International Immunopharmacology 75 (2019) 105747

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Clinical efficacy and safety of synthetic thymic peptides with chemotherapy for non-small cell lung cancer in China: A systematic review and meta-analysis of 27 randomized controlled trials following the PRISMA guidelines

Fen-Lian Zeng[a,d], Zheng Xiao[a,b,c,*,1], Cheng-Qiong Wang[a,b], Yuan Jiang[b,c], Jing-Li Shan[a,b], Shan-Shan Hu[e], Xiao-Rong Huang[e], Yu-Hong Tang[c], Xing-Sheng Yao[f], Tao Zhang[g], Xian-Tao Zeng[i], Ji-Hong Feng[h], Xue Xiao[a,b,**,1]

[a] Department of General Medicine, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou, China
[b] Evidence-Based Medicine Center, MOE Virtual Research Center of Evidence-based Medicine at Zunyi Medical University, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou, China
[c] School of Management, Zunyi Medical University, Zunyi 563003, Guizhou, China
[d] Department of Clinical Nursing, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou, China
[e] GCP Center, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou, China
[f] Department of Immunology, Special Key Laboratory of Gene Detection & Therapy of Guizhou Province, Zunyi Medical University, Zunyi 563000, Guizhou, China
[g] Key Laboratory of Cell Engineering of Guizhou Province and Regenerative Medicine Centre, Affiliated Hospital of Zunyi Medical University, Zunyi 519000, Guizhou, China
[h] Department of Oncology, Medical College of Taizhou University Affiliated Taizhou Municipal Hospital, Taizhou 318000, Zhejiang, China
[i] Center for Evidence-based and Translational Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, Hubei, China

ARTICLE INFO

Keywords:
Non-small cell lung cancer
Synthetic thymic peptides
Thymosin α1
Thymopentin
Randomized controlled trial
Meta-analysis

ABSTRACT

Background: Synthetic thymic peptides (sTPs) are used with chemotherapy to treat non-small cell lung cancer (NSCLC). In this study, we have performed a systematic review and meta-analysis of published trials to confirm the clinical efficacy and safety of sTPs, and determine the optimal types, usages, and sTP/chemotherapy combinations to produce the desired responses.

Materials and methods: We collected all studies regarding combined sTP therapy and chemotherapy for NSCLC from the Chinese and English databases (up to October 2018). Bias risk was evaluated for each. Data for meta-analysis was extracted using a pre-designed form. Evidence quality was rated using the Grading of Recommendations Assessment, Development and Evaluation approach.

Results: We included 27 randomized controlled trials containing 1925 patients, most with unclear bias risk. Combining sTPs with chemotherapy significantly increased the objective response rate [1.28, (1.13 to 1.45)], disease control rate [1.10, (1.01 to 1.18)], quality of life (QOL) [2.05, (1.62, 2.60)], and 1-year overall survival rate [1.43, (1.15 to 1.78)], with decreased risks of neutropenia, thrombocytopenia, and gastrointestinal reactions. Optimal conditions included treatment in combination with gemcitabine or navelbine and cisplatin, twice a week, with one 3-week cycle. In these conditions, thymosin α1 improved both antitumor immunity and tumor response. Most results had good robustness, and their quality ranged from moderate to very low.

Conclusions: The results suggest that treatment with sTPs, especially thymosin α1, and concomitant chemotherapy is beneficial to the patient, and provide evidence for optimal treatment regimens that may increase patient QOL and survival.

* Correspondence to: Z. Xiao, Department of General Medicine, Evidence-Based Medicine Center, MOE Virtual Research Center of Evidence-based Medicine at Zunyi Medical University, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou, China.
** Correspondence to: X. Xiao, Department of General Medicine, Affiliated Hospital of Zunyi Medical University, Zunyi 563003, Guizhou, China.
E-mail addresses: zy426f@163.com (Z. Xiao), xxellen@163.com (X. Xiao).
[1] These authors contributed equally.

https://doi.org/10.1016/j.intimp.2019.105747
Received 10 May 2019; Received in revised form 27 June 2019; Accepted 8 July 2019
Available online 18 July 2019
1567-5769/ © 2019 Elsevier B.V. All rights reserved.

1. Introduction

Lung cancer is the leading cause of cancer deaths in both men and women and a challenging public health problem worldwide [1–4]. Approximately 85% of lung cancers are non-small cell lung cancer (NSCLC). As a result of advanced local invasion and metastasis, most clinically diagnosed patients are ineligible for curative surgery, and forced to receive systematic chemotherapy. Cisplatin, carboplatin, oxaliplatin, or nedaplatin in combination with gemcitabine, vinorelbine, paclitaxel, docetaxel, etoposide, pemetrexed, and other drugs are considered standard first- or second-line treatments for NSCLC [5–8]. However, most patients often undergo multiple adverse drug reactions (ADRs) during the course of treatment, due to hematopoietic, hepatorenal, and gastrointestinal toxicity [9–12]. Moreover, chemotherapeutic agents also damage the host's immune system and down-regulate antitumor immunity. Ultimately, these events reduce the patient's capacity to endure further treatments and result in severe complications, poor quality of life (QOL), and worsened prognosis [13–17]. Thus, improved treatment strategies are urgently needed.

Synthetic thymic peptides (sTPs) are derivatives of peptides isolated from thymus extracts [18]. They include thymosin α1 (Tα1, 28 amino acids long) and thymopentin (five amino acids long), which were first isolated from calf thymuses in 1977 [19] and 1979 [20,21], respectively. These peptides enhance both specific and nonspecific immune functions [22] and are widely used in the treatment of bacterial or viral diseases [23–27]. Furthermore, they have been shown to inhibit the proliferation of malignant tumor cells, induce apoptosis [22,28–32], and prevent carcinogenesis [33,34]. Therefore, sTPs are also used in the treatment of cancer [18,35–37]. For lung cancer, a number of clinical trials evaluating sTPs treatment with chemotherapy have been reported [38–40]. Previous meta-analyses reported that Tα1 treatment with chemoradiotherapy/platinum chemotherapy improved the objective response rate (ORR), with no differences in the risk of neutropenia, thrombocytopenia, and nausea/vomiting [36]. Tα1 with navelbine and cisplatin (NP) or gemcitabine and cisplatin (GP) resulted in significant improvement in the tumor response, 1-year OS rate, and levels of $CD4^+$ T and natural killer (NK) cells [41]. Thymopentin with chemoradiotherapy increased antitumor immunity by increasing the $CD4^+/CD8^+$ T cell ratio, but did not affect the tumor response [37]. Therefore, these studies did not conclusively demonstrate whether sTP administration with chemotherapy improves tumor immunity, tumor response, and survival. Moreover, despite these positive results, the optimal sTPs types, cycle and treatment times, and sTPs/chemotherapy combinations to elicit the best possible antitumor immunity and tumor response have yet to be determined. Therefore, we have performed a systematic review and meta-analysis of trials evaluating combined sTPs/chemotherapeutic treatments for NSCLC, to further confirm the clinical efficacy and safety of the approach, to determine the optimal treatment regimen, and to provide evidence for individualized immunotherapy in NSCLC.

2. Materials and methods

This study was implemented based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [42]. As all data were derived from published studies, ethical approval was not required.

2.1. Inclusion and exclusion criteria

The following inclusion criteria were applied: The study design was randomized controlled trial (RCT). The patients had advanced NSCLC, diagnosed according to histopathological and cytological diagnostic criteria and the TNM staging system [43]. All patients were ineligible for surgery. None of the patients experienced cardiac insufficiency or hepatorenal dysfunction. Interventions studied were sTPs administered via subcutaneous, intravenous, and intramuscular injection, but not through pleural perfusion. The experimental group was administered sTPs with chemotherapy, and the control group was administered chemotherapy alone. None of patients received chemotherapy, radiotherapy, immunotherapy, or traditional Chinese medicine for one month before treatment onset. The main outcomes were tumor response and survival, and the secondary outcomes were QOL, peripheral blood lymphocyte levels, ADRs, and hospital-acquired infections (HAIs). No restrictions were set on follow-up procedures or research institutions.

The following exclusion criteria were applied: (1) duplicates, (2) studies regarding non-lung cancers, small cell lung cancers, and pleural effusion, (3) studies regarding non-thymic peptides and thymopolypeptides, (4) studies where thymic peptides were combined with surgery, radiotherapy, traditional Chinese medicine, or other biological regulators, (5) in vitro/in vivo studies, generic, patents, abstracts, reviews without specific data, and unrelated systematic reviews or meta-analyses, (6) cohort and case control studies, case series, and case reports, and (7) studies without data regarding tumor response, survival, peripheral blood lymphocyte levels, ADRs, or HAIs.

2.2. Search strategy

Two reviewers (Fen-lian Zeng and Cheng-qiong Wang) independently retrieved all related studies from the Chinese Scientific Journals Full-Text Database, China Biological Medicine Database, China National Knowledge Infrastructure Database, Wanfang Database, Medline, Embase, Web of Science (up to October 2018), and Cochrane Central Register of Controlled Trials (CENTRAL, Issue 10 of 12, October 2018). MeSH and free word searches were used in all retrievals. The retrieval form was (“Lung Neoplasms”[Mesh] OR “Carcinoma, Non-Small-Cell Lung”[Mesh] OR “Nonsmall cell lung cancer” OR “Non-small cell lung cancer” OR “Non-small-cell lung cancer” OR “Lung Cancer” OR “Lung Cancers” OR “Lung carcinoma” OR “NSCLC” OR “Pulmonary neoplasms” OR “Lung neoplasm” OR “Pulmonary neoplasm” OR “Pulmonary cancer” OR “Pulmonary carcinoma” OR “Lung neoplasms”) AND (“Thymosin”[Mesh] OR “Thymopentin” OR “Thymalfasin” OR “Thymosin” OR “Thymosins” OR “Thymopentin” OR “Zadaxin” OR “Xinzhuagtai” OR “Maipuxin” OR “Kangsiai” OR “Jitai” OR “Thymopoietin” OR “Thymopoietins” OR “Timunox” OR “Thymic Factor, Circulating” OR “Thymic Serum Factor” OR “Nonathymulin” OR “Thymuline” OR “Serum Thymic Factor” OR “Thymulin” OR “Thymic Factor” OR “Thymustimulin” OR “Active thymus factor” OR “PEG-Tα1” OR “Thymins” OR “Thymopoietin polypeptide” OR “Thymic polypeptide” OR “Prothymosin” OR “THF” OR “Thymic humoral factor” OR “Thymopeptide” OR “Thymopeptides” OR “Thymopetidum” OR “Thymostimulin” OR “Thymopolypeptides” OR “Thymopoietin Pentapeptide”). Additional studies found in the references of related systematic reviews or meta-analyses that met the inclusion criteria were also included.

2.3. Study selection

Two reviewers (Shan-shan Hu and Jing-li Shan) independently selected eligible studies according to the inclusion and exclusion criteria. Any disagreements about selection were resolved through discussion between themselves or with Zheng Xiao.

2.4. Evaluation of methodological bias risk

According to the Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [44], two reviewers (Fen-lian Zeng and Yuan Jiang) independently evaluated the methodological bias risk of all included studies. Any disagreements were resolved through discussion between themselves or with Zheng Xiao. The bias parameters were as follows: random sequence generations, allocation concealments, blinding, incomplete outcome data, selective reporting, and other

biases (e.g., whether the baseline is comparable). We judged each item as three levels (Yes for a low bias, No for a high risk of bias, and Unclear). Then, we evaluated each study and sorted them into three levels: a low risk of bias (Low risk of bias for all key domains), a high risk of bias (High risk of bias for one or more key domains), and an unclear risk of bias (Unclear risk of bias for all key domains).

2.5. Outcome measures

The main outcomes were tumor response and survival. Tumor response was evaluated using the ORR and DCR, according to the World Health Organization (WHO) guidelines for solid tumor response [45] and Response Evaluation Criteria in Solid Tumors [46]. Indicators used were complete response (CR), partial response (PR), no change (NC), and progressive disease (PD). The ORR was equal to CR plus PR, and the DCR was equal to CR plus PR and NC. Survival was evaluated using the overall survival (OS) rate, progression-free survival (PFS) rate, and hazard ratio (HR) of the OS and PFS.

Secondary outcomes were QOL, peripheral blood lymphocyte levels, ADRs, and HAIs. According to the Karnofsky Performance Status (KPS) Scale [47,48], the QOL was considered improved if the KPS score increased by 10 points or more after treatment. Peripheral blood lymphocyte levels were evaluated by measuring T-lymphocyte subsets and NK cells. T-lymphocyte subsets were identified as the proportions of $CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ T cells and the ratio of $CD4^+/CD8^+$ T cells. All indicators were detected using flow cytometry, indirect immunofluorescence test, lactic dehydrogenase release, or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay after treatment. ADRs were evaluated according to the WHO and Common Terminology Criteria for Adverse Events guidelines [45,49], including neutropenia (granulocytes $< 2 \times 10^9$/L) and thrombocytopenia (platelets $< 100 \times 10^9$/L), gastrointestinal reactions, liver dysfunction (serum aminotransferase or alkaline phosphatase $> 1.25 \times$ N), renal dysfunction (serum urea nitrogen or creatinine $> 1.25 \times$ N), and other toxicities, such as neurotoxicity and phlebitis. HAIs were any infection acquired during the course of treatment.

2.6. Data extraction

Two reviewers (Xiao-rong Huang and Fen-lian Zeng) independently extracted the following data: first author and publishing date, demographic characteristics, sample sizes of experimental and control groups, types and uses of sTPs, chemotherapy regimens, evaluation criteria for tumor responses and ADRs, test methods for peripheral blood lymphocytes, follow-up protocols, outcome indicators such as tumor response, survival, peripheral blood lymphocytes, ADRs, and HAIs. The authors of studies without Kaplan-Meier survival curves or other relevant data were contacted to obtain available survival data. When the authors were unavailable, the Kaplan-Meier survival curves were reconstructed into OS, PFS, and HR using Engauge Digitizer 4.1 [50,51].

2.7. Statistical analysis

Two reviewers (Fen-lian Zeng and Cheng-qiong Wang) performed statistical analysis using Review Manager 5.3 (The Cochrane Collaboration, Oxford, UK), as recommended by the Cochrane Collaboration. The ORR, DCR, QOL, OS and PFS rate, ADRs, and HAIs were expressed as risk ratios (RRs) and 95% confidence intervals (CIs); the OS and PFS were expressed as HRs and 95% CIs, and the peripheral blood lymphocyte levels were expressed as the standardized mean difference (SMD) and 95% CI. $P < 0.05$ was considered statistically significant. Statistical heterogeneity among the trials was detected using the Pearson's chi-square test and the I^2 test [52]. When substantial statistical heterogeneity was rejected ($P \geq 0.1$, $I^2 \leq 50\%$), a fixed-effects model (FEM) was used to synthesize the data. Otherwise, a random-effects model (REM) was used. According to sTPs type, treatment frequency, time of cycle, number of cycles, and combination with various chemotherapies, a subgroup analysis model was built to determine the heterogeneity and its influence on tumor response and peripheral blood lymphocyte levels, and reveal the optimal parameters for sTPs therapy [51,53]. Publication bias was determined using funnel plots when there were > 10 studies. A sensitivity analysis model [51,53,54] was built based on rejecting poor trials, trials with over-estimated tumor response, survival, and peripheral blood lymphocytes and trials with under-estimated ADRs and HAIs to further confirm the robustness of the results. Trials were identified as poor when they had at least one domain with a high risk of bias. Trials were identified as over- or under-estimated when their results were statistically significant and beneficial to sTPs use.

2.8. Quality of evidence

Two reviewers (Zheng Xiao and Xian-tao Zeng) independently evaluated the evidence quality for each outcome using the Grades of Recommendation Assessment, Development and Evaluation (GRADE) approach [55]. Any disagreements regarding downgrade or upgrade were resolved by discussion between themselves or with a third reviewer (Xue Xiao). Quality was classified into four levels: high, moderate, low and very low. Quality was down-graded according to five domains: (i) limitations in study design (if most domains had unclear bias risk but no poor studies were present, the evidence was down-rated by one level. If poor studies were present, and the sensitivity analysis confirmed that the results had poor robustness, then the evidence was down-rated by two levels. If no poor study was present, the evidence was only rated down by one level. If all studies were poor, the evidence was down-rated by two levels), (ii) inconsistency (statistical heterogeneity and sensitivity analysis results with poor robustness after rejecting trials with underestimated ADRs or overestimated efficacy), (iii) indirectness (the subjects, interventions, controls, or outcomes did not meet the objectives of this study), (iv) imprecision (number of events for each outcome < 300 cases), and (v) publication bias (publication bias and sensitivity analysis results with poor robustness). For domains ii–iv, the evidence was down-rated by one level.

3. Results

3.1. Search results

After implementing the search strategies, 1290 records were obtained (Fig. 1). Duplicates were excluded, and 659 records remained. We next screened the abstracts, and selected 50 full-texts and 3 systematic reviews [36,37,41], which were rigorously evaluated and included 27 RCTs [22,38–40,56–78] from individual manuscripts and 12 RCTs [22,56–61,64,65,69,70,73] from the references of the systematic reviews [36,37,41]. After removing duplicates [22,56–61,64,65,69,70,73], 27 RCTs [22,38–40,56–78] were included in the meta-analysis.

3.2. Characteristics of the included studies

In this meta-analysis, we included 27 trials involving 1925 patients from China with inoperable stages IIIa and IV NSCLC (Table 1). Patient ages ranged from 26 to 80 years old, and 1306 and 619 patients were male and female, respectively. Combined treatment with sTPs and chemotherapy was administered in 976 cases, and 949 cases were administered chemotherapy alone. The sTPs therapies studied were Tα1 and thymopentin. Tα1 (1.6 mg) was injected subcutaneously either twice a week, once every two days, or three times per week, in 1–24 week per cycle for 1–4 cycles. Thymopentin (1–10 mg) was injected subcutaneously or intramuscularly once every one day, three times per week, in 1–4 week per cycle for 2–3 cycles. Chemotherapies included GP, NP, gemcitabine and carboplatin (GC), docetaxel and cisplatin (DP), paclitaxel and cisplatin

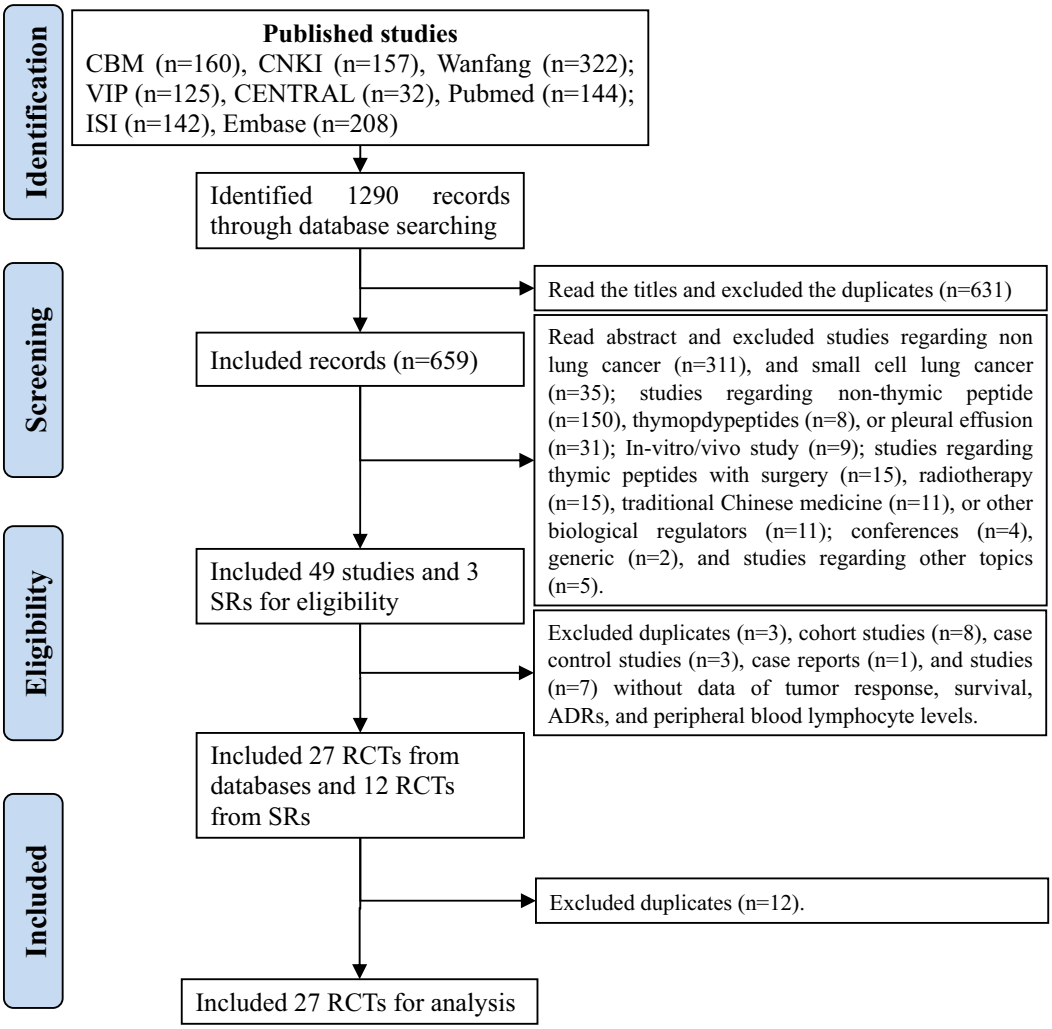


Fig. 1. Flow diagram of study selection and identification.

(TP), taxinol and carboplatin (TC), etoposide and cisplatin (EP), mitomycin, vindesine and cisplatin (MVP) and navelbine, ifosfamide and cisplatin (NIP). Follow-up lasted from 3 weeks to 4 years. Seventeen trials with 1186 cases [22,38,57–60,63,64,67–71,73,75,77,78] reported the tumor response according to the WHO or Response Evaluation Criteria in Solid Tumors guidelines. Seven trials with 382 cases [38,40,57,59,63,71,75] reported the QOL following the KPS Scale [47,48]. Four trials with 269 cases [57,58,71,74] reported the OS rates, and none of the trials reported the PFS. Eighteen trials with 1376 cases [38,39,56,57,60–62,64–66,68,70–74,76,77] reported the peripheral blood T lymphocyte levels and NK cell activity before and after therapy. Peripheral blood T lymphocytes were detected using flow cytometry or indirect immunofluorescence test, and NK cell activity was detected using flow cytometry, lactic dehydrogenase release, or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Sixteen trials with 1122 cases [22,38–40,56,57,60,63,67–71,74,75,78] reported the ADRs according to WHO or Common Terminology Criteria for Adverse Events guidelines [45,49]. Finally, three trials with 182 cases [56,57,74] reported the HAIs.

3.3. Methodological bias in the included trials

Of the 27 trials, only 3 [66,70,73] reported random sequence generation using the random number table. With the exception of one trial [57] with concealment and one trial [72] without it, other trials did not report allocation concealment. Only one trial [66] reported on blinding. Two trials [57,58] had loss to follow-up. Two trials [64,71] failed to completely report the DCR. Ten trials [39,58–60,64,67–69,75,78] failed to completely report the ADRs. Most trials had baseline comparability, except for 2 [40,69]. The bias risk of all trials is shown in Fig. 2.

3.4. Tumor responses

According to the guidelines for solid tumor response, 17 trials with 1186 cases [22,38,57–60,63,64,67–71,73,75,77,78] reported the tumor response (Fig. 3a and b). Pearson's chi-square and I^2 tests demonstrated statistical heterogeneity in DCR (I^2 = 57%) and no heterogeneity in ORR (I^2 = 0%). Therefore, the RR of the ORR was synthesized using an FEM, and the DCR was synthesized using an REM. Compared with chemotherapy alone, the meta-analysis results demonstrated that administration of sTPs with chemotherapy significantly increased the ORR [RR = 1.28, 95% CI (1.13 to 1.45), P = 0.0001] and DCR [RR = 1.10, 95% CI (1.01 to 1.18), P = 0.02] in advanced NSCLC.

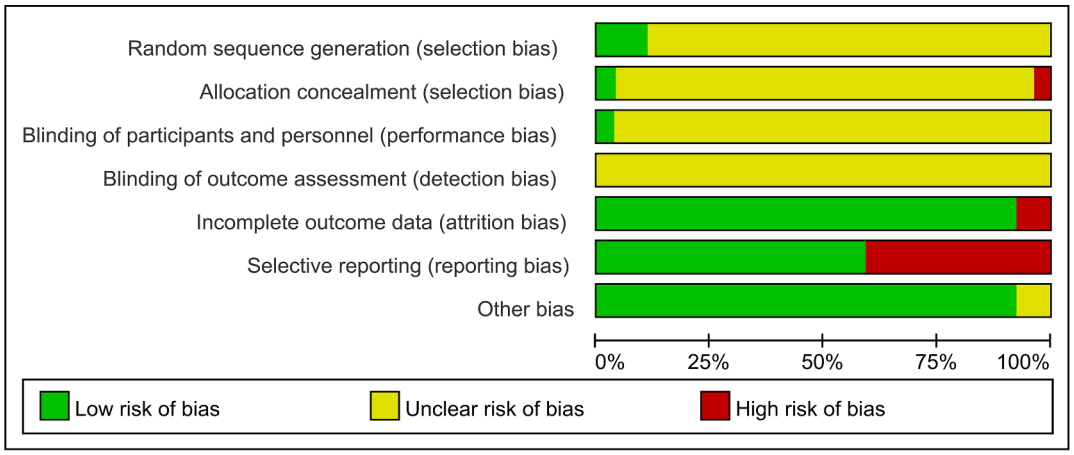
3.5. Quality of life

Seven trials with 382 cases [38,40,57,59,63,71,75] reported the QOL according to the KPS Scale [47,48] (Fig. 4). There was no statistical heterogeneity in QOL (I^2 = 0%); therefore, the data was synthesized using an FEM. The meta-analysis result demonstrated that administration of sTPs with chemotherapy significantly increased the QOL [RR = 2.05, 95% CI (1.62, 2.60), P < 0.00001)].

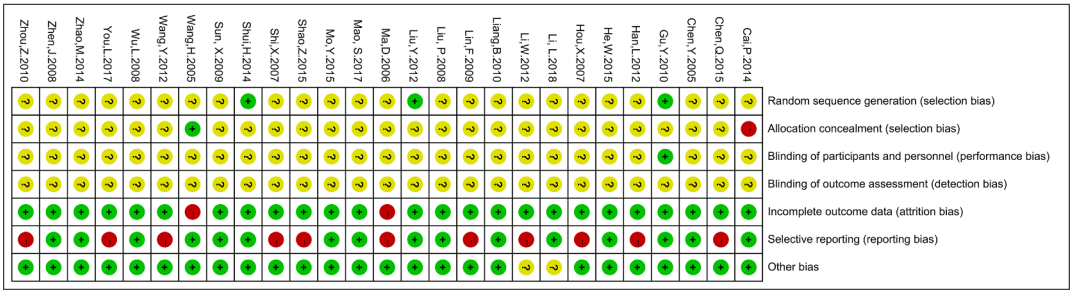
Table 1
Characteristics of the included trials.

First author, year	Non-small cell lung cancer (NSCLC)				Interventions		Criteria A, B, C	Follow-ups	Outcomes
	Stages	E/C	M/F	Age	Synthetic thymic peptides (sTPs) (dose/frequency, treatment time/cycle, cycles)	Chemotherapy			
Chen, Y. 2005 [56]	IIIb–IV	23/19	24/18	38–76	Thymopentin: 1 mg/time, 1–2/d, 15d/C, 2–3 cycles (IV)	NP	No/No/FCM	4–6w	O4, O5
Wang, H. 2005 [57]	III–IV	20/20	26/14	26–74	Thymosin α1: 1.6 mg/d, qd, 4d, change to 3/w, 4w/C, 1 cycle (SI)	MVP/NIP	WHO/NCICTC/FCM	3 years	O1–O5
Ma, D. 2006 [58]	III–IV	36/33	42/27	Un	Thymosin α1: 1.6 mg/d, qd, 7d, change to 1/2d, 3w/C, 4 cycles(SI)	NP	WHO/NCICTC/No	4 years	O1, O3
Hou, X. 2007 [59]	IIIb–IV	34/34	49/19	33–69	Thymosin α1: 1.6 mg/time, 2/w, 3w/C, 3–4 cycles(SI)	NP	WHO/Unclear/No	12w	O1, O2, O3
Shi, X. 2007 [60]	III–IV	29/29	49/9	36–69	Thymosin α1: 1.6 mg/time, 1/2d, 6w/C, 1 cycle (SI)	NP	WHO/WHO/FCM	6w	O1, O4–5
Zhen, J. 2008 [63]	Advanced	28/26	38/16	43–79	Thymosin α1: 1.6 mg/time, 2/w, 10d/C, 3–4 cycles (SI)	NP	WHO/WHO/No	12–16w	O1–2, O4
Liu, P. 2008 [61]	IIIa–IV	32/32	44/20	38–73	Thymopentin: 1 mg/time, qd, 4w/C, 2 cycles (IM)	NP	No/No/FCM	8w	O5
Wu, L. 2008 [62]	Advanced	16/15	19/12	Un	Thymosin α1: 1.6 mg/time, 3/w, 6w/C, 1 cycle (SI)	GP	No/No/Unclear	6w	O5
Lin, F. 2009 [64]	III–IV	100/100	162/38	36–69	Thymopentin: 10 mg/d, qd, 7–10d/C, 2 cycles (IV)	GP	WHO/WHO/Un	Un	O1, O5
Sun, X. 2009 [65]	III–IV	34/34	47/21	34–69	Thymosin α1: 1.6 mg/d, qd, 21d/C, 1 cycle (SI)	GC	No/No/FCM, MTT	3w	O5
Liang, B. 2010 [22]	IIIb–IV	30/28	39/19	65–75	Thymosin α1: 1.6 mg/time, 1/2d, 8w/C, 1 cycle (SI)	Gem	WHO/WHO/No	Un	O1, O4
Zhou, Z. 2010 [67]	IIIb–IV	33/35	51/17	Un	Thymopentin: 1.0 mg/time, 3/w, 4w/C, 3 cycles (SI)	EP	WHO/WHO/No	Un	O1, O4
Gu, Y. 2010 [66]	IV	29/29	30/28	40–75	Thymosin α1: 1.6 mg/d, qd, 4d, change to 2/w, 8w/C, 1 cycle (SI)	GP/DP	No/No/IIT, LDH	8w	O5
Han, L. 2012 [68]	IIIb–IV	32/32	42/22	42–68	Thymosin α1: 1.6 mg/time, 1/2d, 9w/C, 1 cycles (SI)	NP	WHO/WHO/FCM	Un	O1, O4–5
Li, W. 2012 [69]	III–IV	36/28	52/12	36–70	Thymosin α1: 1.6 mg/time, 3/w, 3–4w/C, 2 cycles (SI)	TP	WHO/WHO/No	6–8w	O1, O4
Liu, Y. 2012 [70]	III–IV	45/45	49/41	55–72	Thymosin α1: 1.6 mg/time, 2/w, 4w/C, 1 cycle (SI)	DP	RECIST/WHO/FCM	12w	O1, O4–5
Wang, Y. 2012 [71]	III–IV	30/30	47/13	40–74	Thymosin α1: 1.6 mg/time, 2/w, 3–6 M/C, 1 cycle (SI)	NP, TP, GP	WHO/WHO/FCM	4w	O1–5
Shui, H. 2014 [73]	IIIb–IV	24/25	31/18	43–76	Thymosin α1: 1.6 mg/time, 1/2d, 3w/C, 2 cycles (SI)	GP	RECIST/No/Unclear	Un	O1, O5
Zhao, M. 2014 [74]	IIIb–IV	52/48	59/41	32–68	Thymosin α1: 1.6 mg/time, 1/2d, 2w/C, 2–4 cycles (SI)	TP, NP, GP	No/No/FCM	1 year	O3–5
Cai, P. 2014 [72]	III–IV	40/38	50/28	42–76	Thymosin α1: 1.6 mg/time, 2/w, 6–8w/C, 1 cycle (SI)	NP	No/No/FCM	6w	O5
Chen, Q. 2015 [75]	Advanced	28/28	38/18	40–74	Thymopentin: 1 mg/d, qd, 7d/C, 2 cycles (IV)	GC	WHO/WHO/Unclear	Un	O1–2, O4
Mo, Y. 2015 [77]	III–IV	34/34	43/25	39–74	Thymosin α1: 1.6 mg/time, qd, 2 M/C, 1 cycle (SI)	GP	RECIST/No/Unclear	Un	O1, O5
Shao, Z. 2015 [78]	Advanced	32/32	48/16	36–78	Thymopentin: 1 mg/d, qd, 14d, 3w/C, 3 cycles (IV)	TP	WHO/Unclear/No	Un	O1, O4
He, W. 2015 [76]	IIIb–IV	25/25	31/19	70–80	Thymopentin: 1 mg/time, 2/d, 15d/C, 2 cycles (IV)	GP	No/No/FCM	4w	O5
You, L. 2017 [39]	III–IV	100/100	133/67	36–78	Thymosin α1: 1.6 mg/time, 2/w, 3w/C, 1 cycle (SI)	TP, NP, GP	No/WHO/FCM	Un	O4, O5
Mao, S. 2017 [38]	IIIb–IV	30/26	32/24	65–80	Thymosin α1: 1.6 mg/time, qd, 7–10d/C, 2 cycle (SI)	Pemetrexed	WHO/WHO/FCM	6w	O1–2, O4–5
Li, L. 2018 [40]	IIIb–IV	24/24	31/17	37–70	Thymopentin: 10 mg/time, 2/w, 14d/C, 2 cycles (IV)	TC	WHO	6–8w	O2, O4

Note: E/C: experimental group (synthetic thymic peptides with chemotherapy)/Control group (chemotherapy alone), M/F: male/female, SI: subcutaneous injection, IV: intravenous injection, IM: intramuscular injection, DP: docetaxel and cisplatin, EP: etoposide and cisplatin, NP: navelbine and cisplatin, MVP: mitomycin, vindesine and cisplatin, NIP: navelbine, ifosfamide and cisplatin, GP: gemcitabine and cisplatin, TP: taxinol and cisplatin, TC: taxinol and carboplatin, Gem: gemcitabine, GC: gemcitabine and carboplatin, Criteria.A: evaluation criteria of tumor response, Criteria.B: evaluation criteria of ADRs, RECIST: response evaluation criteria in solid tumors, CTCAE: Common Terminology Criteria for Adverse Events, Criteria.C: test methods of peripheral blood lymphocytes, FCM: flow cytometry, IIT: indirect immunofluorescence test, LDH: lactic dehydrogenase release. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. O: Outcomes, O1: ORR and DCR, O2: QOL, O3: survival, O4: ADRs, O5: peripheral blood lymphocyte level.



Risk of bias graph



Risk of bias summary

Fig. 2. Methodological bias risk of included trials.

3.6. Overall survival

Four trials with 269 cases [57,58,71,74] reported OS rates (Fig. 5). There was no heterogeneity in 1-, 2- or 3-year OS rates ($I^2 = 0\%$). Therefore, all data were synthesized using an FEM. The meta-analysis results demonstrated that administration of sTPs with chemotherapy significantly increased the 1-year OS rate [RR = 1.43, 95% CI (1.15 to 1.78), $P = 0.001$]. However, the 2-, 3-, and 4-year OS rates showed no significant differences between the two groups [RR = 1.68, 95% CI (0.96 to 2.97), $P = 0.07$], [RR = 2.38, 95% CI (1.00 to 5.68), $P = 0.05$] and [RR = 5.50, 95% CI (0.70 to 43.31), $P = 0.11$].

3.7. Peripheral blood lymphocytes levels

Eighteen trials with 1376 cases [38,39,56,57,60–62,64–66,68,70–74,76,77] reported peripheral blood lymphocyte levels (Fig. 6). Pearson's chi-square and I^2 tests demonstrated statistical heterogeneity among the trials in $CD3^+$ T cells ($I^2 = 95\%$), $CD3^+$ $CD4^+$ T cells ($I^2 = 94\%$), $CD3^+$ $CD8^+$ T cells ($I^2 = 97\%$), $CD4^+/CD8^+$ T cell ratio ($I^2 = 92\%$), and NK cell activity ($I^2 = 96\%$). Therefore, all data were synthesized using an REM. The meta-analysis results demonstrated that administration of sTPs with chemotherapy significantly increased the proportions of $CD3^+$ T cells [SMD = 1.81, 95% CI (1.15 to 2.48), $P < 0.00001$], $CD3^+$ $CD4^+$ T cells [SMD = 2.27, 95% CI (1.72 to 2.83), $P < 0.00001$], and NK cells [SMD = 1.97, 95% CI (1.17 to 2.76), $P < 0.00001$], as well as the ratio of $CD4^+/CD8^+$ T cells [SMD = 1.65, 95% CI (1.19 to 2.12), $P < 0.00001$]. However, the proportion of $CD3^+$ $CD8^+$ T cells were not significantly different between the two groups [SMD = −0.63, 95% CI (−1.43 to 0.16), $P = 0.12$].

3.8. Adverse drug reactions and hospital-acquired infections

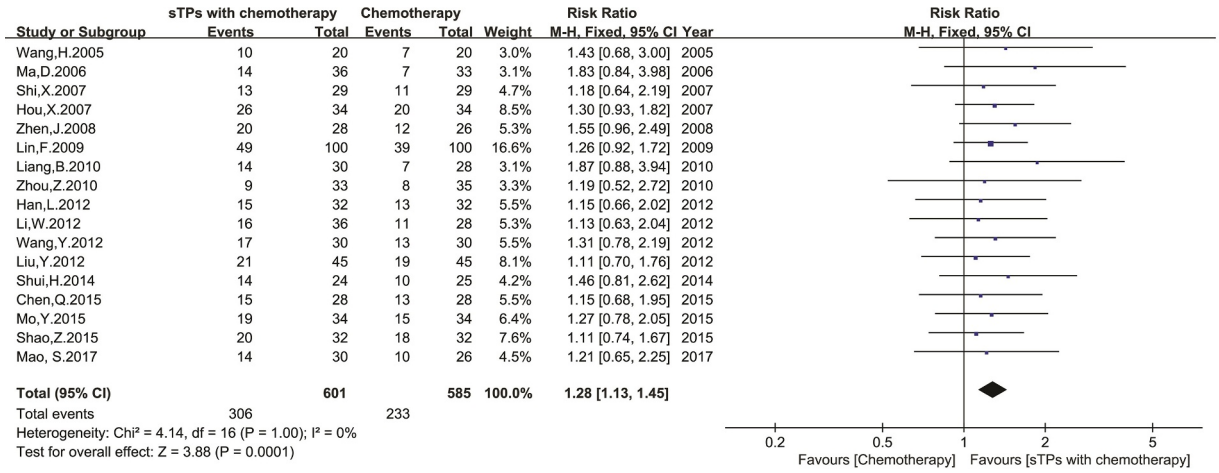
Sixteen trials with 1122 cases [22,38–40,56,57,60,63,67–71,74,75,78] reported the ADRs according to WHO or Common Terminology Criteria for Adverse Events guidelines [45,49] (Table 2 and Fig. S1–6). Pearson's chi-square and I^2 tests demonstrated statistical heterogeneity for neutropenia ($I^2 = 85\%$), minimal heterogeneity for thrombocytopenia ($I^2 = 35\%$), and no heterogeneity for other ADRs ($I^2 = 0\%$). Therefore, the RR of neutropenia was synthesized using an REM, and data for other ADRs were synthesized using an FEM. The meta-analysis results demonstrated that administration of sTPs with chemotherapy resulted in lower risk of neutropenia (RR = 0.75, 95% CI 0.57 to 0.99, $P = 0.04$), thrombocytopenia (RR = 0.68, 95% CI 0.55 to 0.83, $P = 0.0002$), and gastrointestinal reactions (RR = 0.62, 95% CI 0.53 to 0.71, $P < 0.00001$) than with chemotherapy alone. There were no statistical differences in the risk of liver dysfunction (RR = 0.67, 95% CI 0.35 to 1.31, $P = 0.24$), renal dysfunction (RR = 0.70, 95% CI 0.14 to 3.44, $P = 0.66$), neurotoxicity (RR = 0.82, 95% CI 0.37 to 1.79, $P = 0.62$), or phlebitis (RR = 1.40, 95% CI 0.47 to 4.16, $P = 0.54$) between the two groups.

Finally, three trials with 182 cases [56,57,74] reported HAIs. Pearson's chi-square and I^2 tests demonstrated no heterogeneity in HAI ($I^2 = 0\%$); therefore, the data were synthesized using an FEM. The meta-analysis result showed that the risk of HAIs were not significantly different between the groups (RR = 0.69, 95% CI 0.41 to 1.14, $P = 0.15$).

3.9. Subgroup analysis

Subgroup analysis was performed to reveal the influences of sTPs types, treatment frequency, time of cycle, number of cycles, and

a. ORR



b. DCR

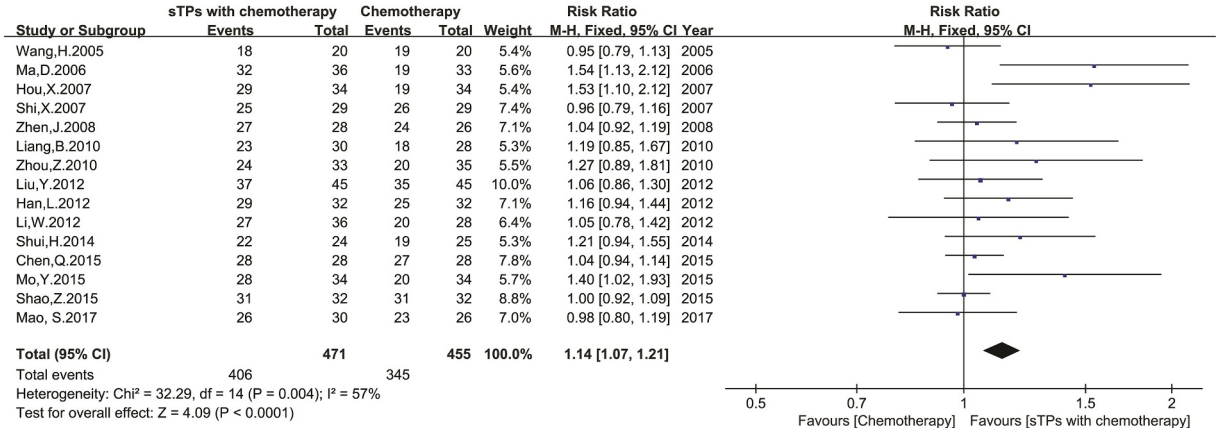


Fig. 3. The analysis of tumor response between the two groups.

combination with various chemotherapies on tumor response (Table 3, Figs. S7–16). This analysis revealed that only Tα1 (via subcutaneous injection) significantly increased the ORR and DCR (Table 3a, Figs. S7–8). With treatment twice per week, sTPs significantly increased the ORR (Table 3b, Figs. S9–10). With three weeks of treatment per cycle, sTPs significantly increased the ORR and DCR (Table 3c, Figs. S11–12). With one cycle of treatment, sTPs significantly increased the ORR, and with four cycles, sTPs significantly increased the DCR (Table 3d, Figs. S13–14). Combining sTPs with gemcitabine chemotherapy (GP/gemcitabine and carboplatin (GC)/gemcitabine (Gem)) and NP significantly increased the ORR (Table 3e, Figs. S15–16).

Subgroup analysis was also performed to reveal the influences of these variables on peripheral blood lymphocyte levels (Table 4, Figs. S17–41). The analysis demonstrated that Tα1 (via subcutaneous injection) and thymopentin (via intravenous/intramuscular injection) significantly increased $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, NK cells, and the $CD4^+/CD8^+$ T cell ratio (Table 4a, Figs. S17–21). With treatment once every two days or twice a week, sTPs significantly increased $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, NK cells, and the $CD4^+/CD8^+$ T cell ratio (Table 4b, Figs. S11–26). With 3- and 6-week per cycle, sTPs significantly increased $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, NK cells, and the $CD4^+/CD8^+$ T cell ratio. With 7–10 days or 2-, 3-, 4-, or 6-week per cycle, sTPs significantly increased NK cells and the $CD4^+/CD8^+$ T cell ratio (Table 4c, Figs. S27–31). With one cycle, sTPs significantly increased $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, NK cells, and the $CD4^+/CD8^+$ T cell ratio (Table 4d, Figs. S32–36). In combination with GP/

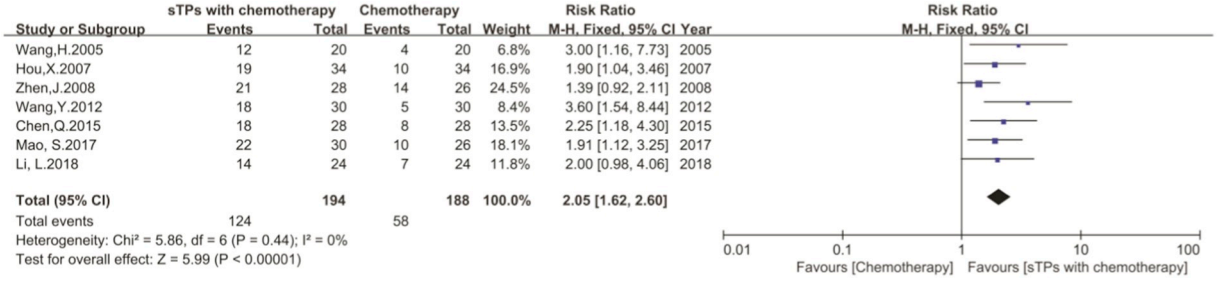


Fig. 4. The analysis of QOL between the two groups.

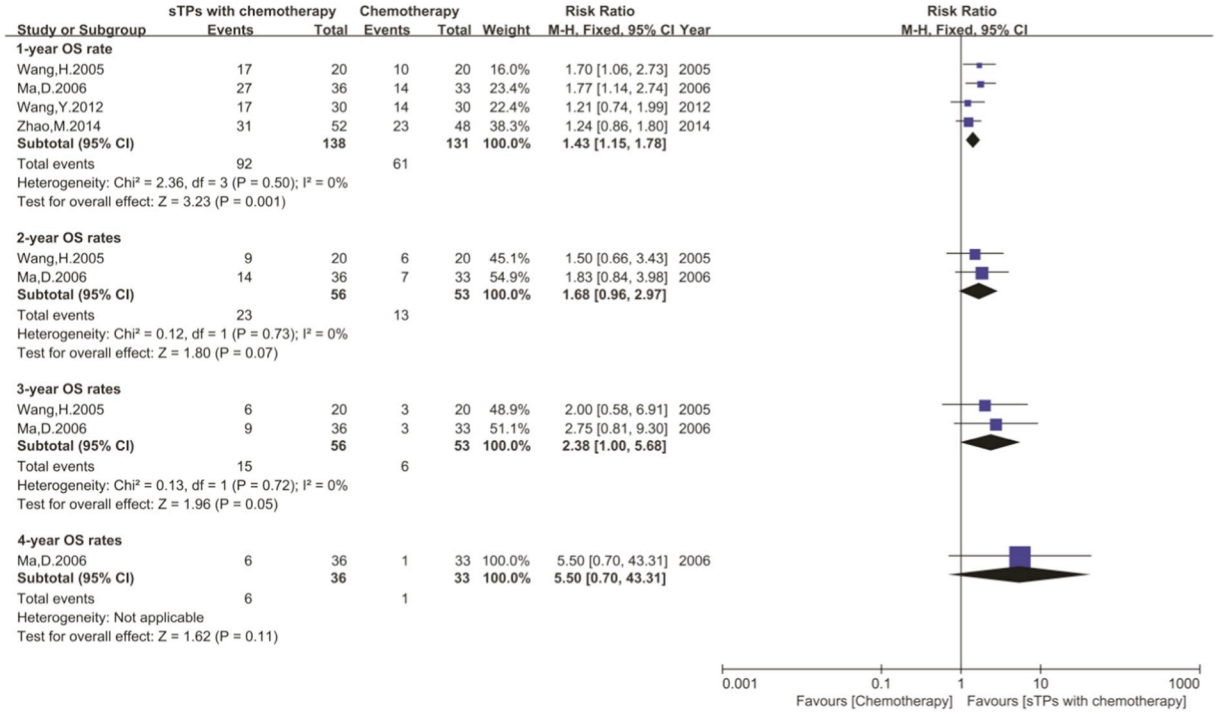


Fig. 5. The analysis of OS rates between the two groups.

GC/Gem, NP, and DP, sTPs significantly increased $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, NK cells, and the $CD4^+/CD8^+$ T cell ratio (Table 4e, Figs. S37–41).

3.10. Publication bias analysis

The funnel plots were symmetric for ORR and gastrointestinal reactions (Fig. 7a and c), indicating no publication bias in these trials for these variables. The funnel plots were significantly asymmetric for the DCR, $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, $CD4^+/CD8^+$ T cell ratio, and NK cells (Fig. 7b, d, e, f, g, and h), indicating publication bias. The DCR was consistently underestimated, while $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, NK cells, and the $CD4^+/CD8^+$ T cell ratio were both under- and over-estimated.

3.11. Sensitivity analysis

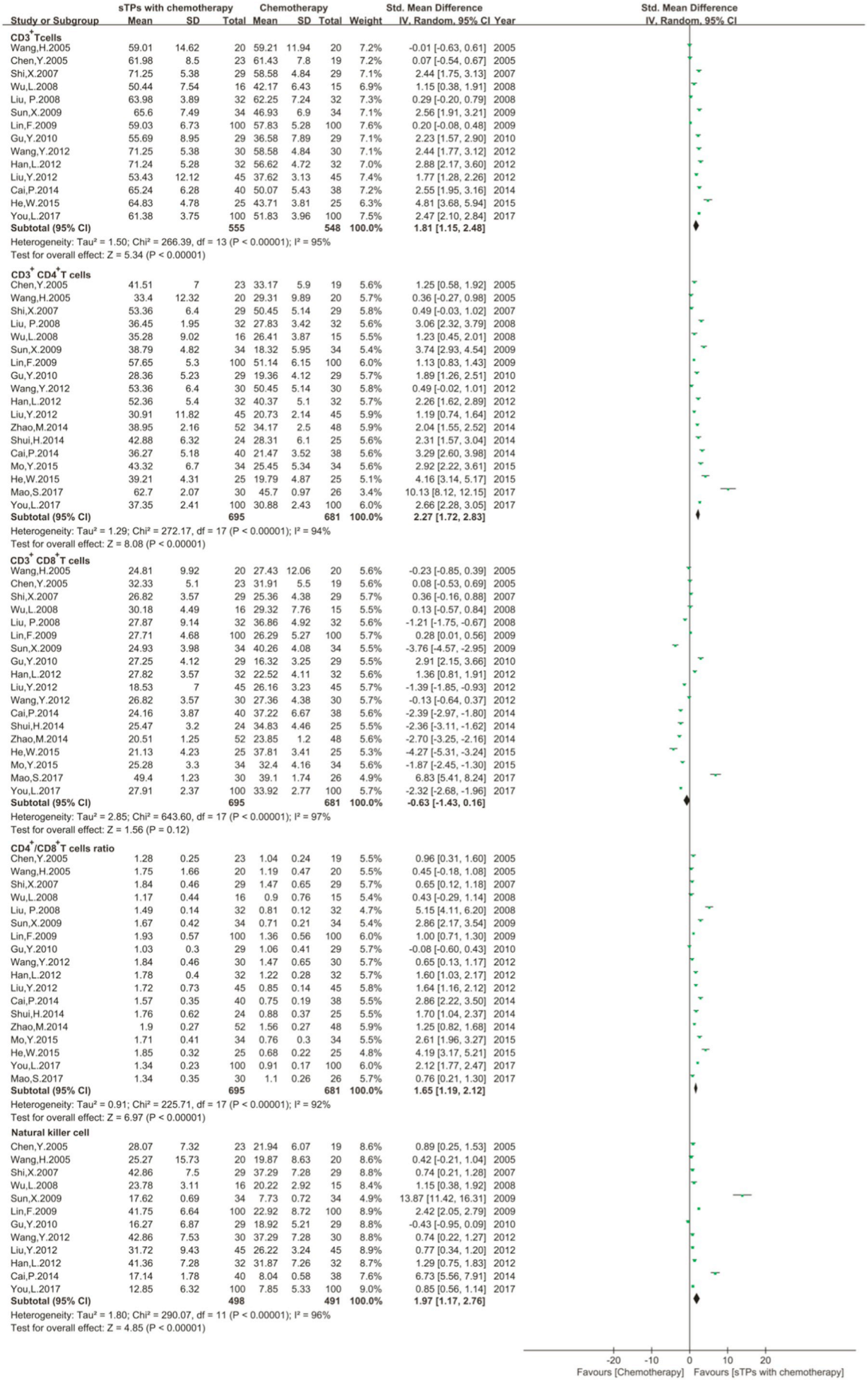
Of the 27 trials, 13 trials [39,57–60,64,67–69,71,72,75,78] had at least one domain with a high risk of bias, including selection bias [72], attrition bias [57,58], and reporting bias [39,58–60,64,67–69,71,75,78], and were designated as poor trials. Poor trials presented in all outcomes. Sensitivity analysis showed that the RRs of the 1-, 2-, 3-, and 4-year OS rates, neurotoxicity, and phlebitis had poor robustness before and after excluding the poor trials. All other results had good robustness (Table 5a and c). There was statistical heterogeneity for the DCR, neutropenia, $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, the $CD4^+/CD8^+$ T cell ratio, and NK cells. Publication bias was evident for the DCR, $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, $CD4^+/CD8^+$ T cell ratio, and NK cells. Therefore, sensitivity analysis was performed by excluding trials over-estimating efficacy and under-estimating ADRs (Table 5b and d). Sensitivity analysis showed that with the exception of neutropenia, all other outcomes had good robustness.

3.12. Quality of evidence

Of the 27 trials, 13 were poor [39,57–60,64,67–69,71,72,75,78], and most trials had unclear bias risk. The RRs for the 1-, 2-, 3-, and 4-year OS rates, neurotoxicity, and phlebitis had poor robustness; therefore, their quality was down-rated by two levels. The RRs for the ORR, DCR, QOL, neutropenia, thrombocytopenia, gastrointestinal reactions, liver dysfunction, renal dysfunction, and HAIs had good robustness, as did the SMDs of $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, NK cells, and $CD4^+/CD8^+$ T cell ratio. Therefore, their quality was down-rated by only one level. There was statistical heterogeneity for the DCR, neutropenia, $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, NK cells, and the $CD4^+/CD8^+$ T cell ratio. The RR for neutropenia had poor robustness, and its quality was down-rated by one level. The number of patients included for 1-, 2-, 3-, and 4-year OS rates, renal dysfunction, neurotoxicity, phlebitis, and HAIs were < 300 cases, and their quality was down-rated by one level. Publication bias was evident for the DCR, $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, NK cells, and $CD4^+/CD8^+$ T cell ratio. The results had good robustness; therefore, their evidence was not down-rated. No outcomes were eligible for upgrade. Taken together, the quality of evidence was moderate for ORR, DCR, QOL, thrombocytopenia, gastrointestinal reactions, liver dysfunction, $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, NK cells, and $CD4^+/CD8^+$ T cell ratio; low for neutropenia, renal dysfunction, and HAIs; and very low for 1-, 2-, 3-, and 4-year OS rates, neurotoxicity, and phlebitis (Table 6).

4. Discussion

Thought to enhance both specific and nonspecific immune functions [22], sTPs are widely used in the treatment of bacterial and viral diseases [23–27]. Furthermore, sTPs have demonstrated the ability to inhibit tumor cell proliferation, induce apoptosis [22,28–32], and prevent carcinogenesis [33,34], instructing their use in cancer treatment [18,37,41,79]. For lung cancer, an increasing number of clinical trials evaluating sTPs use with chemotherapy have been reported [38–40]. However, previous meta-analyses [36,37,41] did not address whether sTPs administration with chemotherapy improves tumor response and survival in patients with NSCLC. Therefore, we expanded the analyses performed in these studies to include 17 additional trials with 1186 cases [22,38,57–60,63,64,67–71,73,75,77,78] to determine whether



(caption on next page)

Fig. 6. The analysis of peripheral blood lymphocyte level between the two groups.

Table 2
Meta-analysis results of ADRs and HAI (Fig. S1–6).

Outcomes	Trials	Experimental groups (Evens/total)	Control groups (Evens/total)	SM	RR (95% CI)	I^2	*P*
Neutropenia (Fig. S1)	8	108/226	142/222	REM	0.75 [0.57, 0.99]	85%	$P = 0.04$
Thrombocytopenia (Fig. S2)	8	69/226	100/222	FEM	0.68 [0.55, 0.83]	35%	$P = 0.0002$
Gastrointestinal reactions (Fig. S3)	11	126/405	201/389	FEM	0.62 [0.53, 0.71]	0%	$P < 0.00001$
Liver dysfunction (Fig. S4)	5	13/153	19/151	FEM	0.67 [0.35, 1.31]	0%	$P = 0.24$
Renal dysfunction (Fig. S4)	3	2/91	3/89	FEM	0.70 [0.14, 3.44]	0%	$P = 0.66$
Neurotoxicity (Fig. S5)	2	9/59	11/59	FEM	0.82 [0.37, 1.79]	0%	$P = 0.62$
Phlebitis (Fig. S5)	2	7/61	5/61	FEM	1.40 [0.47, 4.16]	0%	$P = 0.54$
Hospital-acquired infection (HAI) (Fig. S6)	3	18/95	24/87	FEM	0.69 [0.41, 1.14]	0%	$P = 0.15$

Note: ADRs: adverse drug reactions, HAI: hospital-acquired infection, SM: statistical Method, REM: random-effects model, FEM: fixed-effects model, RR: risk ratios.

Table 3
Subgroup analysis results of ORR and DCR.

Subgroups	Trials	Cases	SM	Objective response rate (ORR)			Trials	Cases	SM	Disease control rate (DCR)		
				RR (95% CI)	I^2	*P*				RR (95% CI)	I^2	*P*
Total	17	1186	FEM	1.28 [1.13, 1.45]	0%	$P = 0.0001$	15	926	REM	1.10 [1.01, 1.18]	57%	$P = 0.02$
Table 3a. Subgroups analysis via sTPs types (Fig. S7–8)												
Thymosin α1 (subcutaneous injection)	13	798	FEM	1.32 [1.14, 1.54]	0%	$P = 0.0003$	12	738	REM	1.11 [1.02, 1.22]	49%	$P = 0.02$
Thymopentin (intravenous or subcutaneous injection)	4	388	FEM	1.20 [0.96, 1.50]	0%	$P = 0.11$	3	188	REM	1.04 [0.93, 1.16]	56%	$P = 0.52$
Table 3b. Subgroups analysis via frequency (Fig. S9–10)												
One time/day	5	444	FEM	1.21 [1.00, 1.47]	0%	$P = 0.05$	4	244	REM	1.04 [0.93, 1.16]	59%	$P = 0.47$
One time/two days	4	229	FEM	1.36 [1.00, 1.85]	0%	$P = 0.05$	4	229	REM	1.09 [0.97, 1.23]	0%	$P = 0.13$
Two times/week	4	272	FEM	1.29 [1.04, 1.61]	0%	$P = 0.02$	3	212	REM	1.14 [0.93, 1.42]	67%	$P = 0.21$
Three times/week	2	132	FEM	1.16 [0.71, 1.87]	0%	$P = 0.56$	2	132	REM	1.14 [0.90, 1.43]	0%	$P = 0.27$
One time/day change to one time/two days	1	69	FEM	1.83 [0.84, 3.98]	No	$P = 0.13$	1	69	No	1.54 [1.13, 2.12]	No	$P = 0.007$
One time/day change to three times/week	1	40	FEM	1.43 [0.68, 3.00]	No	$P = 0.35$	1	40	No	0.95 [0.79, 1.13]	No	$P = 0.55$
Table 3c. Subgroups analysis via treatment time per cycle (Fig. S11–12)												
7–10 days/cycle	4	366	FEM	1.28 [1.03, 1.60]	0%	$P = 0.03$	3	166	3	1.03 [0.96, 1.11]	0%	$P = 0.41$
2 weeks/cycle	1	64	FEM	1.11 [0.74, 1.67]	No	$P = 0.61$	1	64	1	1.00 [0.92, 1.09]	No	$P = 1.00$
3 weeks/cycle	4	250	FEM	1.37 [1.06, 1.77]	0%	$P = 0.02$	4	250	4	1.30 [1.12, 1.52]	23%	$P = 0.0007$
3–4 weeks/cycle	1	64	FEM	1.13 [0.63, 2.04]	No	$P = 0.68$	1	64	1	1.05 [0.78, 1.42]	No	$P = 0.75$
4 weeks/cycle	3	198	FEM	1.19 [0.83, 1.70]	0%	$P = 0.33$	3	198	3	1.04 [0.88, 1.23]	36%	$P = 0.64$
6 weeks/cycle	1	58	FEM	1.18 [0.64, 2.19]	No	$P = 0.60$	1	58	1	0.96 [0.79, 1.16]	No	$P = 0.69$
8 weeks/cycle	2	126	FEM	1.46 [0.97, 2.20]	0%	$P = 0.07$	2	126	2	1.30 [1.03, 1.64]	0%	P = 0.03
12–24 weeks/cycle	1	60	FEM	1.31 [0.78, 2.19]	No	$P = 0.31$	No	No	No	No	No	No
Table 3d. Subgroups analysis via treatment cycles (Fig. S13–14)												
One cycle	7	438	FEM	1.27 [1.03, 1.57]	0%	$P = 0.03$	6	378	REM	1.07 [0.95, 1.20]	35%	$P = 0.25$
Two cycles	5	425	FEM	1.24 [1.00, 1.54]	0%	$P = 0.05$	4	225	REM	1.04 [0.96, 1.13]	0%	$P = 0.29$
Three cycles	2	132	FEM	1.14 [0.78, 1.66]	0%	$P = 0.51$	2	132	REM	1.11 [0.72, 1.69]	82%	$P = 0.64$
Three to four cycles	2	122	FEM	1.39 [1.06, 1.84]	0%	$P = 0.02$	2	122	REM	1.24 [0.74, 2.09]	88%	$P = 0.41$
Four cycles	1	69	FEM	1.83 [0.84, 3.98]	0%	$P = 0.13$	1	69	REM	1.54 [1.13, 2.12]	0%	$P = 0.007$
Table 3e. Subgroups analysis via chemotherapy regimens (Fig. S15–16)												
Gemcitabine chemo* (GP/GC/Gem)	5	431	FEM	1.32 [1.07, 1.63]	0%	$P = 0.01$	4	231	REM	1.17 [0.96, 1.44]	67%	$P = 0.12$
Vinorelbine and cisplatin (NP)	5	313	FEM	1.36 [1.08, 1.71]	0%	$P = 0.008$	5	313	REM	1.18 [0.98, 1.43]	74%	$P = 0.08$
Taxanes and cisplatin (TP/DP)	3	218	FEM	1.11 [0.85, 1.47]	0%	$P = 0.44$	3	218	REM	1.01 [0.94, 1.09]	0%	$P = 0.78$
Etoposide and cisplatin (EP)	1	68	FEM	1.19 [0.52, 2.72]	No	$P = 0.67$	1	68	REM	1.27 [0.89, 1.81]	0%	$P = 0.18$
Pemetrexed chemotherapy	1	56	FEM	1.21 [0.65, 2.25]	No	P = 0.54	1	56	REM	0.98 [0.80, 1.19]	No	$P = 0.84$
Systemic chemotherapy	2	100	FEM	1.35 [0.88, 2.06]	0%	$P = 0.17$	1	40	REM	0.95 [0.79, 1.13]	No	$P = 0.55$

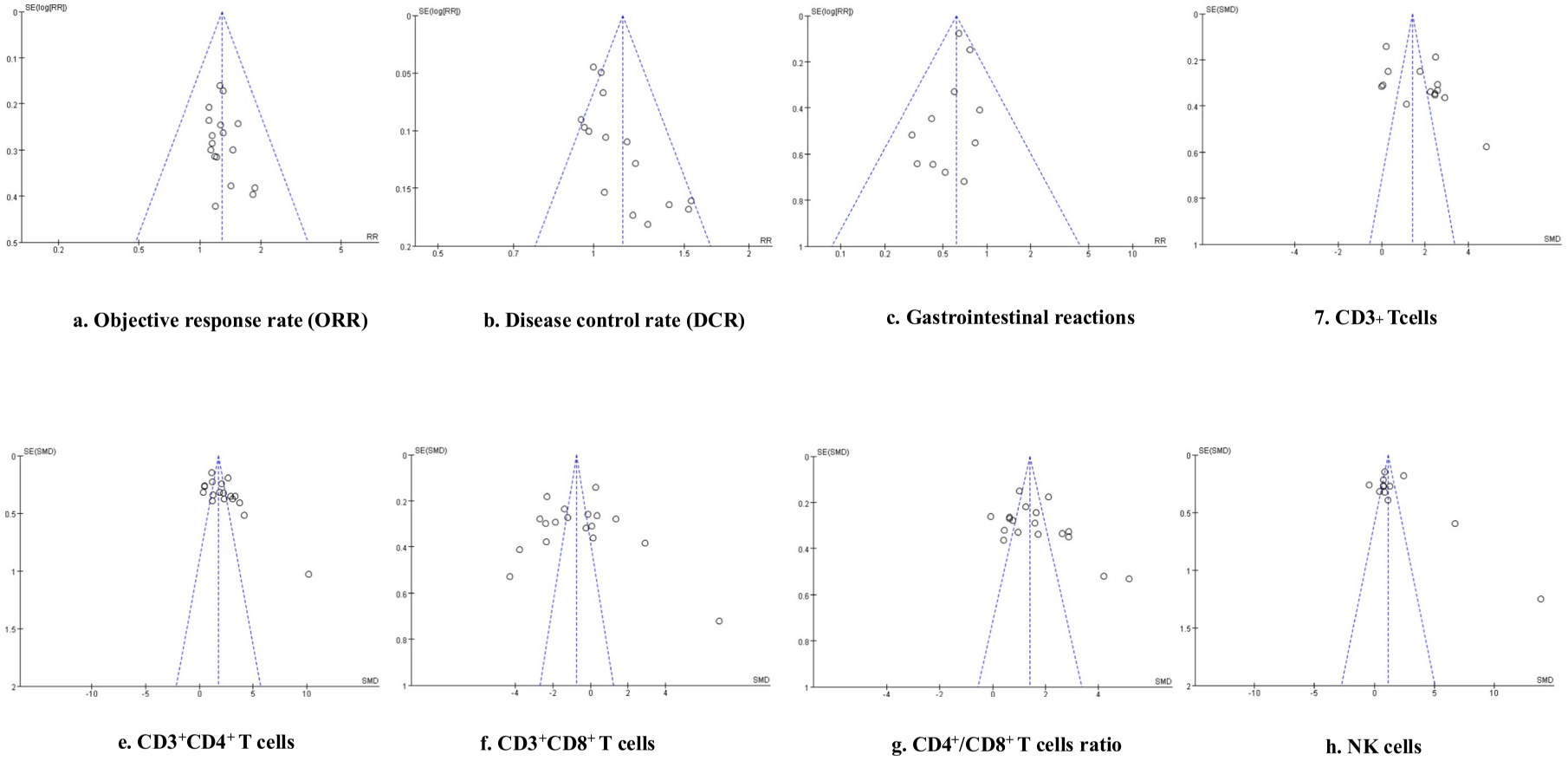
Note: sTPs: synthetic thymic peptides, GP: gemcitabine and cisplatin, GC: gemcitabine and carboplatin, Gem: gemcitabine, TP: taxinol and cisplatin, NP: navelbine and cisplatin, DP: docetaxel and cisplatin, EP: etoposide and cisplatin, SM: statistical method, RR: risk ratios, FEM: fixed-effects model, REM: random-effects model.

sTPs improve tumor response and patient survival compared to chemotherapy alone. The meta-analysis demonstrated that sTPs administration with chemotherapy resulted in significant improvement in the ORR, DCR, and QOL for patients with NSCLC. Most trials had unclear bias risk, and the results had good robustness, with moderate quality. Meta-analysis of 4 trials with 269 cases [57,58,71,74] further demonstrated an improved 1-year OS rate, but none of the trials reported the PFS. The results had poor robustness, and the quality was very low. Jiang et al [41] reported that Tα1 with NP/GP significantly increased the ORR and DCR in NSCLC. However, only Tα1 with NP improved the 1-year OS rate. Lin et al. [36] reported that Tα1 combined with platinum chemotherapy improved the DCR only. Compared with previous studies [36,41], this meta-analysis featured more trials and larger sample sizes for analysis, and revealed that sTPs administration with chemotherapy improves the ORR, DCR, QOL, and 1-year OS rate. Lin et al. [36] also reported that Tα1 with chemoradiotherapy improved the DCR only. In addition, a phase II randomized trial [80] demonstrated that in patients with unresectable advanced hepatocellular carcinoma, thymalfasin with transarterial chemoembolization resulted in higher rates of survival and tumor response than transarterial

Table 4
Subgroup analysis results of peripheral blood lymphocyte level.

Subgroups	$CD3^+$ T cells			$CD3^+$ $CD4^+$ T cells			$CD3^+$ $CD8^+$ T cells			$CD4^+/CD8^+$ T cells ratio			Natural killer cells		
	Trials	Case	SMD (95% CI)	Trials	Case	SMD (95% CI)	Trials	Cases	SMD (95% CI)	Trials	Case	SMD (95% CI)	Trials	Case	SMD (95% CI)
Totality	14	1103	1.81 1.15,2.48]	18	1376	2.27 [1.72, 2.83]	18	1376	−0.63[−1.43,0.16]	18	1376	1.65[1.19,2.12]	12	989	1.97 [1.17,2.76]
Table 4a. Subgroups analysis via sTPs types (Fig. S17–21)															
Thymosin α1 (subcutaneous injection)	10	747	2.05 1.54,2.57]	14	1020	2.28 [1.61, 2.94]	14	1020	−0.45[−1.44,0.53]	14	1020	1.39[0.91,1.86]	12	989	2.05 [1.15, 2.95]
Thymopentin (intravenous or intramuscular injection)	4	356	1.20[0.00,2.40]	4	356	2.34[1.08,3.61]	4	356	−1.21[−2.63, 0.22]	4	356	2.77[0.98, 4.56]	2	242	1.68 [0.17, 3.18]
Table 4b. Subgroups analysis via frequency (Fig. S22–26)															
One time/day	3	332	0.99[−0.25,2.23]	5	456	3.93 [2.21, 5.66]]	5	456	−0.05[−1.96,1.86]	5	456	2.41[1.20, 3.63]	2	268	8.08[−3.14,19.29]
One time/two days	2	122	2.66[2.16, 3.15]	5	313	1.66 [0.94, 2.38]	5	313	−0.65[−2.22, 0.92]	5	313	1.22[0.85, 1.59]	3	164	0.98 [0.64, 1.31]
Two times/day	1	50	4.81[3.68, 5.94]	1	50	4.16 [3.14, 5.17]	1	50	−4.27[−5.31,-3.24]	1	50	4.19[3.17, 5.21]	No	No	No
One to two times/day	1	42	0.07[−0.54,0.67]	No	No	No	No	No	No	No	No	No	No	No	No
Two times/week	4	428	2.30[1.93, 2.67]	4	428	1.90 [0.72, 3.07]	4	428	−1.56[−2.56,-0.55]	4	428	1.81[1.01, 2.61]	4	428	2.11 [0.75, 3.47]
Three times/week	1	31	1.15[0.38, 1.91]	1	31	1.23 [0.45, 2.01]	1	31	0.13 [−0.57, 0.84]	1	31	0.43[−0.29,1.14]	1	31	1.15 [0.38, 1.92]
One time to 2 times/week	1	58	2.23[1.57, 2.90]	1	58	1.89 [1.26, 2.51]	1	58	2.91 [2.15, 3.66]	1	58	−0.08[−0.60,0.43]	1	58	−0.43[−0.95,0.09]
One time to 3times/week	1	40	−0.01[−0.63,0.61]	1	40	0.36 [−0.27, 0.98]	1	40	−0.23[−0.85,0.39]	1	40	0.45[−0.18, 1.08]	1	40	0.42[−0.21, 1.04]
Table 4c. Subgroups analysis via treatment time per cycle (Fig. S27–31)															
7–10 days/cycle	1	200	0.20[−0.08,0.48]	2	256	5.57[−3.25,14.40]	2	256	3.52 [−2.89,9.93]	2	256	0.95[0.69,1.21]	1	200	2.42[2.05,2.79]
2 weeks/cycle	2	92	2.41[−2.24,7.06]	3	192	2.42[1.09,3.75]	3	192	−2.27[−4.62,0.07]	3	192	2.06[0.57,3.56]	1	42	0.89[0.25,1.53]
3 weeks/cycle	3	332	2.56[2.26,2.85]	4	381	2.71[2.16,3.25]	4	381	−1.76[−3.86,0.34]	4	381	2.06[1.58,2.53]	3	332	4.62[1.99,7.25]
4 weeks/cycle	3	194	0.69[−0.41,1.79]	3	194	1.52[0.17,2.87]	3	194	−0.97[−1.63,-0.31]	3	194	2.36[0.28,4.45]	2	130	0.66[0.31,1.01]
6 weeks/cycle	2	89	1.81[0.54,3.08]	2	89	0.80[0.09,1.52]	2	89	0.28[−0.14,0.70]	2	89	0.57[0.14,0.99]	2	89	0.88[0.44,1.31]
8 weeks/cycle	1	58	2.23[1.57,2.90]	2	126	2.39[1.38,3.40]	2	126	0.51[−4.18,5.19]	2	126	1.26[−1.38,3.90]	1	58	−0.43[−0.95,0.09]
6–8 weeks/cycle	1	78	2.55[1.95,3.16]	1	78	3.29[2.60,3.98]	1	78	−2.39[−2.97,-1.80]	1	78	2.86[2.22,3.50]	1	78	6.73[5.56,7.91]
12–18 weeks/cycle	1	60	2.44 [1.77,3.12]	1	60	0.49 [−0.02, 1.01]	1	60	−0.13[−0.64,0.37]	1	60	0.65[0.13,1.17]	1	60	0.74[0.22,1.27]
Table 4d. Subgroups analysis via treatment cycles (Fig. S32–36)															
One cycle	8	643	2.23[1.91, 2.54]	9	711	1.97 [1.21, 2.73]	9	711	−0.94[−2.02, 0.13]	9	711	1.52[0.82, 2.22]	8	643	2.47[1.34, 3.60]
Two cycles	4	354	1.18[−0.03,2.39]	6	459	3.24[1.79,4.70]	6	459	−0.24[−1.86,1.39]	6	459	2.12[1.03,3.22]	2	240	1.44[−0.53,3.40]
Two to three cycles	1	42	0.07[−0.54,0.67]	1	42	1.25[0.58,1.92]	1	42	0.08[−0.53,0.69]	1	42	0.96[0.31,1.60]	1	42	0.89[0.25,1.53]
Two to four cycles	No	No	No	1	100	2.04[1.55,2.52]	1	100	−2.70[−3.25,-2.16]	1	100	1.25[0.82,1.68]	No	No	No
Three cycles	1	64	2.88[2.17,3.60]	1	64	2.26[1.62,2.89]	1	64	1.36[0.81,1.91]	1	64	1.60[1.03,2.17]	1	64	1.29[0.75,1.83]
Table 4e. Subgroups analysis via chemotherapy regimens (Fig. S37–41)															
Gemcitabine chemotherapy	4	349	2.12[0.37, 3.88]	6	466	2.54 [1.51, 3.58]	6	466	−1.95[−3.47,-0.42]	6	466	2.09[1.15, 3.03]	3	299	5.38 [2.09,8.67]
Navebine & cisplatin	5	306	1.64[0.43, 2.84]	5	306	2.06 [0.97, 3.15]	5	306	−0.36[−1.62, 0.91]	5	306	2.19[0.94, 3.45]	4	242	2.33 [0.55,4.10]
Taxanes and cisplatin	1	90	1.77[1.28, 2.26]	1	90	1.19 [0.74, 1.64]	1	90	−1.39[−1.85,-0.93]	1	90	1.64[1.16, 2.12]	1	90	0.77 [0.34,1.20]
Pemetrexed	No	No	No	1	56	10.13[8.12,12.15]	1	56	6.83 [5.41, 8.24]	1	56	0.76[0.21,1.30]	No	No	No
Systemic chemotherapy	4	358	1.79[0.65, 2.93]	5	458	1.50 [0.57, 2.42]	5	458	−0.51[−2.22, 1.19]	5	458	0.89[0.07, 1.71]	4	358	0.41[−0.17, 1.00]

Note: sTPs: synthetic thymic peptides, Taxanes and cisplatin: docetaxel and cisplatin (DP), NP: navebine and cisplatin, Gemcitabine chemotherapy: gemcitabine and cisplatin/carboplatin (GP/GC), Systemic chemotherapy: GP, TP, DP and et.al, REM: random effect model, SMD: Std mean difference, SM: statistical Method.



a. Objective response rate (ORR) **b. Disease control rate (DCR)** **c. Gastrointestinal reactions** **7. CD3+ Tcells**

e. $CD3^+CD4^+$ T cells **f. $CD3^+CD8^+$ T cells** **g. $CD4^+/CD8^+$ T cells ratio** **h. NK cells**

Fig. 7. The analysis of publication bias.

Table 5
Sensitivity analysis.

Indicators	Trials	SM	RR (95% CI)	I^2	Excluded trials (Reference number)	Trials	SM	RR (95% CI)	I^2
Table 5a. Sensitivity analysis by excluding the poor trials									
Objective response rate	17	FEM	1.28 [1.13, 1.45]	0%	Poor* [57–60,64,67–69,71,75,78]	6	FEM	1.35 [1.08, 1.68]	0%
Disease control rate	15	REM	1.10 [1.01, 1.18]	57%	Poor* [57–60,67–69,75,78]	6	FEM	1.13 [1.02, 1.24]	13%
1-year overall survival rate	4	FEM	1.43 [1.15, 1.78]	0%	Poor* [57,58,71]	1	FEM	1.24 [0.86, 1.80]	No
2-year overall survival rates	2	FEM	1.68 [0.96, 2.97]	0%	Poor* [57,58]	0	No	No	No
3-year overall survival rates	2	FEM	2.38 [1.00, 5.68]	0%	Poor* [57,58]	0	No	No	No
4-year overall survival rates	1	FEM	5.50 [0.70, 43.31]	No	Poor* [58]	0	No	No	No
Quality of life	7	FEM	2.05 [1.62, 2.60]	0%	Poor* [57,59,71,75]	3	FEM	1.70 [1.25, 2.30]	0%
Neutropenia	8	REM	0.75 [0.57, 0.99]	85%	Poor* [57,59,68,71,75]	3	FEM	0.67 [0.50, 0.90]	0%
Thrombocytopenia	8	FEM	0.68 [0.55, 0.83]	35%	Poor* [57,59,68,71,75]	3	FEM	0.60 [0.41, 0.88]	0%
Gastrointestinal reactions	11	FEM	0.63 [0.54, 0.72]	0%	Poor* [39,59,60,68,69,71,78]	4	FEM	0.54 [0.34, 0.85]	0%
Liver dysfunction	5	FEM	0.67 [0.35, 1.31]	0%	Poor* [60,68,71,78]	1	No	0.70 [0.17, 2.85]	No
Renal dysfunction	3	FEM	0.70 [0.14, 3.44]	0%	Poor* [60,68]	1	No	0.31 [0.01, 7.35]	No
Neurotoxicity	2	FEM	0.82 [0.37, 1.79]	0%	Poor* [60,71]	No	No	No	No
Phlebitis	2	FEM	1.40 [0.47, 4.16]	0%	Poor* [60,68]	No	No	No	No
Hospital-acquired infection (HAI)	3	FEM	0.69 [0.41, 1.14]	0%	Poor* [57]	2	FEM	0.71 [0.43, 1.18]	19%
Table 5b. Sensitivity analysis by excluding the over- or under-estimated trials									
Disease control rate	15	REM	1.10 [1.01, 1.18]	57%	Over* [58,59,77]	12	FEM	1.07 [1.00, 1.14]	0%
Neutropenia	8	REM	0.75 [0.57, 0.99]	85%	Under* [57,59,63]	6	REM	0.86 [0.69, 1.07]	72%

Peripheral blood lymphocytes	Trials	SM	SMD (95% CI)	I^2	Excluded studies*	Trials	SM	SMD (95% CI)	
Table 5c. Sensitivity analysis by excluding the poor trials									
$CD3^+$T cells	14	REM	1.81 [1.15, 2.48]	95%	Poor* [57,60,64,68,71,72]	8	REM	1.87 [1.03, 2.71]	94%
$CD3^+CD4^+$T cells	18	REM	2.27 [1.72, 2.83]	94%	Poor* [57,60,64,68,71,72]	12	REM	2.77 [2.11, 3.43]	93%
$CD3^+CD8^+$T cells	18	REM	−0.63 [−1.43, 0.16]	97%	Poor* [57,60,64,68,71,72]	12	REM	−0.88 [−1.98, 0.21]	97%
$CD4^+/CD8^+$T cells ratio	18	REM	1.65 [1.19, 2.12]	92%	Poor* [57,60,64,68,71,72]	12	REM	1.90 [1.26, 2.55]	93%
NK cells	12	REM	1.97 [1.17, 2.76]	97%	Poor* [57,60,64,68,71,72]	6	REM	1.99 [0.83, 3.15]	96%
Table 5d. Sensitivity analysis by excluding the over- or under-estimated trials									
$CD3^+$T cells	14	REM	1.81 [1.15, 2.48]	95%	Over* [39,60,65,66,68,70–72,76]	5	FEM	0.24 [0.04, 0.45]	38%
$CD3^+CD4^+$T cells	18	REM	2.27 [1.72, 2.83]	94%	Over* [38,39,56,61,62,64–66,68,70–74,76,77]	2	FEM	0.44 [0.04, 0.84]	0%
$CD3^+CD8^+$T cells	18	REM	−0.63 [−1.43, 0.16]	97%	Under* [39,61,65,70,72–74,76,77], Over* [38,66,68]	6	FEM	0.16 [−0.03, 0.35]	0%
$CD4^+/CD8^+$T cells ratio	18	REM	1.65 [1.19, 2.12]	93%	Over* [30,38,39,56,61,64,65,68,70,72–74,77]	5	FEM	0.41 [0.16, 0.66]	22%
Natural killer cells	12	REM	1.97 [1.17, 2.76]	97%	Over* [64,65,72], Under* [66]	8	FEM	0.85 [0.68, 1.02]	0%

Note: SM: statistical method, FEM: fixed-effects model, REM: random-effects model, RR: risk ratios, SMD: standardized mean difference, CI: confidence interval, Poor trials (Poor*) that had at least one domain were considered to have a high risk of bias; Over* or Under*: over- or under-estimated trials in which the results had significant difference and beneficial to sTP.

Table 6
GRADE evidence profile.

Table 6a. The clinical efficacy and safety

Outcomes (trials)	Quality assessment					NSCLC		Clinical efficacy and safety		Quality
	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	sTP	Chemotherapy	Risk ratios (95% CI)	Absolute effects	
Objective response rate (17)	Serious[a]	No	No	No	None	306/601 (50.90%)	233/585 (39.80%)	1.28 (1.13 to 1.45)	112 more per 1000 (from 52 more to 179 more)	⊕⊕⊕O Moderate
Disease control rate (15)	Serious[a]	No[b]	No	No	None[c]	406/471 (86.20%)	345/455 (75.80%)	1.14 (1.07 to 1.21)	106 more per 1000 (from 53 more to 159 more)	⊕⊕⊕O Moderate
Quality of life (7)	Serious[a]	No	No	No	None	124/194 (63.90%)	58/188 (30.90%)	2.05 (1.62 to 2.6)	324 more per 1000 (from 191 more to 494 more)	⊕⊕⊕O Moderate
1-year overall survival rate (4)	Very serious[d]	No	No	Serious[e]	None	92/138 (66.70%)	61/131 (46.60%)	1.43 (1.15 to 1.78)	200 more per 1000 (from 70 more to 363 more)	⊕OOO Very low
2-year overall survival rates (2)	Very serious[f]	No	No	Serious[e]	None	23/56 (41.10%)	13/53 (24.50%)	1.68 (0.96 to 2.97)	167 more per 1000 (from 10 fewer to 483 more)	⊕OOO Very low
3-year overall survival rates (2)	Very serious[f]	No	No	Serious[e]	None	15/56 (26.80%)	6/53 (11.30%)	2.38 (1.00 to 5.68)	156 more per 1000 (from 0 more to 530 more)	⊕OOO Very low
4-year overall survival rates (1)	Very serious[f]	No	No	Serious[e]	None	6/36 (16.70%)	1/33 (3%)	5.5 (0.7 to 43.31)	136 more per 1000 (from 9 fewer to 1000 more)	⊕OOO Very low
Neutropenia (8)	Serious[a]	Serious[g]	No	No	None	108/226 (47.80%)	142/222 (64%)	0.75 (0.57 to 0.99)	160 fewer per 1000 (from 6 fewer to 275 fewer)	⊕⊕OO Low
Thrombocytopenia (8)	Serious[a]	No	No	No	None	69/226 (30.50%)	100/222 (45%)	0.68 (0.55 to 0.83)	144 fewer per 1000 (from 77 fewer to 203 fewer)	⊕⊕⊕O Moderate
Gastrointestinal reactions (11)	Serious[a]	No	No	No	None	126/405 (31.10%)	201/389 (51.70%)	0.62 (0.53 to 0.71)	196 fewer per 1000 (from 150 fewer to 243 fewer)	⊕⊕⊕O Moderate
Liver dysfunction (5)	Serious[a]	No	No	No	None	13/153 (8.50%)	19/151 (12.60%)	0.67 (0.35 to 1.31)	42 fewer per 1000 (from 82 fewer to 39 more)	⊕⊕⊕O Moderate
Renal dysfunction (3)	Serious[a]	No	No	Serious[e]	None	2/91 (2.20%)	3/89 (3.40%)	0.7 (0.14 to 3.44)	10 fewer per 1000 (from 29 fewer to 82 more)	⊕⊕OO Low
Neurotoxicity (2)	Very serious[f]	No	No	Serious[e]	None	9/59 (15.30%)	11/59 (18.60%)	0.82 (0.37 to 1.79)	34 fewer per 1000 (from 117 fewer to 147 more)	⊕OOO Very low
Phlebitis (2)	Very serious[f]	No	No	Serious[e]	None	7/61 (11.50%)	5/61 (8.20%)	1.4 (0.47 to 4.16)	33 more per 1000 (from 43 fewer to 259 more)	⊕OOO Very low
Hospital-acquired infection (3)	Serious[a]	No	No	Serious[e]	None	18/95 (18.90%)	24/87 (27.60%)	0.69 (0.41 to 1.14)	86 fewer per 1000 (from 163 fewer to 39 more)	⊕⊕OO Low

Table 6b The peripheral blood lymphocytes.

Outcomes (trials)	Quality assessment					Non-small cell lung cancer		Peripheral blood lymphocyte		Quality
	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	sTP	Chemotherapy	Risk ratios (95% CI)	SMD (95% CI)	
$CD3^+$ T cells (14)	Serious[a]	No[b]	No	No	None[c]	555	548	No	SMD 1.81 higher (1.15 to 2.48 higher)	⊕⊕⊕O Moderate
$CD3^+$ $CD4^+$ T cells (18)	Serious[a]	No[b]	No	No	None[c]	695	681	No	SMD 2.27 higher (1.72 to 2.83 higher)	⊕⊕⊕O Moderate
$CD3^+$ $CD8^+$ T cells (18)	Serious[a]	No[b]	No	No	None[c]	695	681	No	SMD 0.63 lower (1.43 lower to 0.16 higher)	

(*continued on next page*)

Table 6 (*continued*)

Table 6b The peripheral blood lymphocytes.

Outcomes (trials)	Quality assessment					Non-small cell lung cancer		Peripheral blood lymphocyte		Quality
	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	sTP	Chemotherapy	Risk ratios (95% CI)	SMD (95% CI)	
$CD4^+/CD8^+$ T cells ratio (18)	Serious[a]	No[b]	No	No	None[c]	695	681	No	SMD 1.65 higher (1.19 to 2.12 higher)	⊕⊕⊕O Moderate
Natural killer cells (12)	Serious[a]	No[b]	No	No	None[c]	498	491	No	SMD 1.97 higher (1.17 to 2.76 higher)	⊕⊕⊕O Moderate

Note: RR: risk ratios, SMD: standardized mean difference, CI: confidence interval.

[a] If most domains had unclear bias risk, with poor trials, and the sensitivity analysis confirmed that the results had good robustness. Therefore, evidence was rated down by only one level.

[b] Considerable heterogeneity. The results had good robustness after excluding the under- or over -estimated trials. Not rated down.

[c] There was publication bias. The results had good robustness after excluding the over- or under-estimated trials. Not rated down.

[d] Most domain had unclear methodological bias risk. There were poor trials and the result had poor robustness. Therefore, evidence was rated down by two levels.

[e] The number of events for each outcome was < 300. Therefore, evidence was rated down by one level.

[f] If all studies were poor, the evidence was down-rated by two levels.

[g] Considerable heterogeneity. The results had poor robustness after excluding the under- or over- estimated trials. Therefore, evidence was rated down by one level.

chemoembolization alone. Based on these results, we believe that sTPs administration with chemotherapy may improve tumor response, QOL, and 1-year OS rate for patients with NSCLC (Fig. 8).

Patients receiving systemic chemotherapy often experience a series of ADRs, due to hematological, hepatorenal, and gastrointestinal toxicity [81–83]. We examined whether sTPs therapy affected the risk of ADRs, including 16 trials with 1122 cases [22,38–40,56,57,60,63,67–71,74,75,78] for analysis. The results demonstrated that sTPs administration with chemotherapy resulted in lower risks of neutropenia, thrombocytopenia, and gastrointestinal reactions than chemotherapy alone. However, the quality for thrombocytopenia and gastrointestinal reactions was moderate, and the quality for neutropenia was low. Jiang et al. [41] reported that only Tα1 plus NP programs resulted in decreased thrombocytopenia. However, patients treated with thymosin plus NP showed no difference in nausea/vomiting to patients treated with NP alone, and likewise, thymosin plus GP was comparable to GP alone with regards to leukopenia and thrombocytopenia. This meta-analysis confirmed that sTPs administration with chemotherapy results in lower risk of neutropenia, thrombocytopenia, and gastrointestinal reactions. In addition, sTPs administration with chemotherapy resulted in no statistical differences in liver and renal dysfunction or HAIs, for which the quality was moderate and low, respectively. These findings reinforce the safety of sTP administration with chemotherapy for NSCLC. Based on the meta-analysis results, we believe that sTPs administration with chemotherapy may decrease the risk of neutropenia, thrombocytopenia, and gastrointestinal reactions (Fig. 8). However, the evidence quality for these variables was moderate to low, and these results will require further confirmation.

As sTPs are thought to enhance specific and nonspecific immune function [22], we also investigated whether sTPs administration with chemotherapy improved the antitumor immunity of patients with NSCLC. Jiang et al. [41] reported that Tα1 plus NP/GP resulted in significant improvements in $CD4^+$ T cells and NK cells. Li et al. [37] reported that thymopentin with chemoradiotherapy increased $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, and the ratio of $CD4^+/CD8^+$ T cells. In this meta-analysis, involving 18 trials with 1376 cases [38,39,56,57,60–62,64–66,68,70–74,76,77], sTPs administration with chemotherapy significantly increased the proportions of $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, NK cells, and the ratio of $CD4^+/CD8^+$ T cells (Fig. 8), confirming and expanding these results. Antitumor immunity plays an important role in enhancing the tumor response. The simultaneous increase in peripheral blood lymphocytes and improvement in tumor response indicates an effective increase in antitumor immunity. This meta-analysis included a diverse range of sTPs types, administration schedules, and combinations with chemotherapy, and subgroup analysis was conducted to determine the optimal conditions for sTPs use. Subgroup analyses revealed that Tα1 increased peripheral blood lymphocytes and improved tumor response, while thymopentin only increased peripheral blood lymphocytes, suggesting that only Tα1 could improve both antitumor immunity and tumor response. Treatment with sTPs twice a week, with one 3-week cycle significantly improved both peripheral blood lymphocytes and tumor response. In terms of chemotherapeutic combinations, sTPs treatment with gemcitabine chemotherapies and NP significantly increased the ORR and peripheral blood lymphocytes. However, under other conditions, sTPs only increased peripheral blood lymphocytes, and not the tumor response. These results suggest that with some therapies, increases in peripheral blood lymphocytes do not necessarily indicate improved antitumor immunity and tumor response. Taken together, our comprehensive analysis found that sTPs, particularly Tα1, improve both antitumor immunity and tumor response, and suggests that combinations with gemcitabine and NP and treatment twice a week for one 3-week cycle are optimal to increase antitumor immunity and tumor response (Fig. 8). Importantly, these finding indicate that sTPs may effectively enhance antitumor immunity and tumor response only with the correct therapy conditions, and therefore

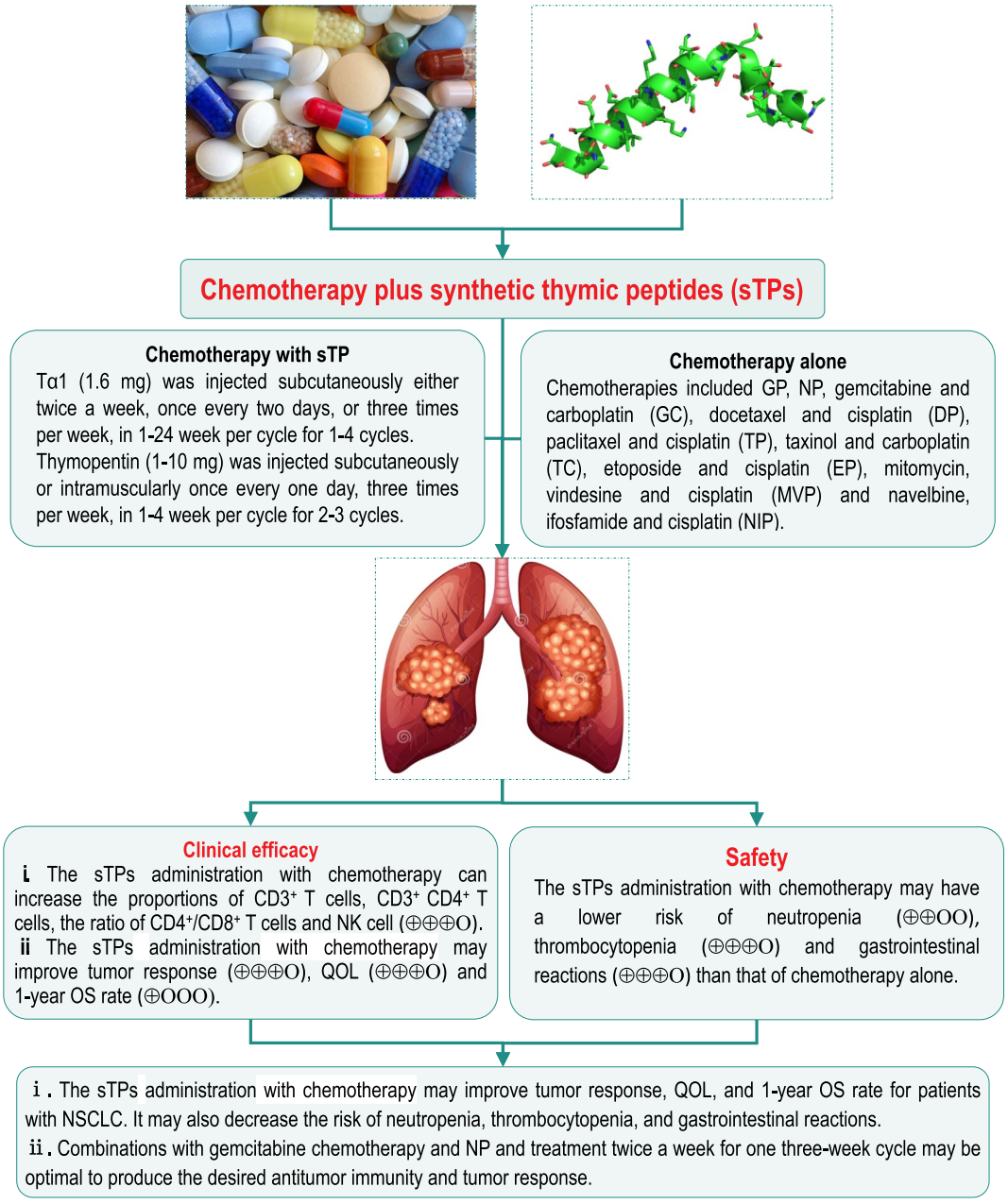


Fig. 8. Clinical efficacy and safety of sTPs with chemotherapy for NSCLC.

may have great value in improving the application efficiency of sTPs and other biological response modifiers. However, conclusions from the subgroup analysis stem from indirect evidence, and will require direct confirmation.

There were some limitations to this study. First, all included studies were published in China, as we did not include the requirement for studies from other regions in the inclusion criteria. Second, the included studies had various individual limitations. Only three trials reported random allocation methods, only one trial provided information about allocation concealments, and none of trials provided information about blinding. Two trials had loss to follow-up, and 11 trials had reporting bias for DCR or ADRs. Third, there were limited available trials and samples to analyze survival and HAIs, and none of the trials reported the PFS. Fourth, based on the GRADE approach, the quality of the main and secondary outcomes was moderate to very low, and the optimal usage conditions are based on indirect evidence. These limitations might have resulted in insufficient assessments of the outcomes.

5. Conclusions

The current evidence indicates that treatment with sTPs, particularly Tα1, with chemotherapy may improve antitumor immunity, tumor response, QOL, and the 1-year OS rate (Fig. 8). It may also decrease the risk of neutropenia, thrombocytopenia, and gastrointestinal reactions. However, with some treatment regimens, increases in peripheral blood lymphocytes may not necessarily improve antitumor immunity. Combinations with gemcitabine chemotherapy and NP and treatment twice a week for one three-week cycle may be optimal to produce the desired antitumor immunity and tumor response. Future studies will be required to test these conditions clinically. Whether sTPs treatment improves survival and prevent HAIs remains unclear. However, taken together, our results confirm the positive effect that sTPs administration with chemotherapy has on patients with NSCLC.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

Acknowledgements

This study was funded by joint funds for science and technology from Science and Technology Foundation of Guizhou Province, Science and Technology Bureau and Affiliated Hospital of Zunyi Medical College [No. (2016)7485], special funds for academic seedlings training and innovation at Zunyi Medical College [Qian Kehe Pingtai Rencai No. (2017) 5733-034], Special Funds for Science and Technology Research of Traditional Chinese and National Medicine in Guizhou (No. QZYY 2017-084), a High-level Innovative Talent Program in Guizhou (No. fzc 120171001), and the Doctoral Fund of Zunyi Medical College (No. F-617).

Author contributions

Conception and design: Zheng Xiao and Xue Xiao. Development of methodology: Zheng Xiao, Cheng-qiong Wang and Xian-tao Zeng. Literature search: Fen-lian Zeng and Cheng-qiong Wang. Study selection: Shan-shan Hu and Jing-li Shan. Risk of bias assessment: Fen-lian Zeng and Yuan Jiang. Data extraction: Xiao-rong Huang and Fen-lian Zeng. Statistical analysis: Fen-lian Zeng and Cheng-qiong Wang, GRADE assessment by Zheng Xiao and Xian-tao Zeng. Writing, review, and/or revision of the manuscript: All authors. Study supervision: Zheng Xiao and Xue Xiao. All authors reviewed the PRISMA guidelines for authorship and agreed with manuscript results and conclusions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105747.

References

[1] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, F. Bray, A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, CA Cancer J. Clin. 66 (2016) 115–132.
[2] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA Cancer J. Clin. 68 (2018) 7–30.
[3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA Cancer J. Clin. 69 (2019) 7–34.
[4] L.A. Torre, R.L. Siegel, A. Jemal, Lung cancer statistics, Adv. Exp. Med. Biol. 893 (2016) 1–19.
[5] A. Clegg, D.A. Scott, P. Hewitson, M. Sidhu, N. Waugh, Clinical and cost effectiveness of paclitaxel, docetaxel, gemcitabine, and vinorelbine in non-small cell lung cancer: a systematic review, Thorax 57 (2002) 20–28.
[6] G.A. Masters, S. Temin, C.G. Azzoli, G. Giaccone, S. Baker Jr., J.R. Brahmer, P.M. Ellis, A. Gajra, N. Rackear, J.H. Schiller, T.J. Smith, J.R. Strawn, D. Trent, D.H. Johnson, American Society of Clinical Oncology Clinical Practice, Systemic therapy for stage IV non-small-cell lung cancer: American Society of Clinical Oncology clinical practice guideline update, J. Clin. Oncol. 33 (2015) 3488–3515.
[7] G. Pilkington, A. Boland, T. Brown, J. Oyee, A. Bagust, R. Dickson, A systematic review of the clinical effectiveness of first-line chemotherapy for adult patients with locally advanced or metastatic non-small cell lung cancer, Thorax 70 (2015) 359–367.
[8] C.M. Rudin, N. Ismaila, C.L. Hann, N. Malhotra, B. Movsas, K. Norris, M.C. Pietanza, S.S. Ramalingam, A.T. Turrisi 3rd, G. Giaccone, Treatment of small-cell lung cancer: American Society of Clinical Oncology endorsement of the American College of Chest Physicians Guideline, J. Clin. Oncol. 33 (2015) 4106–4111.
[9] T. Conroy, P.L. Etienne, A. Adenis, M. Ducreux, B. Paillot, J. Oliveira, J.F. Seitz, E. Francois, E. Van Cutsem, D.J. Wagener, F. Kohser, S. Daamen, M. Praet, T. Gorlia, B. Baron, J. Wils, European Organisation for Research and Treatment of Cancer Gastrointestinal Tract Cancer Cooperative Group, Vinorelbine and cisplatin in metastatic squamous cell carcinoma of the oesophagus: response, toxicity, quality of life and survival, Ann. Oncol. 13 (2002) 721–729.
[10] C.F. Pollera, F. Ameglio, M. Nardi, G. Vitelli, P. Marolla, Cisplatin-induced hepatic toxicity, J. Clin. Oncol. 5 (1987) 318–319.
[11] F. Shahid, Z. Farooqui, F. Khan, Cisplatin-induced gastrointestinal toxicity: an update on possible mechanisms and on available gastroprotective strategies, Eur. J. Pharmacol. 827 (2018) 49–57.
[12] S. Waissbluth, S.J. Daniel, Cisplatin-induced ototoxicity: transporters playing a role in cisplatin toxicity, Hear. Res. 299 (2013) 37–45.
[13] K. Tsung, J.A. Norton, An immunological view of chemotherapy, Immunotherapy 7 (2015) 941–943.
[14] P. Van Houtte, S. Danhier, F. Mornex, Toxicity of combined radiation and chemotherapy in non-small cell lung cancer, Lung Cancer 10 (Suppl. 1) (1994) S271–S280.
[15] R.P. Abratt, G.W. Morgan, Lung toxicity following chest irradiation in patients with lung cancer, Lung Cancer 35 (2002) 103–109.
[16] C.C. Koning, S.J. Wouterse, J.G. Daams, L.L. Uitterhoeve, M.M. van den Heuvel, J.S. Belderbos, Toxicity of concurrent radiochemotherapy for locally advanced non-small-cell lung cancer: a systematic review of the literature, Clin Lung Cancer 14 (2013) 481–487.
[17] L. Yang, L. Wang, Y. Zhang, Immunotherapy for lung cancer: advances and prospects, Am J Clin Exp Immunol 5 (2016) 1–20.
[18] E. Wolf, S. Milazzo, K. Boehm, M. Zwahlen, M. Horneber, Thymic peptides for treatment of cancer patients, Cochrane Database Syst. Rev. 16 (2011) CD003993.
[19] A.L. Goldstein, T.L. Low, M. McAdoo, J. McClure, G.B. Thurman, J. Rossio, C.Y. Lai, D. Chang, S.S. Wang, C. Harvey, A.H. Ramel, J. Meienhofer, Thymosin alpha1: isolation and sequence analysis of an immunologically active thymic polypeptide, Proc. Natl. Acad. Sci. U. S. A. 74 (1977) 725–729.
[20] G. Goldstein, M.P. Scheid, E.A. Boyse, D.H. Schlesinger, J. Van Wauwe, A synthetic pentapeptide with biological activity characteristic of the thymic hormone thymopoietin, Science 204 (1979) 1309–1310.
[21] L. Kisfaludy, O. Nyeki, I. Scho, L. Denes, J. Ember, L. Szporny, G. Hajós, B. Szende, Immuno-regulating peptides, I. synthesis and structure-activity relationships of thymopentin analogs, Hoppe Seylers Z Physiol Chem 364 (1983) 933–940.
[22] B. Liang, Z. Zhou, J.M. Yang, Combination of thymosin-α1 with low-dose gemcitabine for advanced non-small cell lung cancer in elderly patients, J Pract Oncol 25 (2010) 470 + 1–3.
[23] Z. Feng, Q. Shi, Y. Fan, Q. Wang, W. Yin, Ulinastatin and/or thymosin alpha1 for severe sepsis: a systematic review and meta-analysis, J. Trauma Acute Care Surg. 80 (2016) 335–340.
[24] D. Liu, Z. Yu, J. Yin, Y. Chen, H. Zhang, F. Xin, H. Fu, B. Wan, Effect of ulinastatin combined with thymosin alpha1 on sepsis: a systematic review and meta-analysis of Chinese and Indian patients, J. Crit. Care 39 (2017) 259–266.
[25] F. Liu, H.M. Wang, T. Wang, Y.M. Zhang, X. Zhu, The efficacy of thymosin alpha1 as immunomodulatory treatment for sepsis: a systematic review of randomized controlled trials, BMC Infect. Dis. 16 (2016) 488.
[26] H.Y. Mao, T.D. Shi, Treatment with interferon and thymosin alpha-1 versus interferon monotherapy for HBeAg positive chronic hepatitis B: a meta-analysis, Zhonghua Gan Zang Bing Za Zhi 19 (2011) (2011) 29–33.
[27] Y.F. Yang, W. Zhao, Y.D. Zhong, Y.J. Yang, L. Shen, N. Zhang, P. Huang, Comparison of the efficacy of thymosin alpha-1 and interferon alpha in the treatment of chronic hepatitis B: a meta-analysis, Antivir. Res. 77 (2008) 136–141.
[28] D. Liu, X. Xia, Effect of thymosin α1 on apoptosis of lung cancer cell A549, Chin J Clin Pharmacol (2016) 1776–1779.
[29] X. Lao, B. Li, M. Liu, C. Shen, T. Yu, X. Gao, X.H. Zheng, A modified thymosin alpha 1 inhibits the growth of breast cancer both in vitro and in vivo: suppressment of cell proliferation, inducible cell apoptosis and enhancement of targeted anticancer effects, Apoptosis 20 (2015) 1307–1320.
[30] Y. Guo, H. Chang, J. Li, X.Y. Xu, L. Shen, Z.B. Yu, W.C. Liu, Thymosin alpha 1 suppresses proliferation and induces apoptosis in breast cancer cells through PTEN-mediated inhibition of PI3K/Akt/mTOR signaling pathway, Apoptosis 20 (2015) 1109–1121.
[31] T.W. Moody, M. Fagarasan, F. Zia, M. Cesnjaj, A.L. Goldstein, Thymosin alpha 1 down-regulates the growth of human non-small cell lung cancer cells in vitro and in vivo, Cancer Res. 53 (1993) 5214–5218.
[32] F. Pica, M. Fraschetti, C. Matteucci, C. Tuthill, G. Rasi, High doses of thymosin alpha 1 enhance the anti-tumor efficacy of combination chemo-immunotherapy for murine B16 melanoma, Anticancer Res. 18 (1998) 3571–3578.
[33] T.W. Moody, J. Leyton, F. Zia, C. Tuthill, M. Badamchian, A.L. Goldstein, Thymosin alpha1 is chemopreventive for lung adenoma formation in A/J mice, Cancer Lett. 155 (2000) 121–127.
[34] T.W. Moody, Thymosin alpha1 as a chemopreventive agent in lung and breast cancer, Ann. N. Y. Acad. Sci. 1112 (2007) 297–304.
[35] A.Y. Bedikian, Y.Z. Patt, W.K. Murphy, T. Umsawasadi, D.T. Carr, E.M. Hersh, G.P. Bodey, M. Valdivieso, Prospective evaluation of thymosin fraction V immunotherapy in patients with non-small cell lung cancer receiving vindesine, doxorubicin, and cisplatin (VAP) chemotherapy, Am. J. Clin. Oncol. 7 (1987) 99–404.
[36] C. Lin, S. Liu, B. He, W. Lei, T. Liu, Synergistic and protective effect of thymalfasin in treatment of NSCLC: a meta-analysis, Pharm Today 26 (2016) 27–32.
[37] Y. Li, S. Ao, Y. Jin, Evaluation of thymopentin in adjuvant therapy for patients with lung cancer: a meta-analysis of randomized trials, J Integr Technol 4 (2015) 75–81.
[38] S. Mao, X. Kuang, J. Lu, X. Ma, J. Gao, W. Dong, et al., Clinical study of thymosin α1 combined with pemetrexed in the treatment of elderly patients with advanced lung adenocarcinoma, J Basic Clin Oncol 30 (2017) 107–110.
[39] L. You, X. Chen, B. Ye, Efficacy of thymosin combined with chemotherapy in treatment of patients with non-small cell lung cancer and its influence on immune function, J Clin Med Pract 21 (2017) 42–45.
[40] L. Li, X. Chen, Clinical study of thymopentin with TC regimen in the treatment of advanced non-small cell lung cancer, Chin Pract Med (2018) 112–113.
[41] J. Jiang, X. Wang, J. Tian, L. Li, Q. Lin, Thymosin plus cisplatin with vinorelbine or gemcitabine for non-small cell lung cancer: a systematic review and meta-analysis of randomized controlled trials, Thorac Cancer 2 (2011) 213–220.
[42] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman, PRIZMA Group, Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, Int. J. Surg.

8 (2010) 336–341.
[43] C.F. Mountain, Value of the new TNM staging system for lung cancer, Chest 96 (1989) 47S–49S.
[44] J.P.T Higgins, S. Green, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. [updated March 2011]. The Cochrane Collaboration, London, 2011. Available at: http:// wwwhandbookcochraneorg/.
[45] A.B. Miller, B. Hoogstraten, M. Staquet, A. Winkler, Reporting results of cancer treatment, Cancer 47 (1981) 207–214.
[46] Y. Tsuchida, P. Therasse, Response evaluation criteria in solid tumors (RECIST): new guidelines, Med. Pediatr. Oncol. 37 (2001) 1–3.
[47] J.W. Yates, B. Chalmer, F.P. McKegney, Evaluation of patients with advanced cancer using the Karnofsky performance status, Cancer 45 (1980) 2220–2224.
[48] J.K. Clancey, Karnofsky performance scale, J Neurosci Nurs 27 (1995) 220.
[49] A. Trotti, A.D. Colevas, A. Setser, E. Basch, Patient-reported outcomes and the evolution of adverse event reporting in oncology, J. Clin. Oncol. 25 (2007) 5121–5127.
[50] P. Guyot, A.E. Ades, M.J. Ouwens, N.J. Welton, Enhanced secondary analysis of survival data: reconstructing the data from published Kaplan-Meier survival curves, BMC Med. Res. Methodol. 12 (2012) 9.
[51] Z. Xiao, C.Q. Wang, M.H. Zhou, N.N. Li, S.Y. Liu, Y.J. He, Y. Wang, J.H. Feng, X.S. Yao, L. Chen, B. Ma, S. Yu, X.T. Zeng, C.W. Li, J. Ding, Clinical efficacy and safety of CIK plus radiotherapy for lung cancer: a meta-analysis of 16 randomized controlled trials, Int. Immunopharmacol. 61 (2018) 363–375.
[52] J.P.T. Higgins, S.G. Thompson, Quantifying heterogeneity in a meta-analysis, Stat. Med. 21 (2002) 1539–1558.
[53] Z. Xiao, C.Q. Wang, M.H. Zhou, N.N. Li, Y.P. Sun, Y.Z. Wang, S.Y. Liu, H.S. Yu, C.W. Li, X.T. Zeng, L. Chen, X.S. Yao, J.H. Feng, The antitumor immunity and tumor responses of chemotherapy with or without DC-CIK for non-small-cell lung cancer in China: a meta-analysis of 28 randomized controlled trials, J Immunol Res 2018 (2018) 9081938.
[54] Z. Xiao, C. Wang, R. Zhou, S. Hu, N. Yi, J. Feng, M. Zhou, S. Liu, L. Chen, J. Ding, Q. Gong, F. Tang, X. Li, Can AIDI injection improve overall survival in patients with non-small cell lung cancer? A systematic review and meta-analysis of 25 randomized controlled trials, Complement Ther Med 37 (2018) 50–60.
[55] G.H. Guyatt, A.D. Oxman, G.E. Vist, R. Kunz, Y. Falck-Ytter, P. Alonso-Coello, H.J. Schünemann, GRADE Working Group, GRADE: an emerging consensus on rating quality of evidence and strength of recommendations, BMJ 336 (2008) 924–926.
[56] Y. Chen, J. Chang, H. Fu, Y. Xiao, W. Wu, Effect of thymopentin in the prevention of the opportunistic fungal and parasitic infections during treatment of the late cases with lung cancer, Chin J Zoonoses 21 (2005) 309–311.
[57] H. Wang, Y. Chen, M. Liao, B. Han, Effect of thymosin α1 for advanced non-small-cell lung cancer in chemotherapy patients, Chin Oncol 15 (2005) 244–247.
[58] D. Ma, D. Lin, M. Li, P. Yu, Clinical efficacy of thymosin alpha 1 in the treatment of advanced non-small cell lung cancer, Shandong Med J 46 (2006) 77.
[59] X. Hou, Clinical effects of advanced patients with non-small cell lung cancer treatment by thymosin alpha I combined with NP regimens, J Bethune Mil Med Coll 5 (2007) 272–274.
[60] X. Shi, Q. Ding, Q. Yang, Clinical effects of thymosin alpha 1 combined with chemotherapy in the treatment of advanced non-small cell lung cancer, PJCCPVD 15 (2007) 764–766.
[61] P. Liu, G. Liao, H. Wang, Influence of thymopentin on immunologic function in advanced-stage patients with nonsmall-cell lung cancer, J Xinxiang Med Univ 25 (2008) 289–291.
[62] L. Wu, M. Ye, S. Ni, S. Wang, X. Wu, Effect of thymosin alpha 1 on T lymphocyte subsets in lung cancer with chemotherapy, Her Med 27 (2008) 1482–1483.
[63] J. Zhen, Clinical study on the effect and nursing care of bronchogenic carcinoma treated with thymosin alpha1 combined with chemotherapy, Modern Med & Health 24 (2008) 3676–3677.
[64] F. Lin, Clinical effects of thymopentin combined with chemotherapy in the treatment of non-small cell lung cancer, Chongqing Med J 38 (2009) 2060–2062.
[65] X. Sun, F. Gao, X. Liu, G. Li, Effect of thymosin alpha 1 on cellular immune function in patients with non-small cell lung cancer during chemotherapy, Shandong Med J 49 (2009) 83–84.
[66] Y. Gu, Q. Cao, The effect of thymosin alpha 1 on the immune function of patients with advanced lung cancer, Nei Mongol J TCM 29 (2010) 146–147.
[67] Z. Zhou, C. Wang, Comparative study of thymopentin and megestrol acetate P1 us EP regimen with EP regimen alone in the treatment of advanced non-small cell lung cancer, Chin Pract Med 5 (2010) 46–48.
[68] L. Han, Clinical effects of thymosin alpha 1 plus NP in the treatment of advanced non-small cell lung cancer, Chin Healthc Innov. 7 (2012) 58 + 2.
[69] W. Li, Clinical efficacy of thymosin alpha 1 in the treatment of middle and advanced non-small cell lung cancer, J Aerosp Med 23 (2012) 582–583.
[70] Y. Liu, J. Ding, J. Li, Effect of thymosin alpha 1 on advanced NSCLC immune function and bone marrow suppression, Clin Educ Gen Pract 10 (2012) 493–495.
[71] Y. Wang, C. Ba, Y. Yang, J. Li, J. Meng, H. Yu, The clinical study of NSCLC patients with the maintenance treatment of thymopeptides α1 (Tα1) and combined determination of the immune function of the T,B and NK cells, J Diss Monit & Contr 6 (2012) (2012) 9–11.
[72] P. Cai, G. Chen, Xianguo, L. Yu, Effects of thymosin α1 on immune function in patients with non-small cell lung cancer treated by chemotherapy, Chin. J. Clin. Oncol. Rehab. 21 (2014) 261–263.
[73] H. Shui, Clinical efficacy and cellular immunity of thymosin alpha 1 combined with chemotherapy in the treatment of lung cancer, Chin. J. Modern Drug Appl. 8 (2014) 131–132.
[74] M. Zhao, Q. Bi, Q. Tian, M. Bao, Effect of thymalfasin on the immune function of patients with advanced non-small cell lung cancer, Chin. J. Difficult Complicated Cases 13 (2014) (2014) 683–685.
[75] Q. Chen, M. Zhang, X. Wang, X. Li, B. Zhang, J. Song, Thymopentin combined with gemcitabine and carboplatin in the treatment of advanced squamous cell carcinoma of the lung: a clinical study with 56 cases, World Latest Med. Inform. 15 (2015) 17–18.
[76] Z. Wu, S. Lin, Q. Luo, C. Cai, R. Hong, Pharmacoeconomics analysis of Chinese herb injection combined with NP chemotherapy in treatment of non-small cell lung cancer, Chin. J. Exp. Tradit. Med. Formulae 21 (2015) 199–202.
[77] Y. Mo, Influence of immune function of thymosin α1 combined with chemotherapy for patients with advanced non-small cell lung cancer, J. Med. Forum 36 (2015) 41–42.
[78] Z. Sha, Caihong Xiuling, Effect of the combined use of thymopentin and paclitaxel and cisplatin chemotherapy in the treatment of advanced non-small cell lung cancer, J. Baotou Med. Coll. 31 (2015) 9–10.
[79] C. Lin, C. Liu, B. He, W. Lei, T. Liu, Synergistic and protective effect of thymalfasin in treatment of NSCLC: a meta-analysis, Pharm. Today 26 (2016) 27–32.
[80] R.G. Gish, S.C. Gordon, D. Nelson, V. Rustgi, I. Rios, A randomized controlled trial of thymalfasin plus transarterial chemoembolization for unresectable hepatocellular carcinoma, Hepatol. Int. 3 (2009) 480–489.
[81] V. Pinzani, F. Bressolle, I.J. Haug, M. Galtier, J.P. Blayac, P. Balmes, Cisplatin-induced renal toxicity and toxicity-modulating strategies: a review, Cancer Chemother. Pharmacol. 35 (1994) (1994) 1–9.
[82] Z. Cai, J. Yang, X. Shu, X. Xiong, Chemotherapy-associated hepatotoxicity in colorectal cancer, J. BUON 19 (2014) 350–356.
[83] J. Floyd, I. Mirza, B. Sachs, M.C. Perry, Hepatotoxicity of chemotherapy, Semin. Oncol. 33 (2006) 50–67.