Original Article

Iranian Journal of Immunology

March 2026; 23(2):

Heat-sensitive Moxibustion Enhances Immunity in Advanced Non-small Cell Lung Cancer by Modulating the Th1/Th2 and Treg/Th17 Axes

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ABSTRACT

Background: Patients with advanced non-small cell lung cancer (NSCLC) often experience poor clinical outcomes following chemotherapy. Heat-sensitive moxibustion (HSM), a traditional therapeutic intervention, has been reported to improve symptoms and quality of life in patients with cancer.

Objective: To evaluate the effect of HSM in patients with advanced NSCLC following chemotherapy.

Methods: Between January 2023 and February 2024, patients with advanced NSCLC were enrolled. Eligible patients were randomly assigned to either the chemotherapy (control) group or the chemotherapy plus HSM treatment (HSM) group. Following treatment, the percentages of $CD4^+$ T cell subsets were evaluated, and the serum levels of CD4+ T cells-associated cytokines were measured using enzyme-linked immunosorbent assay (ELISA). The expression levels of CD4+ T cells-related factors were assessed by RT-qPCR and Western blot analysis. The overall response rate (OPR) and disease control rate (DCR), Karnofsky Performance Status (KPS) score, and incidence of adverse events were compared between the two groups.

Results: A total of 90 patients with NSCLC were included with 45 patients in each group. The ratio of Th1/Th2 was significantly elevated, whereas the Treg/Th17 ratio was significantly decreased in the HSM group compared with the control group. The HSM group exhibited higher serum levels of IL-2, IL-17A and IFN-γ, and lower levels of IL-4 and IL-10 than the control group. HSM treatment significantly upregulated the expression of T-bet and IL-17A while downregulating GATA3 expression at both the mRNA and protein levels. The HSM group demonstrated superior clinical outcomes, as evidenced by higher KPS score, ORR, DCR, along with a lower incidence of adverse events.

Conclusion: HSM may enhance immune function in patients with advanced NSCLC undergoing chemotherapy by modulating the balance of Th1/Th2 and Treg/Th17 cell subsets.

Keywords: Advanced non-small cell lung cancer; Heat-sensitive moxibustion; CD4+ T cell subsets; Chemotherapy

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Cite this article as:
Luo N, Zan H, Li X, Li P, Zhang L. Heat-sensitive Moxibustion Enhances Immunity in Advanced Non-small Cell Lung Cancer by Modulating the Th1/Th2 and Treg/Th17 Axes. *Iran J Immunol.* 2026; 23(2): ,
doi: 10.22034/iji.2026.110224.3168.

Received: 2025-12-18
Revised: 2026-05-12
Accepted: 2026-06-28

INTRODUCTION

Lung cancer remains a major public health challenge due to its high incidence and mortality worldwide (1). According to the GLOBOCAN 2022 data, lung cancer accounted for approximately 2.21 million new cases (11.4% of all cancer diagnoses) and 1.79 million deaths (18% of all cancer-related deaths) globally (2). Among the histological subtypes of lung cancer, non-small cell lung cancer (NSCLC) is the most common, accounting for approximately 85% of all cases (3). Because early-stage disease is often asymptomatic, many patients are diagnosed at an advanced-stage. Despite advances in diagnosis and treatment, advanced NSCLC remains a major oncological challenge and an important focus of clinical research.

Currently, platinum-based chemotherapy remains a standard treatment option for patients with advanced NSCLC, although its clinical efficacy is often limited by high recurrence rates and treatment-related toxicity (3, 4). Chemotherapeutic agents can also affect the immune system by reducing immune cell population and altering immune function, potentially contributing to treatment-related immunosuppression (5). Accumulating evidence suggests that an immunosuppressive tumor microenvironment is associated with disease progression and poor prognosis in patients with NSCLC (6). Therefore, there is considerable interest in developing therapies that eliminate tumors while mitigating chemotherapy-induced immunosuppression.

As an external therapy in traditional Chinese medicine, heat-responsive moxibustion (HSM) is widely used to treat various diseases due to its simplicity, safety, efficacy and low risk of adverse effects (7). Dazhui (GV14), an acupoint on the Governor Vessel (Du meridian), is situated along the posterior midline in the depression between the spinous processes of the seventh cervical (C7) and first thoracic (T1) vertebrae. As the confluence point where the three *Yang* meridians of the hand and foot meet the Governor Vessel, GV14 pertains to yang and governs the exterior (8). Consequently, GV14 plays a pivotal role in clearing pathogenic factors, regulating *yang qi*, and is widely utilized in clinical practice to treat a variety of diseases (9, 10). Stimulating GV14 via acupuncture may reflexively modulate both the central nervous system and immune function through the neuro-endocrine-immune network and the circulatory system (11). A previous study showed that GV14 stimulation enhances immune responses by reducing splenic regulatory T cell (Treg) levels in tumor-bearing mice (12). Thus, GV14 has proven to be an effective, clinically established acupoint for modulating the immune system. Recently, accumulating evidence has demonstrated that HSM is an effective and safe adjuvant therapy for cancer (13, 14). Recently, accumulating evidence has demonstrated that HSM is an effective and safe adjuvant therapy for cancer However, few studies have systematically investigated the effects of HSM on chemotherapy-induced immunosuppression in lung cancer patients. Therefore, a randomized controlled clinical trial was conducted to evaluate the efficacy and safety of HSM in the treatment of advanced NSCLC and to elucidate the underlying mechanism.

MATERIAL AND METHODS

Patient Recruitment and Eligibility

This study enrolled patients diagnosed with advanced NSCLC between January 2023 and February 2024 at the Weifang Hospital of Traditional Chinese Medicine.

To be eligible for enrollment, patients had to meet the following inclusion criteria: 1. Age ≥18 years; 2. Histologically or cytologically confirmed stage IIIB or IV NSCLC according to the International Association for the Study of Lung Cancer (IASLC) guidelines (15); 3. A Karnofsky Performance Status (KPS) score ≥ 60; and 4. Treatment-naïve prior to study entry. Patients were excluded if they met any

of the following criteria: 1. Cardiovascular and cerebrovascular diseases, hepatic or renal dysfunction, or coagulation abnormalities; 2. Psychiatric disorders, hematological disorders, or autoimmune diseases; 3. Pregnancy or lactation; or 4. Complicated by other concurrent malignant tumors. Additionally, patients presenting with TCM signs of Yin deficiency with heat (e.g., dry mouth and throat, a red tongue with little or no coating, a rapid pulse or a feverish sensation in the palms and soles) or excess heat syndrome were excluded. This investigation was officially approved by the Ethics Committee of Weifang hospital of Traditional Chinese Medicine, and written informed consent was obtained from all participating subject.

Randomization and Treatment Protocol

Eligible patients were randomly assigned to either the control group (chemotherapy alone) or the HSM group (chemotherapy combined with HSM).

Chemotherapy Regimen (Control Group)

To prevent chemotherapy-induced nausea and vomiting, all patients received an intravenous infusion of 5 mL palonosetron hydrochloride prior to chemotherapy administration. Subsequently, a gemcitabine and cisplatin (GP) combination regimen was administered. Cisplatin (Jiangsu Hansoh Pharmaceutical Co., Ltd., Jiangsu, China; National Medicine Permit No. H20040813) was administered via intravenous infusion at a dose of 75 mg/m² on days 1-3. Gemcitabine hydrochloride (Qilu Pharmaceutical Co., Ltd., Hainan, China; National Medicine Permit No. H20113286) was administered via intravenous infusion at a dose of 1,000 mg/m² on days 1 and 8. This regimen was repeated every 21 days for a total of 4 cycles.

Heat-Sensitive Moxibustion Protocol (HSM Group)

Patients in the HSM group received the standard GP chemotherapy regimen combined with HSM treatment. HSM sessions commenced on the first day of each chemotherapy cycle, 30 minutes after the completion of the chemotherapy infusion. During HSM treatment, patients assumed a prone position to fully expose the GV14 acupoint region. The heat-sensitive acupoint was initially located by hovering a burning a moxa stick (diameter: 18 mm; length: 200 mm) at 3-5 cm above the skin surface around GV14. When the patient experienced distinct heat-sensitive sensations—such as penetrating heat, spreading heat, or non-local heat, the precise location was identified as the sensitized acupoint. Mild moxibustion was continuously applied at this site until the heat-sensitive sensation naturally subsided. HSM treatment was performed once every other day for 10 sessions per 20-day cycle, spanning a total of 4 cycles.

Rationale for the HSM Protocol

The rationale for selecting a 4-cycle HSM regimen was threefold. First, it directly aligned with the 4-cycle GP chemotherapy schedule, allowing for a synchronized evaluation of the combined intervention at identical clinical endpoint. Second, according to TCM theory, moxibustion requires a sustained and sufficient dose to achieve therapeutic efficacy; thus, treatment was maintained until the patient's heat-sensitive sensation completely disappeared, satisfying the time-effect and dose-effect relationships established in modern acupuncture immunology research (16). Third, accumulating clinical evidence has demonstrated that a 4-cycle HSM regimen yields stable and durable improvements in immune function and quality of life for cancer patients undergoing chemotherapy (17, 18).

Sample collection and PBMC isolation

Peripheral blood samples were collected at two distinct time points during each chemotherapy cycle. Baseline samples were obtained within 24 hours prior to the initiation of chemotherapy on Day 1 of each cycle. Post-treatment samples were collected on Day 22 of each cycle, representing the end of the current cycle and immediately prior to

the commencement of the subsequent cycle. Blood samples were processed using density gradient centrifugation (400g for 10 min at 4°C) to isolate peripheral blood mononuclear cells (PBMCs). The isolated PBMCs were then collected and stored at -80°C until further until.

Flow cytometry (FCM) Analysis

To analyze the relative frequencies of $CD4^+$ T cell subpopulations, flow cytometry was performed. For intracellular cytokine staining (Th1, Th2, and Th17), PBMCs were stimulated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma Aldrich, Steinheim, Germany) and 1 µM ionomycin (Calbiochem, Darmstadt, Germany) in the presence of a protein transport inhibitor (GolgiStop, BD Biosciences, San Jose, CA, USA). Flow cytometric quantification was conducted using a flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (version 10.8, Ashland, OR, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

To evaluate the CD4+ T cell-associated cytokines before and after treatment, ELISA was conducted. The serum concentrations of pro-inflammatory (IL-2, IFN-γ, IL-17A) and anti-inflammatory cytokines (IL-4, and IL-10) were quantified using commercially available ELISA kit (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions.

Real time quantitative PCR (RT-qPCR)

Total RNA was extracted from PBMCs using Trizol regent (Invitrogen, Waltham, MA, USA). Reverse transcription was performed using ThermoStable V Reverse Transcriptase (Mlbio, Shanghai, China) to synthesize complementary DNA (cDNA. Real-time PCR amplification was subsequently conducted on an ABI PRISM 7300 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). The specific primer sequences for T-bet (Th1 master regulator), GATA-3 (Th2-specific transcription factor), IL-17A (Th17 signature cytokine) and FoxP3 (for Treg cells) are listed in Table 1. Relative gene expression levels were calculated using the comparative Ct (2-ΔΔCt) method and normalized to the endogenous control GAPDH.

Western blot

Total proteins was extracted from PBMCs and quantified as previously described (19). Equal amounts of protein extracts were separated via SDS-PAGE and subsequently electro-blotted onto PVDF membranes. After blocking with 5% skim milk for 1 h at room temperature, the membranes were incubated overnight at 4°C with rabbit primary antibodies against T-bet (1:1000, Abcam), GATA-3 (1:1000, Abcam), IL-17A (1:1000, Abcam), FoxP3 (1:1000, Abcam), and GAPDH (1:5000, Abcam). The membranes were then incubated with an appropriate horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:5000 dilution, Abcam). Protein bands were visualized using an enhanced chemiluminescence (ECL) kit (Bio-Rad, Hercules, CA, USA) and relative protein expressions was quantified by analyzing band intensities using Image J software (NIH, Bethesda, MD, USA), with values normalized to the loading control.

Table 1: Primer sequences for real time PCR assay

Gene symbol	Forward	Reverse
T-bet	5'AGGGAGTGTGTGAACTGTGGG3'	5'CTTCGCTTGGGCTTAATGAGG3'
GATA3	5'GCAATGGAAGTGGTGCTGGTT3'	5'AGGATGCTTTGGCGATGAGTC3'
IL-17A	5'-GAGCTCTGGGGAGCCCACACTCC-CCA-3'	5'-GGGCGAAAATGGTTACGATGT-GAAAC-3'
FoxP3	5'CACGCATGTTTGCCTCTTCAGA3'	5'GTAGGGTTGGAACACCTGCTGGG3'
GAPDH	5'-ATGGGGAAGGTGAAGGTCG-3'	5'-GGGTCATTGATGGCAACAATATC-3'

Karnofsky Performance Status (KPS) Assessment

The Karnofsky Performance Status (KPS) scale is a well-established instrument used to evaluate the functional capacity of patients with advanced malignancies. The KPS is an 11-point scale ranging from 100 (normal activity with no evidence of disease) to 0 (dead), decreasing in 10-point increments (20). Patients were assessed using the KPS scale both before and after treatment. Clinical changes in functional status were categorized as follows: an increase of ≥10 points indicated improvement, a decrease of ≥10 points indicated decline, and a change of less than 10 points indicated stability.

Safety and Adverse Events Monitoring

Throughout the treatment and follow-up periods, standard chemotherapy-related adverse events were monitored and recorded, including hepatic and renal dysfunction, fatigue, insomnia, nausea /emesis, anorexia, and coagulation disorders. Additionally, heat-related symptoms potentially associated with HSM therapy were prospectively monitored. These included dry mouth, sore throat, constipation, oral ulcers, subjective systemic or localized heat sensations, irritability, and alterations in tongue coating and pulse characteristics.

Efficacy assessment

Clinical efficacy was evaluated in accordance with the modified Response Evaluation Criteria in Solid Tumors [14]. Clinical responses were classified into the following categories: Complete Response (CR): Disappearance of all target lesions confirmed by repeat assessments performed at least 4 weeks after the initial documentation of response.

Partial Response (PR): At least a 50% decrease in the sum of the diameters of the target lesions, taking as reference the baseline sum diameters. Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study, or the appearance of one or more new lesions. Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for Progressive Disease (PD). The overall response rate (ORR) was calculated as the percentage of patients who achieved either a CR or a PR relative to the total study population, using the formula: [(number of CR cases + number of PR cases)/total number of patients] × 100%. The disease control rate (DCR) was determined as the proportion of patients demonstrating CR, PR, or SD among the total evaluable population.

Statistical analysis

For clinical data, differences between the two groups were analyzed using the independent-sample t-test for continuous variables and the chi-square test for categorical variables. For laboratory assays (including RT-qPCR, ELISA, and Western blot), all experiments were performed in triplicate. Multi-group comparisons across different time points or cycles were analyzed using one-way ANOVA for multiple group comparisons. Statistical analyses were performed using GraphPad Prism (version 9.5.0; GraphPad Software, San Diego, CA, USA). A $P < 0.05$ was considered statistically significant.

RESULTS

Study Cohort and Baseline Data

A total of 90 eligible NSCLC patients met the inclusion criteria and were randomized equally into the control and HSM group (45 patients per group). There were no statistically significant differences between the groups regarding baseline characteristics, including age, sex, disease stage, and pathological type ($P>0.05$), indicating a well-balanced cohort. The detailed characteristics are presented in Table 2.

Table 2: Comparison of basic information between groups

Variables	Control group (n=45)	HSM group (n=45)	*P* value
Age	55.6±8.9	57.3±9.4	0.38
Sex (male/female)	26/19	25/20	0.83
Pathological stage (n)			0.71
IIIB stage	20	21	
IV stage	25	24	
Histological type (n)			0.67
Squamous cell carcinoma	21	23	
Adenocarcinoma	24	22	

Impact of HSM onCD4+ T cell subsets

Flow cytometric analysis revealed distinct shifts in the peripheral blood Th1/Th2/Th17/Treg profiles between the two groups. As shown in Figure 1A and 1B, the frequencies of Th1 and Th17 cells were markedly increased in the HSM group compared to the control group (P=0.0047 and P=0.016, respectively). Conversely, the percentage of Th2 cells was significantly decreased in the HSM group (P=0.0042, Figure 1C). Meanwhile, Treg proportions remained comparable between the two cohorts (*P*>0.05, Figure 1D). Notably, the Th1/Th2 ratio was significantly elevated (P= 0.0114) whereas the Treg/Th17 ratio was significantly diminished in the HSM group, compared with the control group (P= P=0.022, Figure 1E and F).

Impact of HSM on Circulating Cytokine Profiles

Serum levels of Th1 -related cytokines (IL-2, IFN-γ), Th17-related (IL-17A), and Th2-associated cytokines (IL-4, IL-10) were quantified via ELISA. Baseline concentrations of all evaluated cytokines showed no significant intergroup differences (*P*>0.05). Post-intervention, the HSM group demonstrated significantly higher concentrations of IL-2, IL-17A and IFN-γ relative to the controls (p= 0.0215, p=0.0396, p=0.0057, respectively, Figure 2A, 2B and 2C). Conversely, significantly lower concentrations of IL-10 and IL-4 were observed in the HSM group compared to the control group (all *P*<0.05, Figure 2D and 2E).

mRNA and Protein Expression Profiles of Lineage-Specific Transcription Factors

RT-qPCR analysis revealed significantly elevated transcript levels of *T-bet* and *IL-17A* in the HSM group compared to the control group (P=0.0275, p=0.0054, respectively, Figure 3A and 3B). Conversely, *GATA3* mRNA expression was significantly following adjuvant HSM treatment (P=0.0022; Figure 3C). Despite a downward trend, no statistically significant changes were observed in *Foxp3* expression between the two groups (*P*>0.05, Figure 3D). Consistently, Western blot analysis confirmed that protein expression profiles of T-bet, IL-17A, GATA3 and Foxp3 perfectly mirrored their corresponding mRNA trends is (Figure 3E and F), showing significant variations in T-bet, IL-17A, and GATA-3 levels (P=0.0275, 0.0003 and 0.0103, respectively) but stable FoxP3 expression (P > 0.05).

Incidence of adverse events and Tolerability

Safety outcomes recorded during and after the treatment, courses are summarized in Table 3. The HSM group demonstrated a significantly lower incidence of chemotherapy-induced adverse events compared to the control group, including fatigue (p=0.029), insomnia (p=0.016), nausea/emesis (p=0.011), anorexia (p=0.035) and alopecia (p=0.002).

Regarding therapy-specific safety, HSM was well-tolerated, and no patients withdrew from the study due to intolerable heat-related symptoms. While a mild, transient sensation of localized warmth at the HSM site was

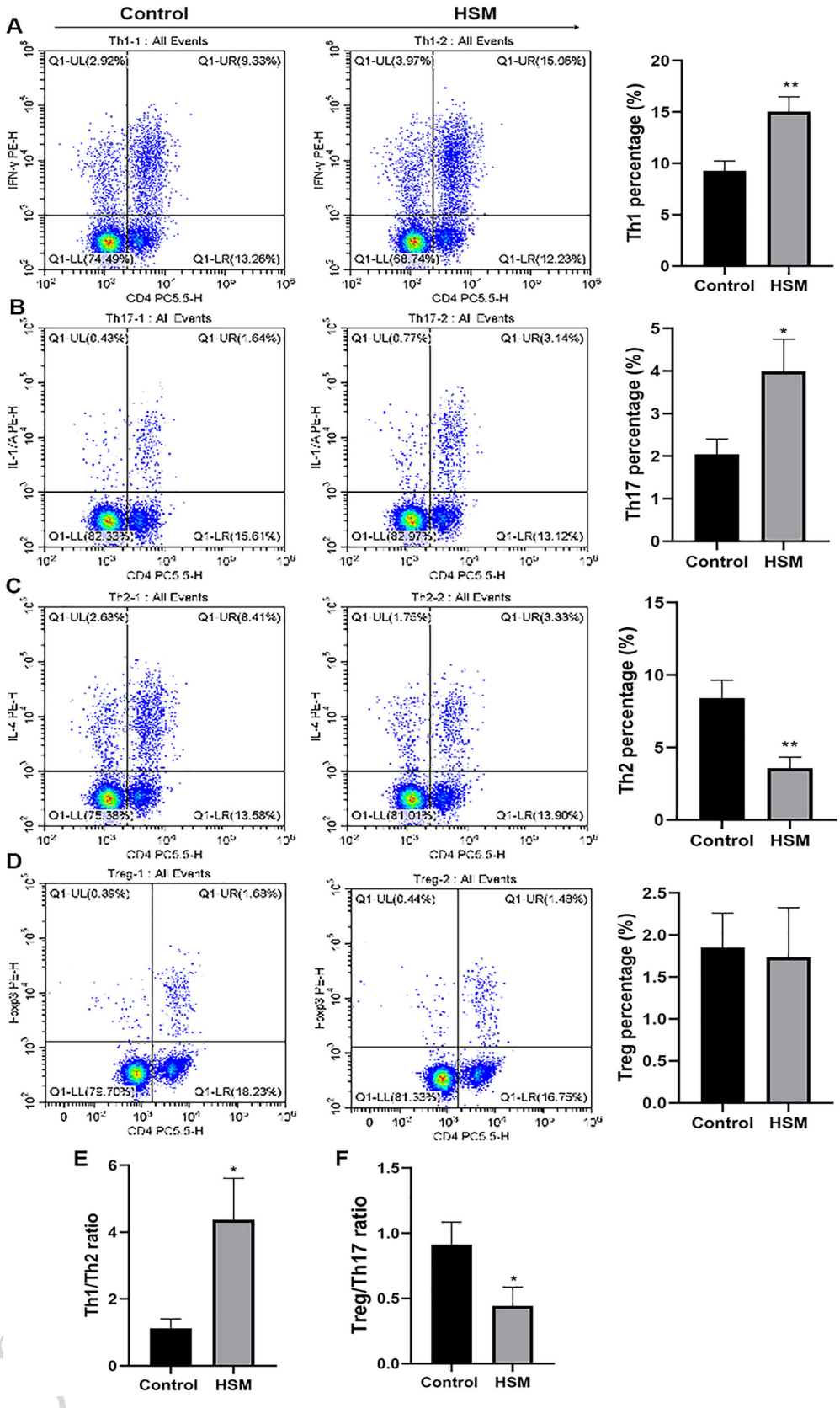

Figure 1: Frequencies of CD4⁺ T cell subsets (Th1, Th2, Th17, and Treg) in peripheral blood measured by flow cytometry. A, Th1 (CD4⁺IFNγ⁺) cells; B, Th17 (CD4⁺IL17A⁺) cells; C, Th2 (CD4⁺IL4⁺) cells; D, Treg (CD4⁺CD25⁺FoxP3⁺) cells; E, Th1/Th2 ratio; F, Treg/Th17 ratio. All percentages are reported as the proportion of CD4⁺ T cells. Data are shown as mean±SD (n = 45 per group). Comparisons between the HSM and control groups were performed using Student's t test. *P < 0.05, **P < 0.01 vs. the control group.

reported by the majority of patients, it resolved spontaneously without intervention. No severe systemic heat-associated syndromes or toxicities requiring medical treatment were observed.

Functional Status and KPS Outcomes

The KPS scale was utilized to monitor improvements or deterioration in patients' functional capacity before and after treatment (21).

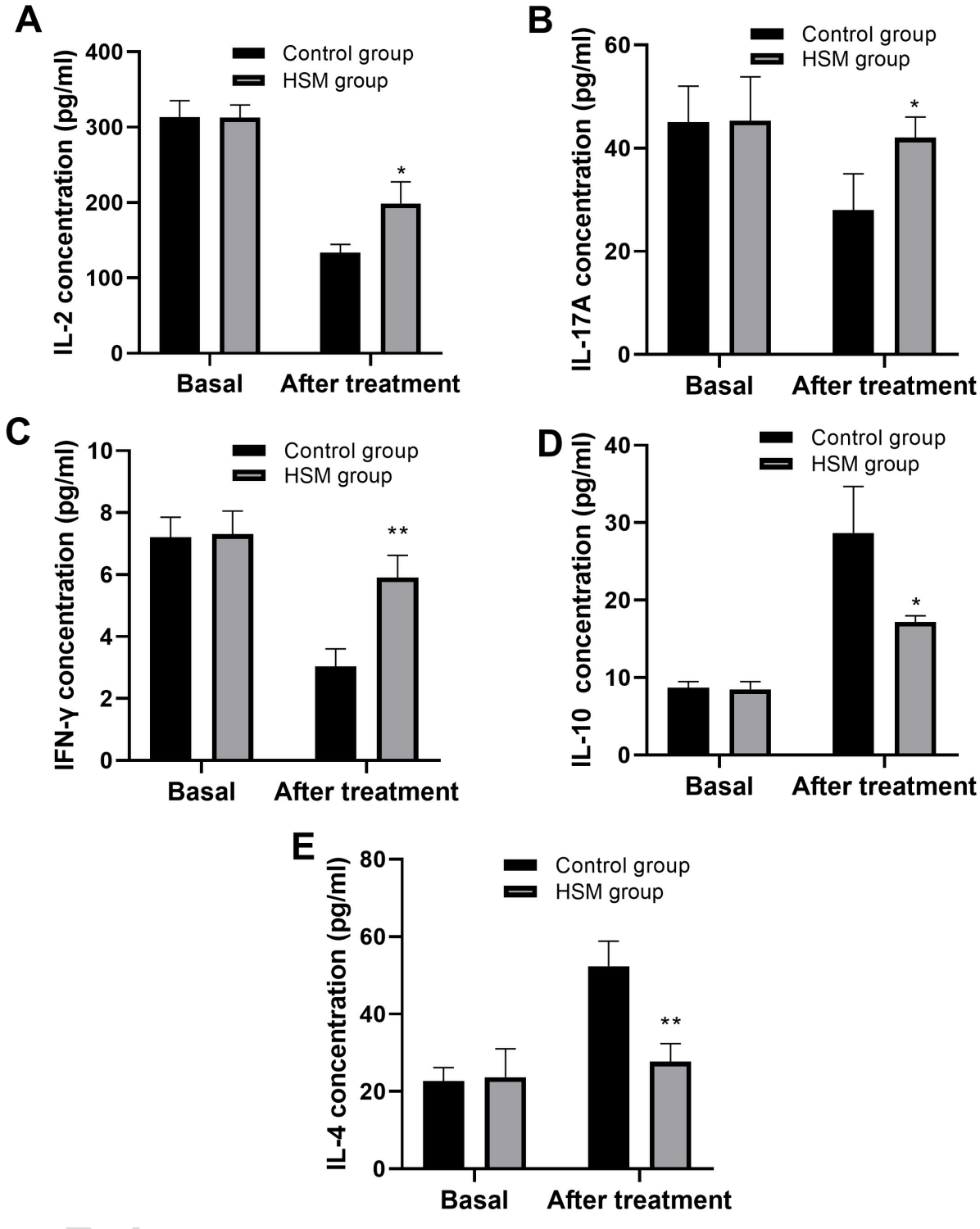

Figure 2: Concentrations of Th cellassociated cytokines in serum measured by ELISA. A, IL2; B, IL17A; C, IFNγ; D, IL10; E, IL4. Data are presented as mean±SD (n = 45 per group). Statistical comparisons between the HSM and control groups were performed using Student's t test. *P < 0.05, **P < 0.01 vs. the control group.

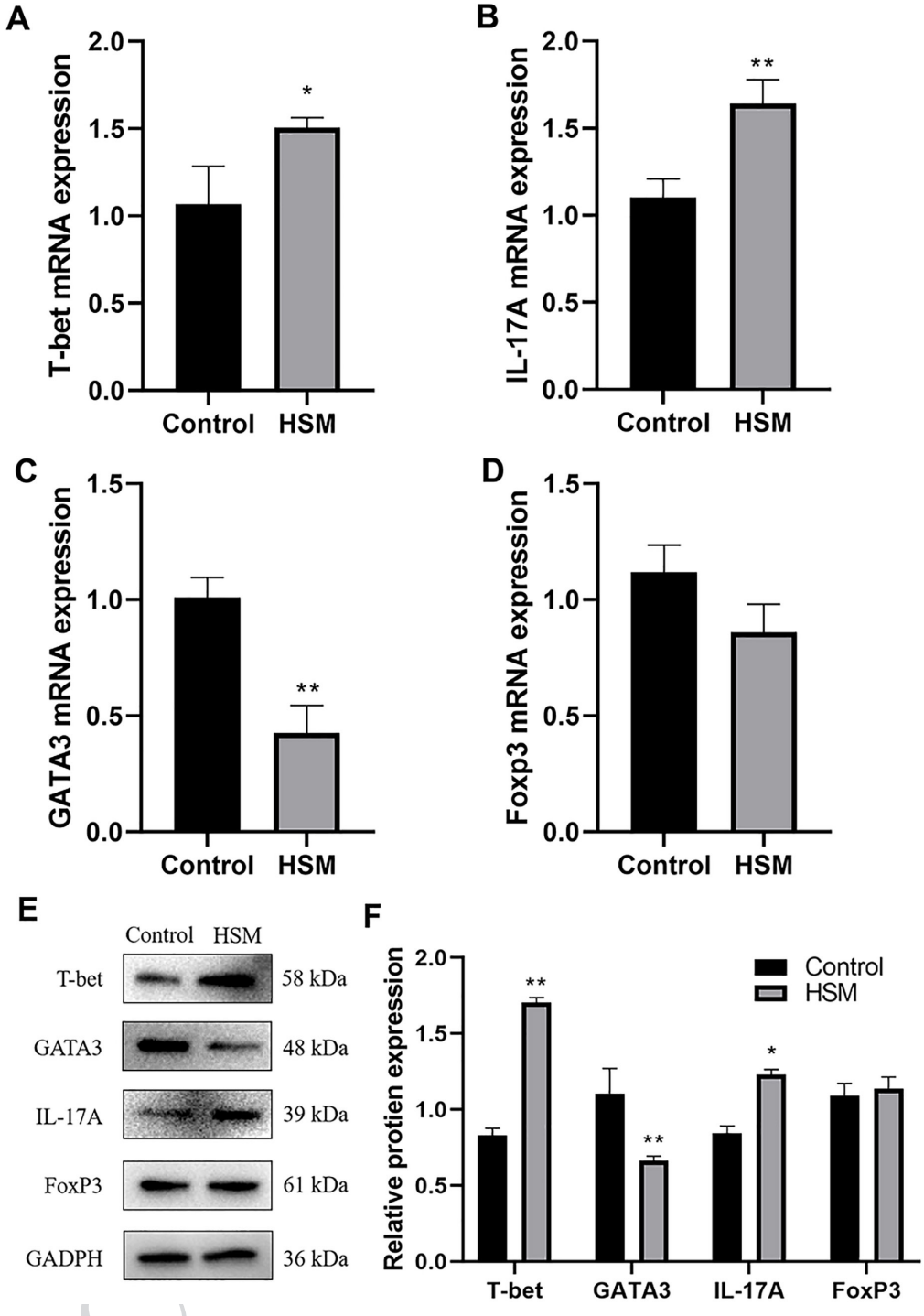

Figure 3: Expression of Th cell-associated transcription factors and cytokines. A-D, RT-qPCR analysis of the mRNA expression levels of T-bet, IL-17A, GATA3, and Foxp3, respectively. E, Representative Western blot images. F, Quantitative analysis of Western blot results. Data are shown as mean±SD (n = 3 independent experiments for Western blot; n = 45 per group for RTqPCR). Comparisons between the HSM and control groups were performed using Student's t test. *P<0.05, **P<0.01 versus the control group.

Following the intervention, the number of patients categorized with improved, stable, or declined KPS score was 26 (57.8%), 10 (22.2%), and 9 (20.0%) in the HSM group, respectively, compared to 14 (31.1%),16 (35.6%), and 15 (33.3%) in the control group. Adjuvant HSM therapy yielded a significantly greater proportion of patients with improved performance status relative to the control group (57.8% vs. 31.1%).

Table 3: Adverse events after treatment

Adverse events	Control group (n=45)	HSM group (n=45)	χ^2 value	*P* value
Hepatic and renal dysfunction (n)	9	6	0.720	0.396
Fatigue (n)	23	13	4.730	0.029
Insomnia (n)	22	11	5.804	0.016
Naupathia/Emesis (n)	30	18		0.011
Anorexia (n)	29	19		0.035
Alopecia (Hair loss) (n)	39	26		0.002

Table 4: Distribution of changes in Karnofsky Performance Status (KPS) scores after treatment (N = 90).

Functional status	Control group (n=45)	HSM group (n=45)	*P* value
Improvement (n, %)	14 (31.1)	26 (57.8)	
Stability (n, %)	16 (35.6)	10 (22.2)	0.162
Decline (n, %)	15 (33.3)	9 (20.0)	0.152

The overall 3 × 2 Pearson chisquare test yielded a χ^2 value of 6.445 (df = 2) with a corresponding P value of 0.040, indicating that the overall distribution was significantly different between the HSM and control groups.

Table 5: Comparison of short-term clinical efficacy between the two groups

Response	Control group (n=45)	HSM group (n=45)	*P* value	X2 value
CR (n)	0	0	——	
PR (n)	12	22	——	
SD (n)	9	15	——	
PD (n)	24	8	——	
ORR (n, %)	12 (26.7)	22 (48.9)	0.029	4.711
DCR (n, %)	21 (46.7)	37 (82.2)	<0.001	12.28

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, overall response rate; DCR, Disease control rate.

Overall, the post-treatment distribution of functional status changes differed significantly between the two cohorts (P=0.036). Detailed KPS changes for both groups are presented in Table 4. 3.7 Comparison of Short-Term Clinical efficacy

Objective tumor responses were evaluated in both cohorts according to the RECIST guidelines. As detailed in Table 5, no patients achieved a CR in either treatment arm. Among 45 patients in the HSM group, 22 patients achieved a PR, 15 maintained SD and 8 experienced PD. Consequently, the ORR and DCR in the HSM group were 48.9% and 82.2%, respectively. In contrast, the control group demonstrated 12 cases of PR, 9 of SD, and 24 of PD, translating to an ORR of 26.7% and a DCR of 46.7%. Statistical analysis confirmed that adjuvant HSM administration was associated with significantly improved ORR (48.9% vs 26.7, p=0.029) and significantly higher DCR (82.2% vs 46.7%, *P*<0.001), compared to conventional chemotherapy alone.

DISCUSSION

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all

pulmonary malignancies, representing the predominant histological subtype worldwide. Despite therapeutic advances, a significant proportion of NSCLC cases remain incurable, primarily due to the frequent absence of clinical manifestation during the early stages of the disease. For decades, conventional cytotoxic chemotherapy has served as a cornerstone treatment for NSCLC patients with unresectable, locally advanced, or metastatic disease. However, these regimens are associated with systemic toxicities which often lead to treatment discontinuation, and compromised quality of life. Consequently, identifying effective interventions to mitigate chemotherapy-induced toxicity remains a critical challenge in NSCLC treatment.

Heat-sensitive moxibustion (HSM) is an innovative therapeutic approach that applies thermal stimulation via ignited moxa to specific, heat-sensitive acupoints. This process activates meridional propagation, channeling thermal energy toward the affected anatomical region, and is maintained until individualized saturated desensitization of the acupoint is achieved (22). Under this localized thermal stimulation, these specialized acupoints elicit distinct, non-superficial sensations, including deep heat permeation, directional heat transmission, and profound internal warming without superficial burning. The successful induction of these heat-sensitive moxibustion sensations is widely recognized as a critical determinant of clinical efficacy (23). A previous study demonstrated that the addition of HSM significantly enhanced the quality of life and alleviated cancer-related pain in patients (24). Consistently, another clinical trial indicated that combining HSM with traditional Chinese medicine effectively suppressed the expression of circulating inflammatory biomarkers while simultaneously improving the quality of life in cancer patients after chemotherapy (13). Recent evidence has further revealed that HSM-integrated palliative care significantly improves the quality of life metrics, even in elderly oncology patients (25). In line with these findings, our data demonstrated that adjuvant HSM treatment significantly enhanced functional performance status, decreased the incidence of chemotherapy-induced adverse events, and notably boosted efficacy in advanced NSCLC patients. Taken together, these insights strongly indicate that incorporating HSM into standard oncology care serves as a powerful therapeutic adjunct, enhancing treatment efficacy while reducing chemotherapy-induced toxicity.

As previously described, conventional cytotoxic chemotherapy is frequently associated with systemic immunosuppression and profound immune dysfunction in patients with malignancies (26). This impairment of host immunity is closely linked to tumor pathogenesis and progression (27). Accumulating evidence demonstrates that homeostatic disruptions within CD4+ T cell subsets, characterized by an altered Th1/Th2/Th17/Treg balance, are responsible for tumor immune tolerance, facilitating immune evasion, and ultimately accelerating tumor progression (28, 29).

Th1 cells drive cell-mediated immunity primarily through the secretion of pro-inflammatory cytokines, including interleukin-2 (IL-2), IFN-γ, and TNF-α. These cytokines activate cytotoxic T lymphocytes (CTLs) and macrophages to orchestrate anti-tumor responses while concurrently modulating B-cell function. In contrast, Th2 cells predominantly secrete IL-4 , which promote B-cell activation, differentiation, thereby driving humoral immune responses (30, 31).

Anti-tumor immunity is primarily mediated by cell-mediated immune responses, with Th1-driven immunity playing a pivotal role in suppressing malignant cell proliferation. In patients with advanced malignancies, a systemic shift toward a Th2-dominant immune status frequently suppresses cellular immune function, thereby facilitating immune evasion and tumor progression. While diminished production of Th1-type cytokine (such as IL-2 and IFN-γ) was observed in lung cancer patients

(32), our data demonstrated that adjuvant HSM treatment significantly elevated post-treatment IL-2 and IFN-ɣ, while concurrently reducing Th2-associated IL-4 and IL-10 levels compared to the control group. Ganoderic acid Me exerts antitumor effects through the upregulation of IL-2 and IFN-γ(33). Collectively, these findings suggest that HSM treatment promotes Th1 cells activation while suppressing Th2-mediated immune responses in patients.

Th17 cells primarily secrete IL-17, a cytokine that stimulates T cell activation and plays a well-characterized role in autoimmune and chronic inflammatory disorders (34). However, the precise function of Th17 and IL-17 in tumor development and progression remains controversial. The upregulation of IL-17 can promote to pulmonary tumor development, as demonstrated in murine models(35). Conversely, enhanced expression of IL-17 can suppress malignant proliferation and exert anti-tumor immunity by recruiting Th1 cells into the tumor microenvironment (36). Our data revealed that IL-17 levels were markedly elevated in the HSM group relative to controls, suggesting that HSM may alleviate chemotherapy-induced toxicity through the modulation of Th17 and IL-17 production. Tregs primarily exert immunosuppressive effects through the secretion of IL-10 and play a key role in tumor immune escape. Notably, targeting or inhibiting Tregs has been shown to confer clinical benefits in NSCLC patients (37). Our analysis of lineage-specific transcription factors demonstrated that Foxp3, the specific transcription factor of Tregs, was significantly downregulated following HSM treatment, directly mirroring the observed decline in serum IL-10 levels. Given that the Th17/Treg axis is frequently skewed toward an immunosuppressive state in NSCLC patients (38), our findings that HSM significantly decreased the Treg/Th17 ratio demonstrates that this adjuvant therapy effectively restores the homeostatic balance of the Treg/Th17paradigm disrupted by cytotoxic chemotherapy.

The chronological synchronization of HSM relative to chemotherapy administration was critical to maximizing its immunomodulatory effects. In our protocol, HSM was initiated on the first day of each chemotherapy cycle, precisely 30 minutes following the completion of the chemotherapy infusion. As recommended by Chinese Anti-Cancer Association (CACA) guidelines, initiating acupuncture-point stimulation on the day of chemotherapy can actively preempt acute adverse reactions and stabilize host immune function (39). The peri-chemotherapy window represents a vital therapeutic opportunity for immune modulation. Early intervention during this specific timeframe has been shown to alleviate bone marrow suppression and accelerate lymphocytes recover (40, 41). Furthermore, acupuncture and moxibustion have been demonstrated to exhibit distinct time- and dose-effects on immune system regulation (42). Our Accordingly, our alternate-day HSM treatment schedule, sustained across four consecutive cycles, was designed to optimize these cumulative therapeutic benefits.

Precise acupoint selection represents the most critical element governing the therapeutic efficacy of HSM. In this study, we selected GV14 as the sole target for HSM monotherapy Previous clinical studies have employed alternative or additional acupoints to regulate immune function in cancer patients. For example, Zhang et al. reported that moxibustion at Zusanli (ST 36) and Feishu (BL 13) significantly reduced the neutrophil-to-lymphocyte ratio and improved quality of life in patients with lung cancer (43). Moxibustion at Geshu (BL-17), Ganshu (BL-18) and Pishu (BL-20) has been shown to alleviate chemotherapy-induced bone marrow suppression (44). However, GV14 is the meeting point of all *Yang* meridians and is therefore considered particularly effective for tonifying *Yang Qi* and correcting the *Qi* and *Yang* deficiencies that commonly arise following cytotoxic chemotherapy. As a single midline acupoint, GV14 is easily accessible with the patient in the supine position, thereby

minimizing treatment burden. In our study, stimulation of GV14 consistently enhanced immune function and reduced chemotherapy-related adverse events in NSCLC patients. Furthermore, combining GV14 with other acupoints, such as ST36 or RN6, may further improve therapeutic efficacy. Future studies are warranted to systematically compare different acupoint selection strategies and determine the most effective and practical treatment regimen for clinical application.

Several limitations of this study should be acknowledged. First, the sample size was relatively small (n=45 per group), which may limit the generalizability of the findings. Most importantly, although patients with TCM heat syndromes at baseline were excluded, no subgroup analyses were performed according to TCM pattern differentiation. Future studies with larger sample sizes and prospective stratification based on TCM pattern are needed to determine which patient subgroups derive the greatest benefit from HSM treatment.

CONCLUSIONS

In conclusion, this study demonstrates that adjuvant HSM treatment effectively restores chemotherapy-impaired immune function in patients with advanced NSCLC. By upregulating the Th1/Th2 and downregulating the immunosuppressive Treg/Th17 ratio, HSM significantly enhances patient health-related quality of life and mitigates the incidence of conventional chemotherapy-induced adverse events. Long-term follow-up clinical trials and in-depth mechanistic analyses are warranted to further validate the persistent therapeutic efficacy, survival benefits, and long-term clinical significance of HSM in advanced NSCLC management.

ACKNOWLEDGMENTS

None.

FUNDING

The mechanism by which heat-sensitive moxibustion regulates chemotherapy immunosuppression in lung cancer by regulating the homeostasis of Th1/Th2 and Th17/Treg (WFZYY2024-3-002).

REFERENCES

1. Brett C B, Charles S DC. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med. 2020;41(1).
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-63.
3. Alduais Y, Zhang H, Fan F, Chen J, Chen B. Non-small cell lung cancer (NSCLC): A review of risk factors, diagnosis, and treatment. Medicine. 2023;102(8):e32899.
4. Pignon J, Tribodet H, Scagliotti G, Douillard J, Shepherd F, Stephens R, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol. 2008;26(21):3552-9.
5. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nature reviews Immunology. 2008;8(1):59-73.
6. Tian X, Li Y, Huang Q, Zeng H, Wei Q, Tian P. High PD-L1 expression correlates with an immunosuppressive tumour immune microenvironment and worse prognosis in ALK-rearranged non-small cell lung cancer. Biomolecules. 2023;13(6):991.
7. Lu S, Wang B, Wang J, Guo Y, Li S, Zhao S, et al. Moxibustion for the Treatment of Cancer and its Complications: Efficacies and Mechanisms. Integrative Cancer Therapies. 2023;22:15347354231198089.
8. Lin X-m. Functional feature of "five yang acupoints" in Governor Vessel. Journal of Acupuncture and Tuina Science. 2008;6(3):184-86.
9. Yan J, Ni Y, Tan L, Zheng S, Zhang Y, Liu J, et al. Efficacy and safety of Dazhui (GV 14) as a single acupoint for managing fever: A systematic review. European Journal of Integrative Medicine. 2022;56:102196.
10. Lee HK, Moon S-K, Jin C, Cho S-Y, Park S-U, Jung W-S, et al. Effects of gv14 acupuncture on cerebral blood flow velocity in the basilar and middle cerebral arteries and co2 reactivity during hypercapnia in normal individuals.

Evidence-Based Complementary and Alternative Medicine. 2021;2021(1):9319413.
11. Li N, Guo Y, Gong Y, Zhang Y, Fan W, Yao K, et al. The anti-inflammatory actions and mechanisms of acupuncture from acupoint to target organs via neuro-immune regulation. Journal of Inflammation Research. 2021:7191-224.
12. Liu Z-D, Pei J, Fu Q-H, Li H-Y, Yu Q-W, Zhang J-Y, et al. [Influence of electroacupuncture and moxibustion and their treated mouse serum on the proliferation of the cultured splenetic CD4+ CD25+ regulatory T cells of tumor-bearing mice]. Zhen ci yan jiu3-002 Acupuncture research. 2009;34(4):219-24.
13. Liu T, Jiang C. Effects of heat-sensitive moxibustion plus Chinese medication on the expression of serum inflammatory indicators and microRNAs in patients with multiple myeloma after chemotherapy. Journal of Acupuncture and Tuina Science. 2023;21(1):51-58.
14. Ji J-F, Ge X-X, Xu C-M, Jiang Y, Gu J-H, Wei G-H, et al. Intradermal needling combined with heat-sensitive moxibustion for moderate to severe cancer pain. 2021.
15. Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WE, et al. The IASLC lung cancer staging project: proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer. Journal of Thoracic Oncology. 2016;11(1):39-51.
16. Chuntao Z, Yuefeng T. Research Progress of Relationship Between Time-Effect and Dose-Effect of Acupuncture and Moxibustion on Immune Regulation. Journal of Hunan University of Chinese Medicine. 2017.
17. Han B, Liu Z, Zhang Y, Zhu J. The curative effect of different acupoints thermosensitive moxibustion and traditional moxibustion on thrombocytopenia after chemotherapy for advanced Lung cancer. Clin J Chinese Med. 2022;14(34):36-40.
18. Yu T. Clinical study on heat-sensitive moxibustion for reducing toxicity and enhancing efficacy in adjuvant tumor chemotherapy. Acupuncture Research. 2026;51(1):122-29.
19. Jameel S, Jahan P, Ramasamy S. A Path to Protein Quantitation: PBMCs to Western Blot. International Journal of Methodology. 2023;2(1):12-27.
20. Thuluvath PJ, Thuluvath AJ, Savva Y. Karnofsky performance status before and after liver transplantation predicts graft and patient survival. J Hepatol. 2018;69(4):818-25.
21. Mor V, Laliberte L, Morris JN, Wiemann M. The Karnofsky performance status scale: an examination of its reliability and validity in a research setting. Cancer. 1984;53(9):2002-07.
22. Wang J, Zhang Q, Deng Y, Deng G, Huang F, Zhou Y, et al. Efficacy and safety of heat-sensitive moxibustion in the treatment of ulcerative colitis: A protocol for a systematic review and meta-analysis. Medicine. 2021;100(4):e24078.
23. Deng H, Shen X. The mechanism of moxibustion: ancient theory and modern research. Evidence-Based Complementary and Alternative Medicine. 2013;2013(1):379291.
24. Ji JF, Ge XX, Xu CM, Jiang Y, Gu JH, Wei GH, et al. [Intradermal needling combined with heat-sensitive moxibustion for moderate to severe cancer pain]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion. 2021;41(7):725-9.
25. Yu T, Liu H, Liu Z, Chen R, Xie D. [Heat-sensitive moxibustion assisted in palliative treatment to improve the quality of life in elderly patients with malignant tumor: a randomized controlled trial]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion. 2025;45(2):167-72.
26. Harris J, Sengar D, Stewart T, Hyslop D. The effect of immunosuppressive chemotherapy on immune function in patients with malignant disease. Cancer. 1976;37(S2):1058-69.
27. Zhang Y-L, Li J, Mo H-Y, Qiu F, Zheng L-M, Qian C-N, et al. Different subsets of tumor infiltrating lymphocytes correlate with NPC progression in different ways. Mol Cancer. 2010;9:1-11.
28. Lin W, Niu Z, Zhang H, Kong Y, Wang Z, Yang X, et al. Imbalance of Th1/Th2 and Th17/Treg during the development of uterine cervical cancer. Int J Clin Exp Pathol. 2019;12(9):3604.
29. Zhou Y, Yu K. Th1, Th2, and Th17 cells and their corresponding cytokines are associated with anxiety, depression, and cognitive impairment in elderly gastric cancer patients. Frontiers in surgery. 2022;9:996680.
30. Hirahara K, Ghoreschi K, Laurence A, Yang X-P, Kanno Y, O'Shea JJ. Signal transduction pathways and transcriptional regulation in Th17 cell differentiation. Cytokine & growth factor reviews. 2010;21(6):425-34.
31. Wei H, Sun R, Xiao W, Feng J, Zhen C, Xu X, et al. Type two cytokines predominance of human lung cancer and its reverse by traditional Chinese medicine TTMP. Cell Mol Immunol. 2004;1(1):63-70.
32. Chen J, Li X, Huang C, Lin Y, Dai Q. Change of serum inflammatory cytokines levels in patients with chronic obstructive pulmonary disease, pneumonia and lung cancer. Technology in Cancer Research & Treatment. 2020;19:1533033820951807.
33. Wang G, Zhao J, Liu J, Huang Y, Zhong J-J, Tang W. Enhancement of IL-2 and IFN-γ expression and NK cells activity involved in the anti-tumor

effect of ganoderic acid Me in vivo. International immunopharmacology. 2007;7(6):864-70.
34. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol. 2009;27(1):485-517.
35. Numasaki M, Watanabe M, Suzuki T, Takahashi H, Nakamura A, McAllister F, et al. IL-17 enhances the net angiogenic activity and in vivo growth of human non-small cell lung cancer in SCID mice through promoting CXCR-2-dependent angiogenesis. The Journal of Immunology. 2005;175(9):6177-89.
36. Nuñez S, Saez JJ, Fernandez D, Flores-Santibañez F, Alvarez K, Tejon G, et al. T helper type 17 cells contribute to anti-tumour immunity and promote the recruitment of T helper type 1 cells to the tumour. Immunology. 2013;139(1):61-71.
37. Xie M, Wei J, Xu J. Inducers, attractors and modulators of CD4+ Treg cells in non-small-cell lung cancer. Frontiers in Immunology. 2020;11:676.
38. Min-Chao D, Wei H, Pei-Wen J, Yu-Ping W, Qiu W, Liang-Ming Z, et al. Disturbed Th17/Treg Balance in Patients with Non-small Cell Lung Cancer. Inflammation. 2015;38(6).
39. Wang J, Association SoLCoCA-C. CACA guidelines for holistic integrative management of lung cancer. Holistic Integrative Oncology. 2024;3(1):10.
40. Li S, Zhao S, Guo Y, Yang Y, Huang J, Wang J, et al. Clinical efficacy and potential mechanisms of acupoint stimulation combined with chemotherapy in combating cancer: A review and prospects. Front Oncol. 2022;12:864046.
41. Fu H, Chen B, Hong S, Guo Y. Acupuncture therapy for the treatment of myelosuppression after chemotherapy: a literature review over the past 10 years. Innovations in Acupuncture and Medicine. 2015;8(3):122-26.
42. Jin C-n, Zhang T-s, Ji L-x, Tian Y-f. [Survey of studies on mechanisms of acupuncture and moxibustion in decreasing blood pressure]. Zhongguo zhen jiu = Chinese acupuncture & moxibustion. 2007;27(6):467-70.
43. Mengxue Z, Ling G, Lili W, Ying L. Effect of scar-producing moxibustion at the acupoints Zusanli (ST 36) and Feishu (BL 13) on neutrophil-to-lymphocyte ratio and quality of life in patients with non-small-cell lung cancer: A randomized, controlled trial. Journal of Traditional Chinese Medicine. 2018;38(3):439-46.
44. Staebler F. The Daily use of Moxibustion to Treat Chemotherapy-Induced Bone Marrow Depression-A practical evaluation based on 20 years of clinical experience. Journal of Chinese Medicine (JCM). 2009(90).