PROTOCOL

Title of research programme: Clinical study on the prevention and treatment of postoperative metastasis in stage IIA-IIIA lung cancer with negative expression of driver genes by Fuzheng Quxie Recipe

Research organization: Shanghai Municipal Hospital of Traditional Chinese Medicine

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Efficacy of Fuzheng Quxie Formula for inhibitting metastasis after surgery in stage IIA-IIIA lung cancer with negative driver genes: study protocol for a multi-centre, double-blind, randomized controlled trial

Abstract:

Background: Metastasis is the main reason for poor prognosis and high mortality of lung cancer. Surgery and postoperative adjuvant chemotherapy are the first choice for patients with driver-negative stage I to IIIA lung cancer. However, the incidence of postoperative recurrence and metastasis did not significantly decrease. In China, traditional Chinese medicine (TCM) is commonly used to treat cancer. TCM has the potential to be used as an adjuvant therapy for lung cancer to reduce treatment related toxicity and improve clinical efficacy. In view of the positive results of the basic study of Fuzheng Quxie Formula against lung cancer metastasis, we plan to evaluate the efficacy of Fuzheng Quxie Formula against postoperative metastasis of stage IIA-IIIA lung cancer that is negative for driver gene expression.

Methods: A multi-centre, double-blind, randomised, placebo-controlled parallel group controlled trial will be conducted. Eligible patients will be randomised into a treatment group (daily Fuzheng Quxie Formula granules + regular chemotherapy) and a control group (daily Chinese medicine placebo granules + regular chemotherapy) in a ratio of 1:1. It is recommended that the participants' chemotherapy be used strictly in accordance with the dosage, duration and usage as recommended in the respective instructions and National Comprehensive Cancer Network (NCCN) guidelines. Fuzheng Quxie Formula was administered orally, twice a day, in the morning and evening, for 6 months. Follow-up after the end of medication, follow-up period of 3 years. After the end of the programme, follow-up was continued until 5 years or until the subject died (or progressed). The primary outcome was disease-free survival (DFS), and the secondary outcomes were overall survival (OS), minimal residual disease (MRD), circulating tumour cells detection, TCM symptom score, assessment of quality of life (QoL), immune indices, tumour markers, peripheral blood systemic immune- inflammation index (SII), prognostic nutritional index (PIN) and prognostic nutritional index (PNI).

Discussion: This will be the first trial to evaluate the efficacy and safety of Fuzheng Quxie Formula in inhibitting metastasis after surgery in stage IIA-IIIA lung cancer with negative driver genes. The results of this randomised controlled trial will fill a gap in this study by showing whether Fuzheng Quxie Formula combined with postoperative chemotherapy improves the prognosis for patients with lung cancer.

Ethics and Dissemination: The Ethics Committee of Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine approved the study protocol (approval no.: 2023SHL-KY-19-01, 2023/02/23). The study will also be supervised and

managed by the Ethics Committee.

Keywords : Fuzheng Quxie Formula, Lung cancer in stage IIA-IIIA, Negative driver genes, Metastasis after surgery

Background

Lung cancer is one of the most common malignant tumours of respiratory system, with the mortality rate perennially ranking first among all kinds of malignant tumours, which greatly threatens the safety of human life[1]. In developed countries (such as the United States), lung cancer is still the malignant tumour with the highest number of deaths[2]. In China, the latest data shows that there are about 787,000 new cases of lung cancer, with an incidence rate of 57.26/100,000, ranking first in the national morbidity and cause of death by gender, causing economic losses of up to 221.4 billion, and this economic and social pressure will continue to increase[3]. In recent years, due to the rapid advances in therapeutic modalities (e.g., molecular targeted therapy, immunotherapy, etc.), the survival time of lung cancer has been extended, but the 5-year survival rate of lung cancer still persists at a low level[4]. Distal metastasis is the main cause of death in 90% of patients and there is a lack of effective treatment options[5]. Surgery is currently the first choice for patients with stage I to IIIA lung cancer, but metastasis after radical lung cancer surgery is a bottleneck limiting its overall efficacy.

Currently, domestic and international guidelines related to lung cancer diagnosis and treatment recommend that patients with stage IB (high-risk type) need to undergo postoperative conventional chemotherapy, and that patients with stage IIA and IIIA must undergo conventional chemotherapy after surgery[6]. However, only about 5% of lung cancer patients have long-term survival benefit[7]. Studies have confirmed that the recurrence rate of patients with stage I lung cancer after adjuvant chemotherapy is around 17.8%, and about 45.5% of patients with stage II-III lung cancer may have recurrent metastasis. Among them, patients with stage I recurrence include : 63% are local recurrence, 40.5% are distant recurrence and 72% are new primary lung cancer[8]. Wang Zezhou et al followed up the survival of 7753 patients operated for lung cancer and confirmed that the 5-year survival rates of patients with stage IB, IIA, IIB, IIIA and IIIB lung cancer were 78.5%, 78.2%, 62.9%, 49.3% and 33.0%, respectively. Therefore, standard chemotherapy administered after surgery presents an overall poor role in the prevention of recurrent metastasis in patients after radical lung cancer surgery. There is a need to develop new diagnostic and therapeutic protocols.

With the development of molecular targeted therapy and immunotherapy, patients with intermediate and advanced lung cancer who are positive for driver genes or have high PD-L1 expression can further benefit from targeted therapy or immunotherapy. Adenocarcinoma and squamous carcinoma are common pathologies in lung cancer. A study that included 371 Chinese lung cancer cases showed that the mutation types in lung adenocarcinoma were EGFR (60%), TP53 (57%), KRAS (13%), PIK3CA (7%), and ALK (7%), whereas those in squamous lung carcinoma included TP53 (87%), PIK3CA (43%), CDKN2A (20%), KMT2D (20%), and EGFR (17%) mutations[9]. For ALK/EGFR/ KRAS/ROS1/MET mutations, there are Several targeted therapeutic agents have been approved, marketed internationally and in the health insurance. Wu Yilong et al. conducted a clinical study confirming the benefit of ositinib in patients after radical surgery for stage IB~IIIA lung cancer with driver gene EGFR positivity[9, 10]. Meanwhile, immunotherapy sequential treatment after chemotherapy for Radical resection of lung cancer

patients with stage IB-IIIA lung cancer with high PD-1/L1 expression (≥1%) may also provide a survival benefit and reduce the rate of metastasis. In addition, a study included 328 patients after resection of stage IA-IIB non-small cell lung cancer (NSCLC) to examine their immune checkpoint expression[11]. The study showed that 62.0% (160/258) of the lung adenocarcinoma group had PD-L1 <1%, and only 38.0% (98/258) had PD-L1 ≥ 1%, suggesting that the expression of immune checkpoints is low in lung adenocarcinoma patients[12]. Therefore, for patients with positive driver gene and high immune checkpoint expression after radical lung cancer surgery, standardised diagnostic and treatment protocols that can benefit patients clinically are already available. However, for patients with negative genes expression and low PD-1/L1 expression (≤1%) after radical lung adenocarcinoma surgery, who have poor chemotherapeutic efficacy in the clinic and lack indications for molecularly-targeted or immunotherapeutic treatments, the development of effective, scalable and specific therapeutic protocols is urgently needed.

In summary, the existing multidisciplinary treatments available for lung cancer metastasis, such as chemotherapy, targeted therapy, and immunotherapy, have been developed based on alterations to the genetic structure and function of the tumour cells themselves in the primary foci. However, as tumour metastasis is often trans-organic and involves imbalances in the function of multiple systems, the overall efficiency of current strategies for the prevention and treatment of lung cancer metastasis is poor [15]. TCM, which stresses the holistic concept and dialectical treatment, and committing to a people-centered approach, has unique advantages in preventing and treating tumour metastasis[13-15]. A large number of basic and clinical studies have confirmed that TCM can significantly inhibit the metastasis of lung cancer, significantly prolong the survival period of patients, and improve their quality of life[16, 17]. Therefore, it is an important breakthrough to overcome the bottleneck of tumour metastasis in clinic under the holistic view and syndrome differentiation of TCM to understand the pathogenesis of lung cancer metastasis from a systemic perspective, and then anchoring the key therapeutic targets to develop effective prevention and treatment strategies[18-20].

Professor Jianhui Tian 's group focuses on lung cancer metastasis research. Under the guidance of a master of Chinese medicine Liu Jiaxiang's academic idea of "treating cancer by reforcing healthy qi", and incorporating Lao Zi’s "Existence and non-existence give birth the one to the other", modern oncology and immunology, the team suggests the core pathogenesis of cancer metastasis in the subclinical stage of cancer --"Deficiency of Vital Qi and Hidden Toxin" theory. According to the theory, immune disorders caused by immune senescence and immune escape are "Deficiency of Vital Qi", while circulating tumour cells, quiescent cancer cells and tumour stem cells are the modern biological connotations of "toxicity". The residual cancer cells after radical treatment of lung cancer are the "Hidden Toxin". After radical lung cancer surgery, residual cancer cells (hidden toxin) are latent in the body and in dormant or quiescent stage. When there is a deficiency of vital qi, the efficiency of immune surveillance and immune clearance decreases, and the cancer cells go from the quiescent phase to the proliferative phase, leading to the occurrence of tumour metastasis. The team further found that myeloid-derived suppressor cells, regulatory T cells, and circulating tumour cells ("hidden toxin") are important factors in the subclinical state of lung cancer metastasis in clinical studies, and analysed their expression patterns[21]. The team took the lead in establishing the world's first human lung adenocarcinoma circulating tumour cell line (CTC-TJH-01), as well as a platform for metastasis-specific studies of lung cancer[22].

Under the theory of "Deficiency of Vital Qi and Hidden Toxin", the group established the treatment method of "supporting vital qi" and "dispelling hidden toxin", and created a Chinese medicine formula for inhibitting postoperative metastasis of early- and middle-stage non-small cell lung cancer. In this formula, Sheng Huang Qi, Fu Ling, Bai Zhu tonify spleen and stomach to "strengthen earth to generate metal"; Bei Sha Shen, Tian Dong, Mai Dong nourishes yin and moistens the lungs; Hai Zao, Xia Ku Cao, Kun Bu, which soften and resolve hard mass, and Bai Hua She She Cao, Shi Jian Chaun, Shi Shang Bai, which clear heat, detoxifying to dispers nodules. Through in-depth clinical and basic research, the team has obtained the scientific basis for Fuzheng Quxie Formula to inhibit metastasis: ①Fuzheng Quxie Formula has the clinical effect of preventing recurrence and metastasis: the team, in cooperation with Shanghai Lung Hospital, carried out a prospective clinical study, which confirms that the DFS rates of early and middle stage NSCLC patients after intervention with Fuzheng Quxie Formula were 98.8% and 96.5% in 1 and 2 years, respectively, which were higher than 95.3% of the patients in the pure follow up group. (ii) The mechanism of inhibiting metastasis by Fuzheng Quxie Formula may be to regulate the immune function to promote apoptosis of circulating tumour cells: intra-group comparison of immune indexes before and after the study suggests that the peripheral blood NK, CD3+ and IgM content of the treatment group after intervention of Fuzheng Quxie Formula increased significantly ($P < 0.01$), and at the same time, it can reduce the number of circulating tumour cells in the blood ($P<0.01$), suggesting that Fuzheng Quxie Formula has the tendency to regulate patients' immunity and inhibit circulating tumour cells to control tumour progression; (iii) Mechanism study found that Fuzheng Quxie Formula can improve immune function and restore the state of body immune balance: Fuzheng Quxie Formula can up-regulate the expression of positive immune cells, such as NK cells and T-cell subpopulations, which have an inhibitory and killing effect on tumour cells, and down-regulate positive immune cell expressions, which promote tumour cell proliferation, metastasis, immune escape, and so forth, Down-regulate the expression of negative immune cells such as myeloid-derived suppressor cells and regulatory T cells, which promote the proliferation, metastasis and immune escape of tumour cells; inhibit and remove circulating tumour cells in the peripheral circulation; and influence and regulate the metabolic reprogramming of tumour cells in terms of sugar, fat and amino acids. Meanwhile, the theory of "Deficiency of Vital Qi and Hidden Toxin" guided the construction of clinical efficacy prediction and metastasis risk assessment model for TCM treatment of lung cancer (Journal of Traditional Chinese Medicine, 2022), which found that maintaining peripheral immune homeostasis is an important basis for TCM to inhibit lung cancer metastasis, and optimised and enriched the prognostic assessment system of lung cancer. Fuzheng Quxie Formula has been applied as an in-hospital preparation for many years, and has successfully entered the process of joint development of new drugs with enterprises.

Therefore, in response to the urgent clinical needs of postoperative patients with IIA-IIIA non-small cell lung cancer who are negative for driver gene expression, we attempted to objectively evaluate the efficacy of Fuzheng Quxie Formula in inhibitting metastasis of IIA-IIIA non-small cell lung cancer who are negative for driver gene expression, and to actively explore the establishment of a combined prevention and treatment programme of TCM and Western medicine by designing a clinical study protocol in line with the international norms.

1 Methods

1.1 Trial Design

A prospective clinical study of combined Chinese and Western medicine for the prevention and treatment of metastasis after radical lung adenocarcinoma in postoperative stage IIA-IIIA non-small cell lung adenocarcinoma patients with negative driver gene expression was conducted in a randomised, double-blind, placebo-parallel controlled, multicentre clinical trial design. Participants from 7 study centres: Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai Pulmonary Hospital, Shanghai Chest Hospital, Shanghai General Hospital, and Shanghai TCM-Integrated Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. Firstly, the clinical study will obtain written informed consent from the participants. Eligible participants will be randomly assigned in a 1:1 ratio to the treatment group (daily Fuzheng Quxie Formula granules + regular chemotherapy) and the control group (daily Chinese medicine placebo granules + regular chemotherapy). The treatment group was Fuzheng Quxie Formula granules, and Components of Fuzheng Quxie Formula is shown in Table 1. The drug was supplied by Jiangyin Tianjiang Pharmaceutical Co. 5% of the drug content of the treatment group was made into a control placebo drug, provided by Jiangyin Tianjiang Pharmaceutical Co., Ltd, which had the same odour, colour and appearance as the test drug, but had no significant drug treatment effect.

Table 1. Components of Fuzheng Quxie Formula.

Chinese name	Latin name	Parts of the substances	Amount (g)
Sheng Huang Qi	Udis Astragalus	Root	15
Jiao Bai Zhu	Frixum Atractylodes	Root tuber	15
Bai Fu Ling	Alba Poria	Root tuber	15
Bei Sha Shen	Adenophora Septentrionalis	Root	15
Zhe Mai Dong	Ophiopogon japonicus	Root tuber	15
Dang Shen	Codonopsis pilosula	Root	15
Shu Yang Quan	Shuyang Spring	Whole herb	15
Xia Ku Cao	Prunella vulgaris	Flowering fruit spike	15
Sheng Mu Li	Udis Ostreis	Shell	15
Shi Jian Chuan	Ishimi Chuan	Above-ground parts	15
Shi Shang Bai	Cupressus Lapis	Whole herb	15
Hai Zao	Alga	Dried algae	15

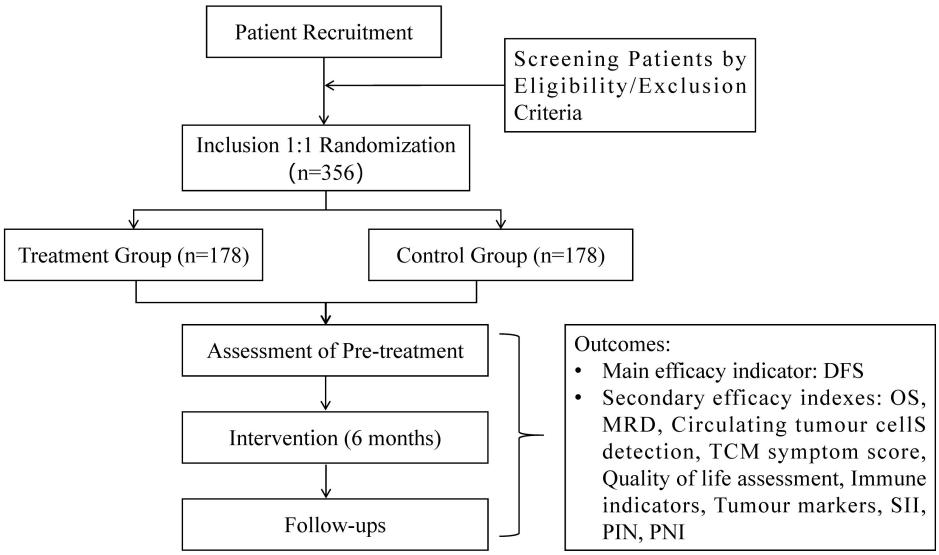

Figure 1. Study flow chart. DFS, disease-free survival; OS, overall survival; MRD, minimal residual disease; SII, systemic immune- inflammation index; PIN, prognostic nutritional index; NSCLC, non-small cell lung cancer; PNI, pharmaceutical news index;

1.2 Study Population and Eligibility Criteria

The sources of cases for this study were inpatients and outpatients admitted from 1 July 2022 to 30 June 2025 with negative expression of driver genes in postoperative stage IIA-IIIA lung adenocarcinomas with qi and yin deficiency. Inclusion for observation should be no later than 6 weeks postoperatively. Screening will be based on inclusion and exclusion criteria (Table 1).

Table 1. Study Inclusion and Exclusion Criteria.

Inclusion criteria	Exclusion criteria
① Patients with clinical staging of stage IIA-IIIA lung adenocarcinoma after radical surgery, patients within 6 weeks after surgery; ② Driver gene negative (no EGFR, ALK, ROS1 mutations), PD-1/L1 expression <1%; ③ Fulfilling the diagnostic criteria for the lung qi and yin deficiency type, the primary symptoms are cough, low sputum, shortness of breath, low or muffled voice, weakness, and thirst without wanting to drink. Secondary symptoms include spontaneous sweating, nocturnal sweating, five heartburns, red tongue or teeth marks on the sides of the tongue, and a weak pulse. At least two primary symptoms and one of the secondary symptoms are present; ④ Patients aged between 18 and 75 years; ⑤ Patients with basically normal blood and biochemical indicators, without serious viral or bacterial infections; without organ failure and serious	① Patients with incomplete resection or whose cancer has undergone recurrence or metastasis; ② Patients who are being treated with other drugs or therapies (including other Chinese herbal medicines, immunological drugs, radiotherapy, etc.); ③ Patients who are themselves mentally ill and have a lack of autonomous behaviour; ④ Women who are pregnant, preparing for pregnancy or breastfeeding; ⑤ Combined heart, lung, brain, liver, kidney and haematopoietic system and other serious diseases, psychiatric patients; ⑥ Allergic or known hypersensitivity to the components of the drug; ⑦ Patients who are participating in other clinical trials or have participated in other clinical trials within 3 months; ⑧ Alcohol and/or psychoactive substances abuse, drug

heart disease (TBIL <68 μ mol/L, AST <90 IU/L, CREA <350 μ mol/L, WBC >3.5 × 10^9/L and < 12 × 10^9/L, PLT >80 × 10^9/L, HCT >0.20) ⑥ Those with a tumour PS score ≤ 2 and no other serious comorbidities; ⑦ The patients themselves gave informed consent to participate in the study by signing an informed consent form with good compliance; ⑧ Non-pregnant and lactating patients; ⑨ Passing the chemotherapy-related indicators; ⑩ Those who have no allergic reaction to the ingredients in the formula.	abusers and dependent persons; ⑨ Other pathologies or conditions that, in the judgement of the investigator, have the effect of reducing the likelihood of enrolment or complicating enrolment, e.g., frequent changes in the work environment, unstable living conditions, and other conditions that predispose to loss of visits.

TBIL: Blood bilirubin, AST: aspartate aminotransferase, CREA: creatinine, WBC: white blood cell count, PLT: platelet count, HCT: erythrocyte pressure, PS: Performance staus.

1.3 Concurrent Treatment

On the one hand, during the trial, drugs that affect the evaluation of therapeutic efficacy, such as chemotherapeutic drugs, targeted drugs, bio-immune agents, Chinese medicine injections, and other drugs that are clearly indicated in the instructions to be used for anti-tumour purposes, shall not be used in combination. On the other hand, detailed records should be made of the combined diseases or symptoms that existed before the start of the trial, and the drugs or other therapies that need to be continued or added must be recorded in the case report form with the generic name of the drug or the name of the other therapies, the dosage, the reason for use, the number of times of use, and the time of use, etc., so that they can be analysed and reported at the time of the summary.

1.4 Sample Size

The calculation of the sample size of this clinical trial is based on the statistical results of the "Chinese Medicine Clinical Research on Common Malignant Tumours (Lung Cancer)" conducted by this group in the Third Round of Shanghai Three-Year Action Plan of Traditional Chinese Medicine. Based on the group's previous clinical research on the survival and immune function effects on patients after radical NSCLC surgery, the 5-year disease-free survival rate was 90.2% in the treatment group and 78.3% in the control group. Sample size was calculated using PASS.15 software, and Survival-Two Survival Curves-Test(Inequality)-Logrank Tests were applied. set Power=0.8, α = 0.025 (unilateral), according to the control group and experimental group 1:1, combined with the basis of the previous research, taking the Proportion Surviving as Input Type, S1(Proportion Surviving - Control) = 0.783, S2(Proportion Surviving - Treatment) = 0.90, T0 (Survival Time) = 5 years, Accrual Time (Integers Only) = 2 years, uniform enrolment, the rate of shedding in each group is 10%, the calculation can be calculated to obtain a total sample size of 317 cases, 158 cases in the treatment group and 159 cases in the control group. At the same time, there are five clinical trial centres in this study, according to the sample size estimation requirements of multifactorial analysis, the introduction of one more confounding factor needs to increase the sample size of each group by 15-25 cases, and this study considers an increase of 20 cases in each group. Therefore, 178 cases need to be collected in each group, and the total sample size of the two groups is 356 cases (Table 2).

Table 2. Sample size estimation for each centre.

research centre	treatment group	control group	total
Shanghai Municipal Hospital of Traditional Chinese Medicine	38	38	76
Shanghai Pulmonary Hospital	35	35	70
Shanghai Chest Hospital	35	35	70
Shanghai General Hospital	35	35	70
Shanghai TCM-Integrated Hospital	35	35	70
total	178	178	356

1.5 Subject Withdrawal

Trial suspension refers to the stopping of a clinical trial in the middle of a trial that has not yet been completed as planned. The purpose of trial suspension is mainly to protect the rights and interests of the subjects, ensure the quality of the trial and avoid unnecessary economic losses. Discontinuation of the trial is required in the following cases: 1. Serious safety problems occurred in the trial, the trial should be promptly suspended; 2. The trial found that the drug treatment effect is too poor, or even ineffective, does not have clinical value, the trial should be discontinued, on the one hand, to avoid delaying the effective treatment of subjects, while avoiding unnecessary economic losses; if there is a disease progression or serious adverse reactions can be initiated by the withdrawal of the protection mechanism, i.e., the inclusion of evaluable cases, the later assessment of efficacy data to invalid for carry-over. The lack or loss of efficacy is assessed by the investigator (not the subject). 3. Significant errors in the clinical trial protocol are identified during the trial, making it difficult to evaluate drug effects; or a well-designed protocol has undergone important deviations in its implementation, and further continuation makes it difficult to evaluate drug effects; 4. The sponsor requests suspension (e.g., financial reasons, management reasons, etc.); 5. Withdrawal of the trial by the administrative authority, etc.

1.6 Compliance

We will record the number of medications returned by participants to calculate utilisation rates. We will take measures to improve adherence, including asking them to complete a statement as part of the informed consent process, emphasising the importance of completing the study.

2 Trial Procedures

2.1 Stratified randomisation and blind implementation

The stratified random grouping scheme used in this study was prepared by a statistician. The entire study population that met the inclusion criteria was allocated according to the stratified random grouping scheme. The sample size for each stratum was a multiple of the group size. The stratification factors were different sub-centres. A teacher of medical statistics, who was not related to the data management and statistical analysis of this trial, generated a random table through Statistical Product and Service Solutions (SPSS) software, and the test group: control group was randomly coded in a ratio of 1:1 using the block randomisation method, and the length of the selected block (block) and the random seed parameter, etc., were sealed together as confidential data in a blinded bottom. After blinding, the blinders of the statistical unit handed over the sealed blinds to the unit in charge of the clinical study, including the first-level blinds and

second-level blinds made according to the random number, and the blinds were sealed in duplicate at the unit in charge of the clinical study.

A two-level blinded design was used, with the first level being the group corresponding to each case number (e.g., group A or group B) and the second level being the treatment corresponding to each group (test or control). The randomised coding table was created by the statistical unit, and the two levels of blinded bottoms were individually sealed in duplicate and stored in the team leader's unit and in the quality control office, respectively. When all the case report forms (CRFs) were entered into the database, and after questioning, verification, and blinding audit, the data were locked (Locked), and the first unveiling of blinding (i.e., clarifying Groups A and B) was carried out by the staff member who kept the blinded bottoms, which was handed over to biostatisticians to enter into the computer, and then statistically analysed after linking up with the data files. When the statistical analyses were completed a second blinding was carried out to clarify the test and control groups.

Blinding may be broken in emergency situations when the investigator believes that knowledge of the medication taken by the subject is beneficial for the management of adverse events. This should be done by the investigator and a detailed record of the reason, time and place of blind breaking should be recorded and signed. Within 24 hours of the blinding, the responsible (lead) unit of the clinical trial and the clinical supervisor as well as the statistically relevant personnel should be notified and the reason for the blinding should be explained. Once the contingency letter has been opened, the numbered subject will be withdrawn from the trial and treated as a dropout case, and the investigator should record the reason for withdrawal on the case report form. All contingency letters will be retrieved at the end of the trial along with the case report form for blinded audit at the end of the trial.

2.2 Method of administration

Chemotherapy regimen selection: chemotherapy regimens will be selected according to NCCN guidelines, and patients will be treated with platinum-based chemotherapy according to one of the following regimens for 3 weeks each for 4 cycles: (1) NP regimen: vincristine, 25mg/m2 on day 1, cisplatin, 75mg/m2 on day 1; (2) GP regimen: gemcitabine, 1,250mg/m2 on days 1 and 8, and 75mg/m2 on day 1; (3) DP regimen: docetaxel, 75 mg/m2 on day 1 and cisplatin, 75 mg/m2 on day 1; (4) AP regimen: pemetrexed, 500 mg/m2 and cisplatin, 75 mg/m2 on day 1; and (5) TC regimen: paclitaxel, 200 mg/m2 on day 1 and carboplatin, area under the curve (AUC) 6 on day 1, and patients intolerant of cisplatin will be given area under the curve (AUC) 5-6 on day 1 (AUC) 5-6 given carboplatin. All chemotherapeutic agents will be administered intravenously in both groups. The chemotherapeutic agents were administered strictly according to the appropriate instructions and NCCN guidelines for recommended dosage, duration and usage.

For oral administration of traditional Chinese medicine, each bag was soaked in 400 ml of boiling water at 100°C for 20 minutes, divided into 2 portions, each with a content of 200 ml. 200 ml was taken orally twice a day, in the morning and in the evening.

2.3 Test period

Chinese medicine treatment period is 6 months. Follow-up period: 3 years after the end of dosing. After the end of the programme, follow-up will continue until 5 years or until the subject dies (or progresses).

2.4 Discontinuation Criteria

First, during the course of medication, if the subject develops an aggravation of the condition

he/she should discontinue the use of the test drug, complete the post-evaluation of the efficacy and the relevant laboratory tests to end the trial, and the subject will be counted as an invalid eligible case for inclusion in the PPS. Second, if the investigator believes that other circumstances make it inappropriate for the subject to continue using the test drug.

2.5 Data Collection and Management

2.5.1 Data management system

All the original data in the case report form will be backed up and entered into the database by two people, proofread each other and correct the entry errors, and after checking the accuracy with the original data in the case report form, the data will be locked and then statistically analysed. Construct a high-quality clinical research database, incorporate it into the unified management of the big data platform of the Oncology Research Centre, and conduct long-term tracking and follow-up of the research subjects.

2.5.2 Establishment of Data Steering Committee

The subject leader, statisticians and clinical scholars will work together to formulate standard treatment protocols, design trial protocols, organise the formulation and approval of various standard operating procedures, organise the formulation and approval of research plans, approve and decide whether to continue the trial, as well as approve the dissemination of trial results and write reports on the results.

2.5.3 Establishment of a Data Safety and Monitoring Board (DSMB)

Investigators (clinicians, not involved in the inclusion of participants) and statisticians were responsible for designing the CRF, assessing the adequacy of the participant recruitment process, evaluating data quality, outcome safety assessment, outcome and effect assessment, recommending continuation of the trial, and reviewing and approving the dissemination of the results and the draft report: a database administrator (full-time) was responsible for the management of the database; an ombudsman was responsible for the monitoring of the data: a data entry clerk 2 persons responsible for data entry.

2.5.4 Data capture and database management

Accurately collect and record process management information data and resultant measurement indicators and inspection data of observation items. A two-person, two-track entry into the electronic case information system (EDC) system is used, and after the entry is completed, the double data are checked and logical consistency verified. The database uses EDC for data management.

2.5.5 Data security and monitoring

Data monitoring before the start of the clinical trial is generally on-site verification; real-time online monitoring and online quality control of the EDC system during the trial; the final data monitoring is carried out after the completion of the trial data collection, and the external data materials are reviewed and accepted.

2.5.6 Database Locking

After the completion of the interim analysis of the clinical trial and the data monitoring at the end of the trial, report to the DSMB, the subject leader, with a list of data monitoring, and lock the database after obtaining a written signature of approval.

2.6 Outcomes

2.6.1 Baseline data observation

Demographic data: gender, age, height, weight, etc.; Vital signs: such as blood pressure, heart rate,

respiration, etc.; General clinical data: co-morbidities and medications, etc.; Other data: such as education level, marital status, etc.

2.6.2 Baseline tumour assessment and risk factors

Time of diagnosis, pathological type, degree of differentiation, tumor node metastasis (TNM) classification of tumour, gene mutation status, etc.; Comorbidities; Surgical procedure, history of preoperative adjuvant therapy; Smoking status; family history of illness.

2.6.3 Safety indicators

Blood routine; Liver and kidney functions: alanine aminotransferase (ALT), menthionine aminotransferase (AST), glutamyl transpeptidase (γ-GT), blood creatinine (CREA), urea nitrogen (BUN), urinary protein (UPr), etc.; Electrocardiogram; Adverse events and serious adverse events (detailed records at any time). The above examinations were tested once before the test, once every month during the treatment period, and once every 6 months during the follow-up period.

2.6.4 Efficacy indicators

Main efficacy indicator is DFS. Refers to the time between the start of randomisation and disease recurrence or death (from any cause). Imaging: Chest CT examination before the start of treatment and every 6 months after surgery. Patients found to have recurrent metastases were discharged from the group for standardised treatment when the pathological diagnosis was clear. Observation and follow-up until 5 years after surgery, disease-free survival rate, disease-free survival and median survival calculation. Secondary efficacy indexes includs OS, MRD, Circulating tumour cellS detection, TCM symptom score, QoL, Immune indicators, Tumour markers, SII, PIN, PNI.

OS: Endpoint indicator, defined as the time from the start of randomisation to death due to any cause. Overall survival and median survival calculations were performed with observational follow-up up to 5 years postoperatively. MRD: Second-generation sequencing NGS method is used to detect MRD in peripheral blood of the study subjects to obtain the superior population screening model for TCM treatment; Circulating tumour cell detection: Detect 1 time each before and after intervention. Specimen collection method: 10ml of venous blood was taken from the patient's median elbow vein with EDTA blood collection tube, the blood collection tube was repeatedly inverted and mixed, centrifuged at 2000g for 10min, the supernatant was retained, and in order to further remove the redundant cellular components, it should be centrifuged again at 8000g for 10min. separated plasma was frozen and stored at -80℃ or extracted immediately according to the extraction kit instruction steps respectively. The remaining blood cells should be added to saline to replenish 10ml and then immediately isolate the circulating tumour cells. TCM symptom score: According to the "Shanghai TCM disease diagnosis and treatment routine", 10 TCM symptoms of cough, sputum, chest pain, chest tightness, shortness of breath, fatigue, loss of appetite, insomnia, dry mouth and throat and spontaneous sweating were observed, and the severity was scored according to 0-3, which was recorded once before and 6 months after the intervention. Significant effect: the reduction of TCM symptom score is greater than or equal to 70% after the intervention; Effective: the reduction of TCM symptom score is greater than or equal to 30% and less than 70% after the intervention; Ineffective: the reduction of TCM symptom score is less than 30% after the intervention or elevated compared with the previous one. Quality of life assessment: The Quality of Survival Scale for Lung Cancer Patients EORTC QLQ-LC43 was used, which consists of EORTC QLQ-C30 (core scale) and EORTC QLQ-LC13 Th (characteristic subscale of lung cancer). It mainly scores lung cancer patients on 5 domains related to functioning, general clinical symptoms, characteristic sub-symptoms, general health status and

financial difficulties, and is recorded once before and 6 months after the intervention. The higher the score of function-related domains, the worse the function of the organism; the higher the score of general clinical symptoms and characteristic sub-symptoms, the more serious the symptoms; the higher the score of general health status, the better the status; the higher the score of economic hardship status, the more difficult the economic hardship. Immune indicators: Cellular immunity (CD3, CD4, CD8, CD16, CD56), regulatory T cells, myeloid-derived suppressor cells MDSCs, natural killer cells NK, IL-1, IL-2, IL-6, IL-8, IL-10. 1 test before and 6 months after intervention. Tumour markers: CEA, CA125, CA153, Cyfra21-1, SCC before and after treatment. At least 1 test before intervention and 6 months after intervention. SII and PIN: The inflammatory response, immune status and nutritional status of the body affect the tumour microenvironment and prognosis of tumour patients. Studies have shown that tumour-associated inflammation, especially host-associated systemic inflammation, is closely related to tumour development and progression, as well as survival of cancer patients. SII is a comprehensive immune-inflammatory index, calculated based on the results of platelet, neutrophil, and lymphocyte counts. SII is a more objective predictor of the prognosis of patients with various types of tumours. It represents the balance between inflammation and immunity in tumour patients. Inflammation, in turn, can influence tumour progression by affecting the host's immunity and response to anti-tumour therapy. PNI is a nutritional prognostic indicator that can be used to reflect the nutritional and immune status of tumour patients and is calculated from serum albumin and circulating lymphocyte counts. PNI is a nutritional prognostic indicator that can be used to reflect the nutritional and immune status of tumour patients. Malnutrition is very common in patients with malignant tumours, especially advanced tumours Day plays a crucial role in the process of tumour progression. There is also a relationship between tumour-associated inflammation and nutritional status. Studies have shown that tumour-secreted inflammatory mediators, including tumour necrosis factor (TNF) and IL-6, lead to a loss of appetite and affect nutritional intake. And nutritional and immune status is associated with tumour progression and prognosis. The formula is as follows: SII=peripheral blood platelet count (*10/L) x peripheral blood neutrophil count (*10/L)/peripheral blood lymphocyte count (*10%/L). PNI:Serum albumin(g/L)+Lymphocyte count(10/L)*5. Critical values were calculated based on ROC curves, and based on the critical values, the above indexes were divided into high level and low level groups.

2.7 Safety Assessments

2.7.1 Safety background information related to the medicines used in the trial

According to the preclinical study, clinical trial information and drug composition, it is necessary to pay attention to the observation of the changes in the haematopoietic system, digestive system and urinary system of the patients during the trial, such as the number of red blood cells, haemoglobin content, blood creatinine and urea nitrogen, liver function and so on.

2.7.2 Observation and recording of adverse events

Observation and recording: The investigator should carefully observe any adverse events occurring in the subjects during the clinical study period, and require the subjects to truthfully reflect the changes in their condition after the administration of the drug, avoiding induced questions. While observing the efficacy, pay attention to the observation of adverse events or unanticipated toxic side effects (including symptoms, signs and laboratory tests). Regardless of whether the adverse event is related to the test drug or not should be recorded in detail in the CRF form, including the time of the appearance of the adverse event, symptoms, signs, degree, duration,

laboratory test indexes, treatment methods, after, results, follow-up time, etc., and should be recorded in detail in the case of the combined use of medication, in order to analyse the relevance of the adverse event to the test drug, the record should be signed and dated. Medical treatment of subjects: When adverse events are detected, the investigator may take necessary treatment measures according to the condition, such as adjusting the dosage, temporarily interrupting the medication, etc., and decide whether to terminate the trial. In the event of serious adverse events, the unit undertaking the trial study must immediately take the necessary treatment measures to protect the safety of the subjects.

2.7.3 Judgement of severity

Mild: mild discomfort, the subject can tolerate does not affect the treatment, do not need special treatment, no effect on the subject's recovery. Medium: moderate discomfort, intolerable to the subject, requiring special treatment, and having a direct impact on the subject's recovery. Severe: severe discomfort, endangering the life of the subject, death or disability, requiring immediate emergency treatment.

2.7.4 Judgement of causal relationship with drugs

Indicators of causal judgement in the determination of adverse events include: 1. whether the time of initiation of the drug is reasonably related to the appearance of the suspected adverse reaction; 2. whether the suspected adverse reaction corresponds to the type of adverse reaction known for the drug; 3. whether the suspected adverse reaction can be explained by the effect of the combination of the drugs, by the patient's clinical condition, or by the effect of other therapies; 4. whether the suspected adverse reaction disappears or is alleviated by discontinuation or reduction of the dose; and 5. whether the suspected adverse reaction disappears or is alleviated after re-exposure to the drug. Whether the same reaction recurs after re-exposure to the suspected drug. Criteria for determining causality: according to the above 5 judgement indicators in order (Table 3). Based on Table 4, determine the relationship between the following 5 levels of adverse events and the medicinal product: 1-certainly related, 2-maybe related, 3-cannot be determined, 4-maybe not related, 5-certainly not related. The incidence of adverse events was calculated using the total number of cases 1+2+3 as the numerator and all the enrolled cases available for adverse event evaluation as the denominator.

Table 3. Adverse event causation judgements.

Judgement results	Judgement indicators				
	1	2	3	4	5
Certainly relevant	+	+	-	+	+
Possibly relevant	+	+	-	+	?
Undetermined	+	+	±	±	?
Possibly irrelevant	+	-	±	±	?
Definitely irrelevant	-	-	+	-	-

Description: + affirmative, - negative, ± difficult to affirm or deny, ? Circumstances unknown.

2.7.9 Reporting and Handling of Adverse Events

The occurrence of any adverse event, such as the subjective discomfort of patients and abnormal laboratory tests, should be taken seriously, carefully analysed, and immediate measures should be taken to protect the safety of the subjects. Record in detail in the CRF form and retest in 24 hours

and 7 days and 14 days as appropriate. Record its persistence, regression and disappearance. Handling of Serious Adverse Events: Any serious adverse event that occurs during the trial must be reported immediately to the Medical Ethics Committee of the lead unit or the principal investigator by completing the Serious Adverse Event Report Form (SARF), and in the case of serious adverse reactions, to the State Food and Drug Administration (SFDA) within 24 hours. Notify the telephone number and contact person of the unit listed in the CRF form. When a patient has an emergency, the principal investigator of the research unit can open the emergency letter of the corresponding number taken by the patient (but there must be two witnesses present, and make corresponding records), according to the drug and the symptoms of the appropriate treatment, and notify the clinical monitor of the results of the treatment, and the researcher should be recorded in the case report form in detail on the reason for the breakthrough of blinding, date, treatment, results and sign. All adverse events should be followed up until they are properly resolved or the condition is stabilised. The method of follow-up can be inpatient, outpatient, home visit, telephone, or newsletter, depending on the type and severity of the adverse event.

Statistical Analysis

Statistical software such as SPSS 25.0 and GraphPad Prism were used for data analysis and statistical graphing in this study, with a test level of α taken as 0.05 and two sided test, giving point estimates and 95% confidence intervals.

Case data collectively: full analysis set (FAS): all cases who were randomised, took the study drug at least once and had at least one post-dose efficacy assessment constituted the FAS population of the study.Missing data in the efficacy-related part of the FAS population will be supplemented using a carry-over of data from the last previous observation.FAS will be used for the All analyses. Compliance with the protocol set (PPS), the criteria for the PPS and its population will be finalised in the data and will include at least the following criteria: 1. Compliance with the enrolment criteria set out in the trial protocol; 2. Completion of all scheduled visits; 3.No medication or treatment used during the trial that could have affected the evaluation of efficacy. The PPS is a secondary population for the evaluation of efficacy in this study.

Measurement data obeying normal distribution were expressed as mean and standard deviation; two independent samples t-test was used for inter-group differences, and paired t-test was used for pre- and post-treatment comparisons within the group; if the data as a whole did not obey the normal distribution of measurement data, the median (1st quartile, 3rd quartile) was used, Wilcoxon rank-sum test was used for inter-group differences, and paired samples rank-sum test was used for pre- and post-treatment comparisons within the group; count data were expressed as frequencies and numbers; and PPS was the population of this study. and test; count data were expressed as frequency (n), constitutive ratio (%), and rate (%), and differences between groups were tested by chi-square test (Fisher's exact probability test was used if the chi-square test was not satisfied); grade data were expressed as frequency and constitutive ratio, and differences between groups were tested by the Wilcoxon rank-sum test for independent samples.

Survival data DFS, OS were obtained using the Kaplan-Meier method and by plotting Kaplan-Meier curves to provide a visually intuitive depiction of differences between treatment groups. The estimation of treatment effect will be expressed by the hazard ratio (HR) and its 95% Confidence interval (CI) estimated by stratified COX model. The dataset included in the statistics is based on Intention-to-treat (ITT) analysis.

Discussion

Lung cancer is the leading malignant tumour in China in terms of deaths, and metastasis is the key cause. With the advances in diagnostic and treatment technologies (e.g., postoperative chemotherapy, targeted therapy, immunotherapy), the survival time and quality of life of postoperative patients have been improved to some extent. However, there is still a lack of effective standardised diagnostic and treatment protocols for postoperative radical lung cancer patients with negative driver gene expression, which has led to this specific group of patients becoming a challenge in the clinic that restricts the improvement of the overall efficacy of lung cancer, and at the same time, causes the society and families to incur huge medical costs.

This research programme inherits the theoretical advantages of Chinese medicine's "the Holistic Concept" and the thought of "Precentive Treatment of Diease", inherits the academic thought of "Treating Cancer by Reforcing Healthy Qi", and gives full play to the advantages of systems biology, Precision medicine and Chinese medicine. Based on the theory of "Deficiency of Vital Qi and Hidden Toxin", the research target is located in IIA-IIIA stage IIA-IIIA lung cancer patients who are negative for the expression of driver genes after radical surgery, and carry out a multi-centre clinical study on the combination of chemotherapy with Chinese medicine to prevent and control the recurrence and metastasis of IIA-IIIA stage IIA-IIIA driver gene-negative NSCLC after radical surgery. The study clarified the comprehensive efficacy of TCM in regulating the internal environment of the body's immune function state to reduce recurrence and metastasis, with a view to obtaining high-level evidence-based medical evidence and forming a standardised diagnostic and treatment protocol of TCM for preventing and treating recurrence and metastasis of postoperative cancer that can be promoted and applied, so as to promote the improvement of the therapeutic efficacy of postradical treatment of lung cancer patients. Secondly, based on the results of the study, the advantageous population of TCM for the prevention of recurrence and metastasis was screened out by combined MRD detection, and histopathology, circulating tumour cells, tumour-associated exosomes, immune indexes and other related indexes of postoperative patients with stage IIA-IIIA lung adenocarcinoma who were negative for the expression of driver genes were detected, and the therapeutic efficacy prediction model of postoperative patients with stage IIA-IIIA lung adenocarcinoma who were negative for the expression of driver genes was jointly established, so as to To improve the precise treatment level of lung cancer after radical surgery and promote the overall prevention and control efficiency of lung cancer.

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List of Abbreviation

TCM, traditional Chinese medicine

NCCN, National Comprehensive Cancer Network

DFS, disease-free survival

OS, overall survival

MRD, minimal residual disease

QoL, quality of life

SII, systemic immune- inflammation index

PIN, prognostic nutritional index
NSCLC, non-small cell lung cancer
PNI, pharmaceutical news index
TBIL, blood bilirubin,
AST, aspartate aminotransferase
CREA, creatinine
WBC, white blood cell count
PLT, platelet count
HCT, erythrocyte pressure
PS, performance staus.
ALT, alanine aminotransferase
AST, menthionine aminotransferase
γ-GT, glutamyl transpeptidase
BUN, urea nitrogen
UPr, urinary protein
SPSS, Statistical Product and Service Solutions
CRFs, the case report forms
EDC, electronic case information system
DSMB, data safety and monitoring board
TNM, tumor node metastasis
TNF, tumour necrosis factor
SARF, the Serious Adverse Event Report Form
SFDA, the State Food and Drug Administration
FAS, full analysis set
PPS, compliance with the protocol set
HR, the hazard ratio
ITT, Intention-to-treat
GCP, Good Clinical Practice

Availability of Data and Material

The results of our study will be presented at relevant scientific conferences and/or magazines. The raw trial data will be published on the China Clinical Trial Registration website within 6 months of trial completion.

Ethics Approval and Consent to Participate

The trial will be conducted in accordance with Good Clinical Practice (GCP) guidelines; it will be supervised and managed by The Ethics Committee of Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine. The investigational plan was approved by Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine (approval no.: 2023SHL-KY-19-01). All participants would sign the informed consent before joining the trial.

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