alone group. Meta-analysis for the incidence of adverse reaction including nausea, vomit, allergic and dizzy. "Test for overall effect" represents pooled estimate of risk ratio after comprehensive analysis of all studies. Blue boxes indicates the dichotomous data in the forest plots. CI, confidence interval; M-H, Mantel-Haensel.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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