International Journal of General Medicine

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

The Synergistic and Attenuated Mechanism of Action of the Xihuang Pill in Dual Immunotherapy After Stenting for Advanced Cholangiocarcinoma: A Controlled Clinical Trial

Peng Wang[1,2], Yu-Huan Wang[2], Yun Tao[1], Xiang-Long Zheng[2], Wan-Chun Wang[3]

[1]Department of Interventional and Vascular Surgery, Affiliated Hospital of Jiangnan University, Wuxi, People's Republic of China; [2]Clinical Medical College, Jiangxi University of Chinese Medicine, Nanchang, People's Republic of China; [3]Department of Traditional Chinese Medicine Surgery, Affiliated Hospital of Jiangxi University of Chinese Medicine, Nanchang, People's Republic of China

Correspondence: Wan-Chun Wang, Department of Traditional Chinese Medicine Surgery, Affiliated Hospital of Jiangxi University of Chinese Medicine, 445 Bayi Avenue, Nanchang City, Jiangxi, 330006, People's Republic of China, Email wwjx7469108@163.com

Purpose: This study aims to investigate the synergistic and attenuation mechanism of Xihuang pill in the treatment of cholangiocarcinoma (CCA), thereby providing a reliable scientific basis for the selection of postoperative treatment strategies in cholangiocarcinoma patients.

Methods: In total, 120 patients with advanced CCA who underwent stent implantation were divided into control group I (n = 40), control group II (n = 40), and observation group (n = 40). The patients in control group I were only treated with a tumor immunosuppressant (tislelizumab injection), the patients in control group II were administered tumor double immunotherapy (tislelizumab injection + thymalfasin injection), and the patients in the observation group were treated with Xihuang pill combined with tumor double immunotherapy. The therapeutic effect, side effects, coagulation function, tumor markers, and immune function were compared among the three groups.

Results: Compared to the patients in control groups I and II, those in the observation group showed significantly longer activated partial thromboplastin time (APPT) and prothrombin time (PT), and lower fibrinogen (FIB) levels and platelet count (PLT) after treatment ($P < 0.05$). In the observation group, the levels of CD3+, CD4+, and CD4+/CD8+ increased, but the level of CD8+ decreased. The levels of CEA, CA125, CA19-9, CA242, and CA50 in serum decreased. The adverse reactions in the observation group were lower, while the objective remission rate (ORR) was significantly higher than their corresponding values in control groups I and II (42.5%vs17.5%, 27.5%) ($P < 0.05$). The 1-year overall survival rates of the control group I, control group II and observation group were 42.5%, 50% and 60%, and the difference was not statistically significant ($P > 0.05$).

Conclusion: Xihuang Pill combined with dual immunotherapy can synergistically enhance anti-tumor efficacy and reduce treatment-related toxicity in patients with advanced CCA by regulating coagulation function and immune mechanisms.

Keywords: cholangiocarcinoma, stent implantation, Xihuang pill, immunotherapy, enhanced efficacy and attenuated toxicity

Introduction

Cholangiocarcinoma (CCA) is a malignant tumor originating from the epithelial cells of the bile duct. A study showed that the global incidence of CCA increased by 25% between 2007 and 2017.[1] The incidence of CCA in China has also increased considerably in the last few years. The early stage of the disease has no specific symptoms, and most patients diagnosed are at an advanced stage. The treatment of advanced CCA mainly includes minimally invasive intervention, radiotherapy, chemotherapy, immunosuppressive agents, and targeted combination therapy. Interventional therapy is promising and is the main method of treating elderly and sick patients and those with poor general conditions, refractory CCA, and recurrent jaundice after resection. After solving the problem of cholestasis by internal and external biliary

Received: 5 December 2024
Accepted: 6 May 2025
Published: 12 June 2025

drainage and biliary stent angioplasty, intra-arterial catheter infusion chemotherapy is a mature scheme for treating CCA.[2,3] However, chemotherapy is also associated with serious toxic effects and side effects, poor tolerance of patients, and drug resistance of tumors caused by long-term chemotherapy, which often fails to yield the expected results.[4] As tumors often lack blood supply, interventional embolization of tumor blood supply arteries can be combined with percutaneous transhepatic cholangio-drainage (PTCD), thermal ablation, cryoablation, radioactive particle implantation, and other technologies to administer synergistic treatment.[5,6] Such interventions may delay disease progression and prolong survival. However, postoperative fever, abdominal pain, fatigue, embolism syndrome, low appetite, liver function damage, and even failure often occur.

Immunosuppressive agents/checkpoint inhibitors, particularly, programmed cell death protein (PD-1)/programmed cell death protein receptor 1 (PD-L1), are effective in treating malignant tumors. They manipulate the immune system of the patient to target cancer antigens or overcome the barrier of T-cell infiltration.[7,8] PD-1 inhibitor has high affinity, long-lasting stability, and high target occupancy rate, and thus, is suitable for treating CCA. By binding to PD-1 and blocking the interaction of PD-1 with PD-L1 and PD-L2, PD-1 monoclonal antibodies can relieve the immunosuppressive effect, activate the function of T cells, enhance immune surveillance, increase the ability of T cells to kill tumor cells, and produce tumor immune response. However, PD-1 drugs have adverse reactions.[9–11] Thymosin is injected to strengthen the immunity of the patient, and administering it can lead to viral remission (loss of serum HBV-DNA) and normalization of aminotransferase levels. In some patients who respond to this treatment, serum surface antigen can be removed effectively.

Traditional Chinese medicine (TCM) has a unique effect in the treatment of advanced CCA. According to TCM, the etiology and pathogenesis of this disease include the accumulation of dampness and heat, prolonged stagnation of qi and liver depression, the change in heat and fire, and the stagnation of fire into poison. Liver stagnation is worsened by spleen, transport, and chemical disorders, endogenous phlegm dampness, dampness and heat condensation, liver enlargement, liver dispersion disorders, influence, and biliary excretion function disorders. These problems increase the chances of bile overflow and jaundice. The Chinese patent medicine "Xihuang pill" is a detoxicant and detumescent, composed of bezoar, artificial musk, frankincense, and myrrh. It can remove heat and toxic substances and also inhibit tumor growth and spread. It is used for treating cancer caused by heat and toxicity. It is administered as adjuvant therapy, as it can improve the clinical symptoms of patients with advanced cancer and improve the quality of life.[12]

The combination of PD-1 inhibitors, thymalfasin injection, and Xihuang pill may yield synergistic antitumor effects. Previous studies have demonstrated that Xihuang pills, either as monotherapy or in conjunction with other anticancer agents, have achieved promising outcomes in the treatment of solid tumors such as breast cancer, esophageal cancer, ovarian cancer, and primary liver cancer. Yu D and An GY reported that the combination of Xihuang pills with chemotherapy increased the objective response rate (ORR) to 46.88% in patients with advanced rectal cancer.[13] However, the potential efficacy of this TCM compound in combination with immunotherapy for patients with advanced CCA after percutaneous transhepatic insertion of biliary stent (PTIBS) remains unclear. Therefore, this study proposes an innovative therapeutic hypothesis: the triple regimen of Xihuang pill combined with tirelizumab injection and thymosin injection may represent a promising treatment approach for advanced CCA. This study was designed to evaluate the clinical efficacy and safety of this combination regimen in patients with advanced CCA after PTIBS. The findings from this study are anticipated to provide a novel theoretical foundation for the development of combined immunotherapy strategies for advanced CCA and offer valuable insights for future treatment protocols.

Methods

Participants

This study is a prospective, single-center, randomized controlled clinical trial. From October 2023 to March 2024, a total of 120 patients with advanced CCA after interventional therapy were recruited. The patients were randomly divided into three groups (control group I, control group II and observation group) at a ratio of 1:1:1, with 40 cases in each group. Patients flow diagram is shown in Figure 1. The study was approved by the Ethics Committee of the Affiliated Hospital

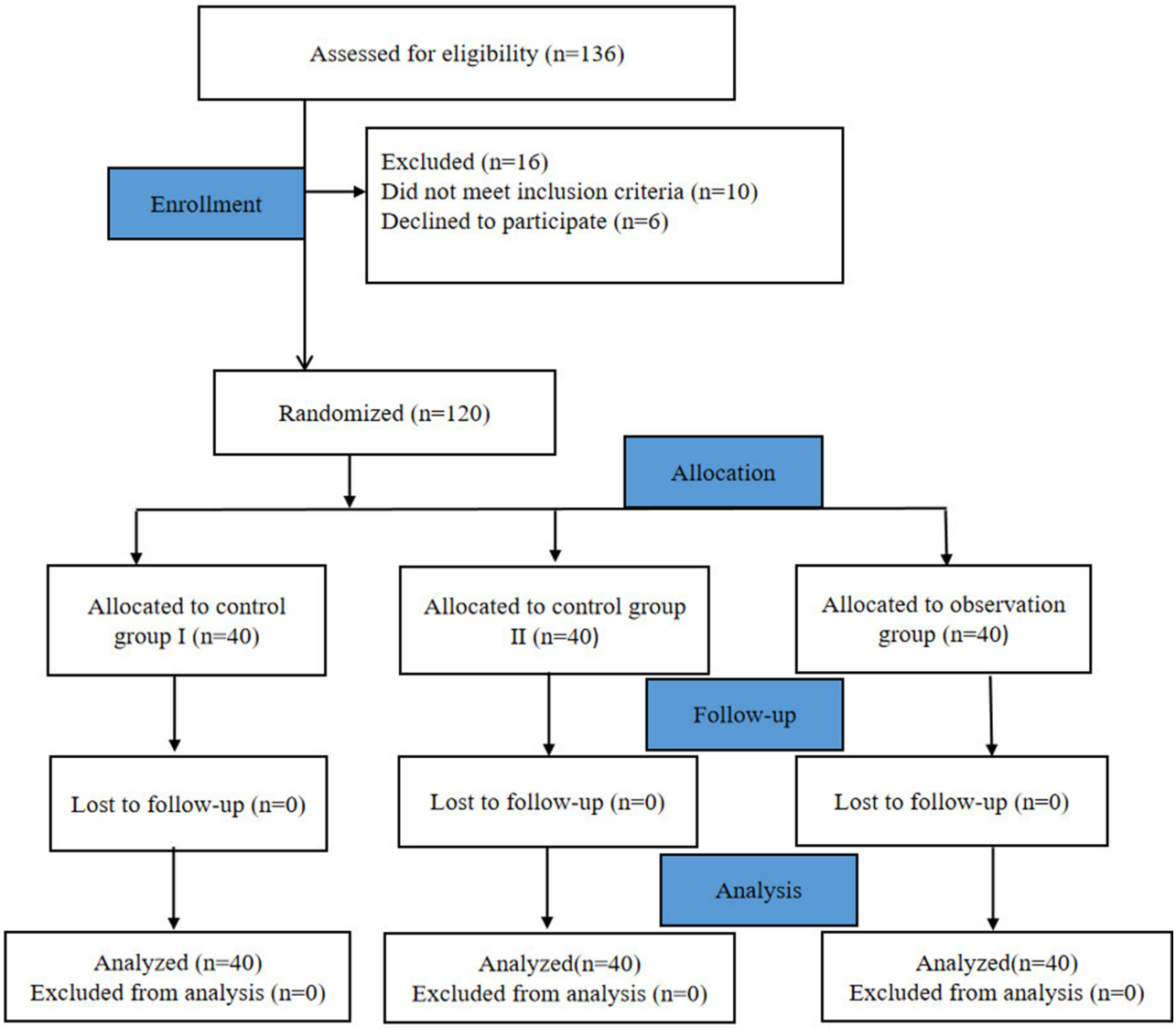

Figure 1 Patients flow diagram.

of Jiangnan University and complies with the Declaration of Helsinki. This study was registered in the Chinese Clinical Trial Center (registration number: ChiCTR2300076873, date: 23/10/2023).

The inclusion criteria were as follows: (I) TNM stage IV according to the American Joint Committee on Cancer (AJCC) 2010; (II) TCM syndrome differentiation classified as damp heat accumulation syndrome; (III) expected survival time of at least three months; (IV) meeting the requirements of therapeutic indications; (V) Karnofsky score ≥60.

(V) The exclusion criteria were as follows: (I) patients with serious diseases of the heart, lungs, liver, kidneys, and other organs; (II) complicated with other malignant tumors; (III) age < 18 years; (IV) history of drug allergy or contraindication.

Treatment Options

Patients in the control group I were only treated with tumor immunosuppressive agents. Tislelizumab (Sinopharm Huaizi S20190045, 100 mg, Guangzhou Baiji Shenzhu Biopharmaceutical) was administered via intravenous infusion, and its recommended dose was 200 mg every three weeks until disease progression or until intolerable toxicity occurred.

Patients in the control group II were administered tumor double immunotherapy (tislelizumab injection + thymalfasin injection). Tislelizumab (Sinopharm Huaizi S20190045, 100 mg, Guangzhou Baiji Shenzhu Biopharmaceutical) was administered via intravenous infusion, and its recommended dose was 200 mg every three weeks until disease progression or until intolerable toxicity occurred. Thymalfasin injection (HJ20171177, 1.6 mg, Patheon Italia S.p.A) was subcutaneously injected at a dose of 1.6 mg twice a week at intervals of 3–4 days. The treatment was administered for four weeks (eight injections in total).

Observation group: Xihuang pill was administered in combination with double immunotherapy. Xihuang Pill (Tongrentang Pharmaceutical, batch number 20130619). Xihuang Pill was administered orally at a dose of 3 g, twice a day. The pill was administered for four weeks.

General Information Questionnaire

The investigator developed a self-designed general information questionnaire, which included data on demographic characteristics (such as gender, age, BMI, smoking, drinking, etc.) and clinical symptoms (KPS score and Child-Pugh classification).

Evaluation Indicators

Coagulation Function

Before and after treatment, 5 mL of fasting venous blood was collected from the patients. Then, activated partial thromboplastin time (APPT), prothrombin time (PT), and fibrinogen (FIB) were detected using an LB-2A automatic blood coagulation analyzer (Jinan Hanfang Medical Equipment Co., Ltd.), and platelet count (PLT) was detected using an HF-3800P blood cell analyzer (Jinan Hanfang Medical Equipment Co., Ltd.)

Immune Function

From each patient, 5 mL of fasting venous blood was collected before and after treatment. After incubating for 1 h at room temperature, the serum was separated by centrifugation. A FACSCanto II flow cytometer was used to detect peripheral blood T lymphocyte subsets CD3 +, CD4+, and CD8+, and the CD4+/CD8+ ratio was calculated.

Tumor Markers

Before and after two months of treatment, 5 mL of fasting venous blood was collected from the patients in the three groups and centrifuged at 3500 r/min for 10 min. Then, the upper serum solution was stored in a low-temperature refrigerator at −80 °C. Serum CEA, CA125, CA19-9, CA242, and CA50 levels were analyzed within 4 h of collection.

Side Effects

The occurrence of gastrointestinal reactions, thrombocytopenia, leukopenia, hemoglobin reduction, liver toxicity, hypothyroidism, rash, fatigue, and other symptoms during the treatment were recorded.

Clinical Efficacy

According to the RECIST solid tumor response evaluation criteria, the maximum long diameter and vertical diameter of the tumor were measured by contrast-enhanced CT. Complete remission (CR): the lesions disappeared and were absent for ≥4 weeks. Partial remission (PR) was defined as a reduction of ≥30% in the sum of the maximum and long diameters of the lesions and maintained for ≥4 weeks. Stable disease (SD): the sum of the maximum and long diameters of the lesions decreased by less than 30% or increased by less than 20%. Progression (PD): the sum of the maximum and long diameters of the lesions increased by ≥20% or new lesions appeared. Objective response rate (ORR) = (CR+ PR)/total cases ×100%. Disease control rate (DCR) = (CR+PR+SD)/total cases ×100%.

Statistical Analysis

The data were statistically processed by SPSS 26 (IBM SPSS, USA), and count data were expressed as rate (%) and analyzed by the χ^2 test. Continuous data were expressed as the mean ±standard deviation, and the differences among the three groups were determined by a one-way analysis of variance (ANOVA). The differences between the data before and

after treatment in each group were determined by paired sample *t*-tests. The survival curve was drawn by Kaplan-Meier, and the difference was compared by Log rank test. All differences among and between groups were considered to be statistically significant at $P < 0.05$.

Results

Basic Demographic Characteristics of the Patients

The differences in gender, age, BMI, KPS, primary tumor site, and Child-Pugh classification among the three groups were not significant ($P > 0.05$), indicating that the basic demographic characteristics of the three groups were similar (Table 1).

Comparison of Coagulation Function Indices Before and After Treatment in the Three Groups

After treatment, the APPT and PT increased significantly, whereas the FIB level and PLT decreased significantly in the observation group ($P < 0.05$). The coagulation function indices of the control groups I and II were not significantly different before and after treatment ($P > 0.05$) (Table 2).

Table 1 Basic Demographic Characteristics of the Patients

	Control Group I (n=40)	Control Group II (n=40)	Observation Group (n=40)	F/χ^2	P
Gender				0.205	0.903
Male	26 (65.0)	21 (52.5)	23 (57.5)		
Female	14 (35.0)	19 (47.5)	17 (42.5)		
Age (years)	62.88±7.24	63.75±5.58	63.58±6.04	0.214	0.807
BMI	20.60±2.48	21.03±2.39	21.29±2.70	0.782	0.460
KPS	70.38±6.49	70.45±5.53	71.68±6.16	0.577	0.563
Primary site				1.297	0.862
Intrahepatic	25 (62.5)	21 (52.5)	24 (60.0)		
Porta hepatis	8 (20.0)	10 (25)	10 (25.0)		
Extrahepatic	7 (17.5)	9 (22.5)	6 (15.0)		
Child Pugh classification				0.933	0.627
A	27 (67.5)	23 (47.5)	26 (65.0)		
B	13 (32.5)	17 (27.5)	14 (35.0)		
Smoking				0.753	0.686
No	32 (80.0%)	31 (77.5%)	34 (85.0%)		
Yes	8 (20%	9 (22.5%)	6 (15.0%)		
Alcohol drinking				0.84	0.657
No	35 (87.5%)	33 (82.5%)	32 (80%)		
Yes	5 (12.5%)	7 (17.5%)	8 (20%)		

Table 2 Comparison of Coagulation Function Indexes Before and After Treatment in the Three Groups

		Control Group I (n=40)	Control GroupII (n=40)	Observation Group (n=40)	F	P
Pre-treatment	APPT(s)	30.17±2.35	29.67±1.66	29.52±1.43	1.307	0.275
	PT(s)	11.56±1.95	12.20±1.76	11.87±2.16	1.056	0.351
	FIB(g/L)	3.81±0.89	3.79±0.81	3.85±1.00	0.032	0.969
	PLT($\times10^9$/L)	261.65±60.76	267.95±51.46	272.77±56.82	0.390	0.678

(*Continued*)

https://doi.org/10.2147/IJGM.S496375

Table 2 (Continued).

		Control Group I (n=40)	Control GroupII (n=40)	Observation Group (n=40)	F	P
Post-treatment	APPT(s)	29.65±2.00	30.10±1.71	32.67±1.74*■▲●	31.997	0.000
	PT(s)	11.21±1.46	11.71±1.37	13.59±1.68*■▲●	27.727	0.000
	FIB(g/L)	3.90±0.76	3.87±0.74	3.21±0.85*■▲●	9.696	0.000
	PLT($\times 10^9$/L)	244.35±54.62	250.78±48.99	208.30±52.52*■▲●	7.726	0.001

Notes: Compared with Pre-treatment, *P < 0.05; Compared with Control group I, ■P< 0.05; Compared with Control group II, ▲P< 0.05; Comparison between groups, ●P < 0.05.

Comparison of Immune Function Indices Before and After Treatment in the Three Groups

After treatment, the levels of CD3+, CD4+, and CD4+/CD8+ in the three groups increased, but the levels of CD8+ decreased, and their corresponding levels were significantly different from those recorded before treatment (P < 0.05). Among them, the levels of CD3+, CD4+, CD8+, and CD4+/CD8+ in patients who received the combination of the Xihuang pill and tumor immune double therapy were higher than those of patients who did not receive the combination therapy (P < 0.05) (Table 3 and Figure 2).

Comparison of Tumor Markers Before and After Treatment in the Three Groups

The levels of CEA, CA125, CA19-9, CA242 and CA50 in the three groups after treatment were significantly lower than their respective levels before treatment, and the levels of CEA, CA125, CA19-9, CA242 and CA50 in the observation group after treatment were significantly lower than their respective levels in the control groups I and II (P < 0.05) (Table 4 and Figure 3).

Comparison of Adverse Reactions Among the Three Groups

The incidence of hemoglobin reduction, liver toxicity, hypothyroidism, and fatigue in the observation group was higher than that in the control groups I and II. The difference between the two groups was not significant (P > 0.05). The incidences of leukopenia, thrombocytopenia, gastrointestinal reaction, and rash in the observation group were significantly lower than those in the control groups I and II (P < 0.05) (Table 5).

Table 3 Comparison of Immune Function Indexes Before and After Treatment Among the Three Groups

		Control Group I (n=40)	Control GroupII (n=40)	Observation Group (n=40)	F	P
Pre-treatment	CD3+	50.87±4.28	51.13±4.14	51.03±4.31	0.038	0.963
	CD4+	32.45±2.80	31.97±2.37	32.66±2.32	0.793	0.455
	CD8+	33.06±1.56	32.47±1.31	32.91±1.57	1.693	0.188
	CD4+/CD8+	0.98±0.09	1.00±0.08	1.00±0.09	0.260	0.771
Post-treatment	CD3+	54.14±4.83*	57.16±4.15*■	62.91±4.84*■▲●	37.264	0.000
	CD4+	35.52±3.07*	39.02±2.48*■	44.64±3.31*■▲●	95.705	0.000
	CD8+	32.23±1.54*	31.22±1.40*■	31.16±1.72*■●	5.950	0.003
	CD4+/CD8+	1.11±0.11*	1.25±0.11*■	1.44±0.16*■▲●	67.379	0.000

Notes: Compared with Pre-treatment, *P < 0.05; Compared with Control group I, ■P< 0.05; Compared with Control group II, ▲P< 0.05; Comparison between groups, ●P < 0.05.

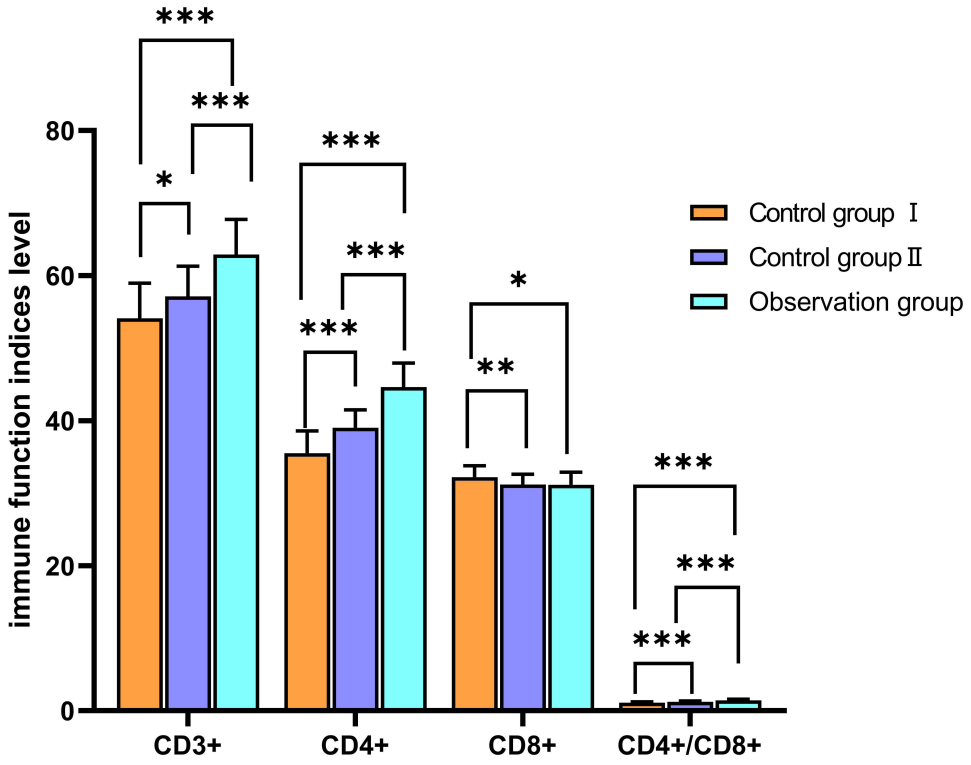

Figure 2 The level of immune indexes after treatment in three groups. (*P<0.05, **P<0.01, ***P<0.001).

Comparison of Clinical Efficacy Among the Three Groups

The ORR of the observation group was significantly higher than that of the control groups I and II (42.5%vs17.5%, 27.5%), and the difference between the three groups was significant (P < 0.05). The difference in the DCR among the three groups was not significant (P > 0.05) (Table 6).

Table 4 Comparison of Tumor Markers Before and After Treatment in the Three Groups

		Control Group I (n=40)	Control GroupII (n=40)	Observation Group (n=40)	F	P
Pre-treatment	CEA (ng/mL)	27.08±8.73	25.63±10.89	26.36±8.82	0.230	0.795
	CA125(U/mL)	69.61±12.83	70.28±15.63	69.79±23.54	0.015	0.985
	CA19-9(U/mL)	356.00±55.87	361.10±46.78	359.49±32.18	0.129	0.879
	CA242(U/mL)	74.61±14.40	74.80±12.94	76.65±14.18	0.265	0.768
	CA50 (μg/L)	59.74±16.12	58.51±19.98	60.09±18.79	0.081	0.922
Post-treatment	CEA (ng/mL)	20.02±8.85*	16.12±9.52*▪	10.93±5.99*▪▲•	12.186	0.000
	CA125(U/mL)	61.01±10.03*	56.99±13.54*▪	45.86±15.61*▪▲•	14.020	0.000
	CA19-9(U/mL)	301.64±42.64*	283.92±43.55*▪	229.36±33.77*▪▲•	35.075	0.000
	CA242(U/mL)	61.73±12.24*	57.06±11.19*▪	53.77±11.98*▪•	4.583	0.012
	CA50 (μg/L)	51.66±13.83*	48.35±16.68*▪	36.13±10.18*▪▲•	14.016	0.000

Notes: Compared with Pre-treatment, *P < 0.05; Compared with Control group I, ▪P< 0.05; Compared with Control group II, ▲P< 0.05; Comparison between groups, •P < 0.05.

https://doi.org/10.2147/IJGM.S496375

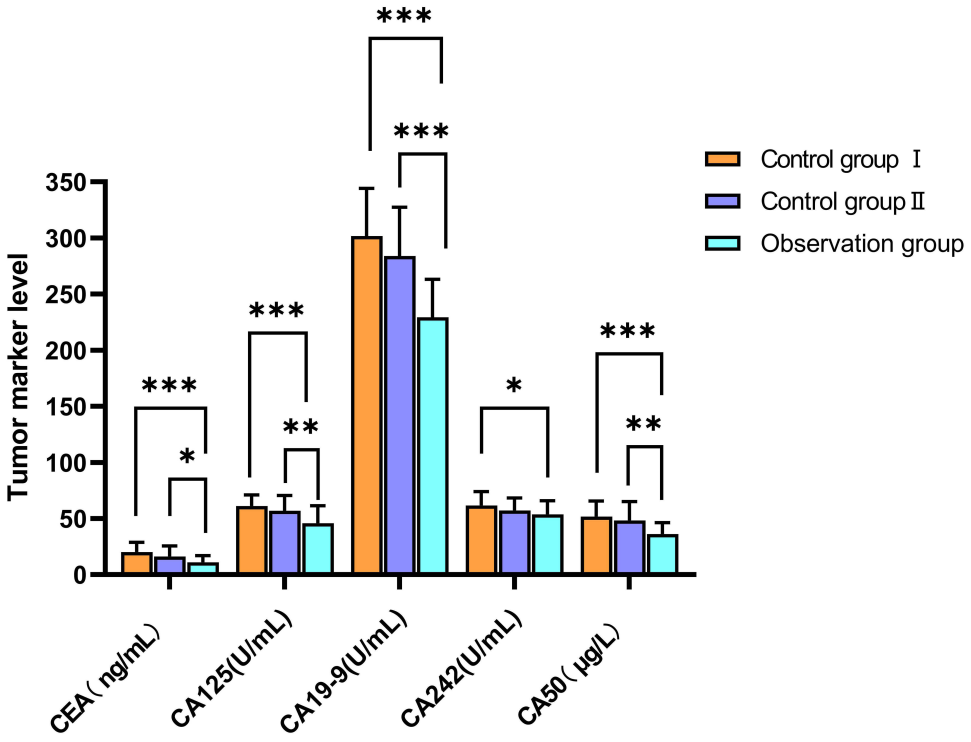

Figure 3 The level of tumor marker after treatment in three groups. (*P<0.05, **P<0.01, ***P<0.001).

Comparison of 1-year Overall Survival Rate Among Three Groups

The 1-year overall survival rates of the control group I, control group II and observation group were 42.5%, 50% and 60%, respectively, and the difference was not statistically significant (P > 0.05) (Figure 4).

Table 5 Comparison of Toxicity and Side Effects Among the Three Groups

	Control Group I (n=40)	Control Group II (n=40)	Observation Group (n=40)	χ^2	P
Leukopenia	10 (25.0)	7 (17.5)	2 (5.0)	6.128	0.047
Decreased hemoglobin	6 (15.0)	5 (12.5)	2 (5.0)	2.243	0.326
Thrombocytopenia	8 (20.0)	4 (10.0)	1 (2.5)	6.384	0.041
Gastrointestinal reactions	15 (37.5)	11 (27.5)	5 (12.5)	6.611	0.037
Liver toxicity	5 (12.5)	3 (7.5)	1 (2.5)	2.883	0.236
Hypothyroidism	8 (20.0)	6 (15.0)	2 (5.0)	4.114	0.127
Rash	7 (17.5)	4 (10.0)	0 (0.0)	7.406	0.025
Fatigue	9 (22.5)	6 (15.0)	3 (7.5)	3.502	0.174

Table 6 Comparison of Clinical Efficacy Among the Three Groups

	Control Group I (n=40)	Control GroupII (n=40)	Observation Group (n=40)	χ^2	P
CR	0	0	0		
PR	7 (17.5)	11 (27.5)	17 (42.5)	6.131	0.047
SD	12 (30.0)	11 (27.5)	9 (22.5)	0.597	0.742
PD	21 (52.5)	18 (45.0)	14 (35.0)	4.095	0.129
ORR	7 (17.5)	11 (27.5)	17 (42.5)•	6.131	0.047
DCR	19 (47.5)	22 (55.0)	26 (65.0)	2.501	0.286

Note: Comparison between groups, •P < 0.05.

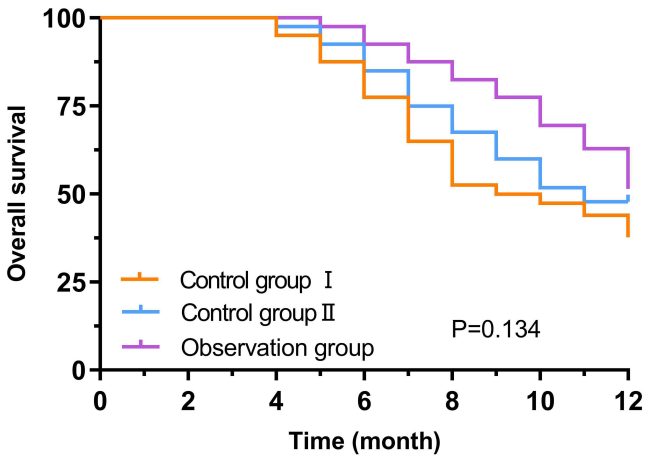

Figure 4 Kaplan-Meier:1-year overall survival rate of patients.

Discussion

The advancement in integrated traditional Chinese and Western medicine has facilitated the use of the Xihuang Pill in the treatment and adjuvant treatment of tumors. Many clinical studies have found that the Xihuang pill can be used for treating various solid tumors and hematopoietic system malignant tumors, which can decrease the toxicity of chemotherapy and radiotherapy, alleviate clinical symptoms and cancer pain caused by tumors, and prolong the survival of patients with advanced cancer.[12,14]

Xihuang pill is composed of four Chinese herbs, including bezou, musk, frankincense, and myrrh. It can clear heat and detoxify, promote blood circulation, eliminate blood stasis, decrease swelling, and relieve pain. Modern pharmacological studies have confirmed that the main components of the Xihuang pill have certain anti-tumor activity and can boost the immunity of patients. Bezoar contains bilirubin, bile acids, fatty acids, amino acids, and other components. It has sedative, anticonvulsant, antipyretic, analgesic, antihypertensive, and cardiovascular protection effects.[14] Some studies have shown that natural and artificial bezoar can increase the phagocytic function of monocytes/macrophages and strengthen humoral immunity.[15] Musk is a non-specific anti-inflammatory drug that can exert anti-inflammatory effects by inhibiting reactive lipid formation, reducing neutrophil release, and reducing free calcium levels. In addition, musk can also kill tumor cells by enhancing the body's non-specific immunity and inhibiting tumor cell respiration. Frankincense has the effects of anti-infection, promoting blood circulation, relieving pain and anti-tumor, while myrrh extract can inhibit platelet aggregation activity and play an anticoagulant role.

It has been reported that most patients with malignant tumors have a hypercoagulable state of blood, and interventional therapy may aggravate abnormal coagulation function in patients with malignant tumors and increase the risk of thrombosis.[16,17] Blood viscosity, platelet aggregation, and microcirculation disorders caused by the hypercoagulable state of blood are similar to those caused by blood stasis in traditional Chinese medicine. The dynamic changes in concentration, adhesion, aggregation, and coagulation are the common pathological basis of both diseases and occur throughout the process of tumor occurrence and progression.[18] Some studies have found that the development of "congestion" is an important pathogenesis of tumor metastasis.[19] Xihuang Pill, which activates congestion, can not only improve the blood hyperviscosity state of tumor patients but also effectively inhibit or kill tumor cells that escape from the primary lesion and enter the blood circulation; thus, it can prevent tumor invasion, metastasis, and damage to the body after tumor embolus formation.[20]

We found that after two months of treatment, APTT and PT in the observation group were significantly prolonged, and FIB and PLT decreased significantly, compared to that recorded in the control groups II and II. These findings indicated that the Xihuang Pill can inhibit platelet aggregation, decrease blood viscosity, and improve blood microcirculation disorders in patients with advanced CCA. Vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) play an irreplaceable role in tumor formation, metastasis, and invasion.[20,21] Studies have shown that the Xihuang Pill can inhibit proliferation and induce the apoptosis of Tumor Necrosis Factor-related Apoptosis-

https://doi.org/10.2147/IJGM.S496375

inducing Ligand (TRAIL) in human CCA cell line RBE. It can also inhibit the growth of tumor cells by affecting MMP-2 secreted by the liver cancer cells SMMC7721. The mechanism may be related to the upregulation of the expression of TRAIL death receptor in RBE cells by Xihuang pill-containing serum. It is noteworthy that the active ingredients of Xihuang Pill (eg, frankincense) may further synergistically enhance its apoptosis-inducing effects. Agrawal[22] et al showed that boswellic acid can increase the expression of Bcl-2-associated X protein (Bax) and cysteinyl aspartate proteolytic enzyme 3 (Caspase-3) and induce tumor cell apoptosis in mice with solid tumors.

T lymphocyte-mediated cellular immune dysfunction is common in patients with CCA, which is mainly manifested as the decrease in the number of T lymphocytes, the imbalance of T lymphocyte subsets (such as the decrease of CD4+/CD8+ ratio) and functional inhibition. This phenomenon may be related to the excessive secretion of immunosuppressive factors (such as TGF-β and IL-10) in the tumor microenvironment, which can inhibit the proliferation of T cells and induce the expansion of regulatory T cells (Tregs), eventually leading to immune escape. Immunotherapy has become an important means to improve the prognosis of malignant tumors by improving the immunogenicity of tumor cells and enhancing the sensitivity of effector cells to kill.[23,24] Among them, CD4+/CD8+ balance is an important indicator to evaluate the effect of immunotherapy for malignant tumors. The levels of CD3+, CD4+, and CD4+/CD8+ in the peripheral blood of the observation group increased, and the levels of CD8+ decreased after the treatment cycle ended, and the improvement was better than that of the control group. This finding indicated that the Xihuang Pill combined with immunotherapy can effectively regulate the imbalance of T lymphocyte subsets and restore the body ‘s anti-tumor immune response. A study found that the volatile oil of the Xihuang pill can help in the immune clearance function of tumor-bearing rats by upregulating the levels of interleukin-2 (IL-2) and interferon-γ (IFN-γ) in peripheral blood and the content of CD3+ T cells, CD8+ T cells, and B7-1 cells in the T lymphocyte population.[25] IL-2 plays a pivotal role in promoting T cell proliferation, while IFN-γ augments macrophage phagocytosis and antigen presentation, thereby enhancing antitumor immune responses.[26] Additionally, Xihuang Pill can also inhibit the PI3K/AKT signaling pathway in Tregs, reduce the number of Treg cells in tumor tissues, improve the immunosuppressive state in the tumor microenvironment, and reverse immune escape, thereby inhibiting tumor growth.[27–29]

CEA, CA125, CA19-9, CA242, and CA50 are important serological markers related to CCA. Combined detection can enhance the sensitivity and specificity of the diagnosis of advanced CCA and can help in evaluating the curative effect and prognosis of CCA. This finding suggested that the Xihuang pill can improve the coagulation and immune function of patients with advanced CCA and improve the ability of chemotherapeutic drugs to kill tumor cells.

We also found that the incidence of leucopenia, thrombocytopenia, gastrointestinal reaction, and rash in the observation group was significantly lower than that in the control groups I and II, suggesting that Xihuang pill can increase the effect of immune drugs, stimulate the death of tumor cells, alleviate the toxic side effects of chemotherapy, enhance immune function, decrease the symptoms of gastrointestinal discomfort, and improve the tolerance of patients.

Our study had some limitations. First, the sample size was small. Second, our study lacked the support of evidence-based medical research, and the assessment of its mechanism of action was not comprehensive. Specifically, the efficacy and mechanism of combination therapy need to be further studied based on evidence-based standards to objectively evaluate its anti-tumor effect.

Conclusion

Based on the concept of integrated traditional Chinese and Western medicine, this study innovatively proposed and validated the clinical value of Xihuang pill combined with a dual immunotherapy regimen (tislelizumab + thymalfasin injection) in patients with advanced CCA following stent implantation. This combination therapy exerts synergistic anti-tumor effects by modulating coagulation function and immune mechanisms, while also reducing toxic side effects and significantly improving the objective response rate. This synergistic and attenuated property reflects the unique advantages of integrated traditional Chinese and western medicine in the regulation of complex tumor microenvironment, which provides new ideas and strategies for the treatment of patients with advanced CCA.

Data Sharing Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement

The study was conducted per the Declaration of Helsinki and approved by Ethics Committee of Affiliated Hospital of Jiangnan University, and informed consent was taken from all the patients.

Funding

This project was approved and supported by the Wuxi Chinese Medicine Administration Science and Technology Project Plan (No. ZYYB07); and Jiangsu Medical Association Interventional Medicine Third Phase Research Special Fund (Project Approval No. SYH-3201140-0090, 2023037); and Jiangxi University of Chinese Medicine Science and Technology Innovation Team Development Program (No. CXTD22009); and High level talents of Affiliated Hospital of Jiangnan University, 2023; and Top Talent Support Program for young/middle-aged people of Wuxi Health Committee (BJ2023044 for H.Y.Q).

Disclosure

The authors have no conflicts of interest to declare for this work.

References

1. Roth GA, Abate D, Abate KH, et al. Global, regional, and national age - sex - specific mortality for 282 causes of death in 195 countries and territories, 1980 - 2017: a systematic analysis for theGlobal burden of disease study 2017. *Lancet*. 2018;392(10159):1736–1788.
2. Ottaiano A, Santorsola M, Diana A, et al. Treatments, prognostic factors, and genetic heterogeneity in advanced cholangiocarcinoma: a multicenter real-world study. *Cancer Med*. 2024;13(4):e6892. doi:10.1002/cam4.6892
3. Afshar M, Khanom K, Ma YT, et al. Biliary stenting in advanced malignancy: an analysis of predictive factors for survival. *Cancer Manag Res*. 2014;6:475–479. doi:10.2147/CMAR.S71111
4. Wang Y, Wei Z, Zhang Z, et al. Hepatic arterial infusion chemotherapy with or without lenvatinib for unresectable cholangiocarcinoma: a single-center retrospective study. *Hepatol Oncol*. 2023;10(2):HEP49. doi:10.2217/hep-2023-0006
5. Pang L, Wu S, Kong J. Comparison of efficacy and safety between endoscopic retrograde cholangiopancreatography and percutaneous transhepatic cholangial drainage for the treatment of malignant obstructive jaundice: a systematic review and meta-analysis. *Digestion*. 2023;104(2):85–96. doi:10.1159/000528020
6. Li ZM, Jiao DC, Han XW, et al. Preliminary application of brachytherapy with double-strand 125I seeds and biliary drainage for malignant obstructive jaundice. *Surg Endosc*. 2022;36(7):4932–4938. doi:10.1007/s00464-021-08848-6
7. Suriyo T, Fuangthong M, Artpradit C, et al. Inhibition of T-cell-mediated immune response via the PD-1/ PD-L1 axis in cholangiocarcinoma cells. *Eur J Pharmacol*. 2021;897:173960. doi:10.1016/j.ejphar.2021.173960
8. Qian T, Liu D, Cao G, et al. Neoadjuvant PD-1 plus chemotherapy for locally advanced esophageal squamous cell carcinoma. *Technol Cancer Res Treat*. 2024;23:15330338241231610. doi:10.1177/15330338241231610
9. Li T, Niu M, Zhou J, et al. The enhanced antitumor activity of bispecific antibody targeting PD-1/PD-L1 signaling. *Cell Commun Signal*. 2024;22 (1):179. doi:10.1186/s12964-024-01562-5
10. Li Y, Liang Z, Tian Y, et al. High-affinity PD-1 molecules deliver improved interaction with PD-L1 and PD-L2. *Cancer Sci*. 2018;109 (8):2435–2445. doi:10.1111/cas.13666
11. Viricel C, Ahmed M, Barakat K. Human PD-1 binds differently to its human ligands: a comprehensive modeling study. *J Mol Graph Model*. 2015;57:131–142. doi:10.1016/j.jmgm.2015.01.015
12. Chen Z, Li Z, Yang S, et al. The prospect of Xihuang pill in the treatment of cancers. *Heliyon*. 2023;9(4):e15490. doi:10.1016/j.heliyon.2023.e15490
13. Yu D, An GY. Clinical effects of Xihuang pill combined with chemotherapy in patients with advanced colorectal cancer. *Evid Based Complement Alternat Med*. 2017;2017(1):5936086. doi:10.1155/2017/5936086
14. Xu HB, Chen XZ, Wang X, et al. Xihuang pill in the treatment of cancer: TCM theories, pharmacological activities, chemical compounds and clinical applications. *J Ethnopharmacol*. 2023;316:116699. doi:10.1016/j.jep.2023.116699
15. Zheng A, Moritani T. Effect of the combination of ginseng, oriental bezoar and glycyrrhiza on autonomic nervous activity and immune system under mental arithmetic stress. *J Nutr Sci Vitaminol*. 2008;54(3):244–249. doi:10.3177/jnsv.54.244
16. Xiao B, Ma LL, Zhang SD, et al. Correlation between coagulation function, tumor stage and metastasis in patients with renal cell carcinoma: a retrospective study. *Chin Med J*. 2011;124(8):1205–1208.
17. Lind SM, Sletten M, Hellenes M, et al. Coagulation factor V in breast cancer: a p53-regulated tumor suppressor and predictive marker for treatment response to chemotherapy. *J Thromb Haemost*. 2024;22(6):S1538–7836(24)00106–5. doi:10.1016/j.jtha.2024.02.008
18. Xin QQ, Chen X, Yuan R, et al. Correlation of platelet and coagulation function with blood stasis syndrome in coronary heart disease: a systematic review and meta-analysis. *Chin J Integr Med*. 2021;27(11):858–866. doi:10.1007/s11655-021-2871-2
19. Jin L, Tang B, Liu X, et al. Blood stasis syndrome accelerates the growth and metastasis of breast cancer by promoting hypoxia and immunosuppressive microenvironment in mice. *J Immunol Res*. 2022;2022:7222638. doi:10.1155/2022/7222638
20. Zheng P, Huang Z, Tong DC, et al. Frankincense myrrh attenuates hepatocellular carcinoma by regulating tumor blood vessel development through multiple epidermal growth factor receptor-mediated signaling pathways. *World J Gastrointest Oncol*. 2022;14(2):450–477. doi:10.4251/wjgo.v14.i2.450

21. Teng YJ, Deng Z, Ouyang ZG, et al. Xihuang pills induce apoptosis in hepatocellular carcinoma by suppressing phosphoinositide 3-kinase/protein kinase-B/mechanistic target of rapamycin pathway. *World J Gastrointest Oncol*. 2022;14(4):872–886. doi:10.4251/wjgo.v14.i4.872
22. Agrawal SS, Saraswati S, Mathur R, et al. Antitumor properties of boswellic acid against Ehrlich ascites cells bearing mouse. *Food Chem Toxicol*. 2011;49(9):1924–1934. doi:10.1016/j.fct.2011.04.007
23. Gaggero S, Witt K, Carlsten M, et al. Cytokines orchestrating the natural killer-myeloid cell crosstalk in the tumor microenvironment: implications for natural killer cell-based cancer immunotherapy. *Front Immunol*. 2021;11:621225. doi:10.3389/fimmu.2020.621225
24. Ling SP, Ming LC, Dhaliwal JS, et al. Role of immunotherapy in the treatment of cancer: a systematic review. *Cancers*. 2022;14(21):5205. doi:10.3390/cancers14215205
25. Ma J, Wang YY, Yang W, et al. Experimental study on anti-tumor effect of xihuang pill and its immune clearance function. *Zhongguo Zhong Yao Za Zhi*. 2014;39(8):1499–1501.
26. Cheng L, Tang X, Xu L, et al. Interferon-γ upregulates Δ42PD1 expression on human monocytes via the PI3K/AKT pathway. *Immunobiology*. 2019;224(3):388–396. doi:10.1016/j.imbio.2019.02.009
27. Wang J, Hou D, Peng Y, et al. Xihuang pill induces pyroptosis and inhibits progression of breast cancer cells via activating the cAMP/PKA signalling pathway. *Medicine*. 2021;100(19):e25726. doi:10.1097/MD.0000000000025726
28. Li XY, Su L, Jiang YM, et al. The antitumor effect of Xihuang pill on treg cells decreased in tumor microenvironment of 4T1 breast tumor-bearing mice by PI3K/AKT~AP-1 signaling pathway. *Evid Based Complement Alternat Med*. 2018;2018(1):6714829. doi:10.1155/2018/6714829
29. Su L, Jiang Y, Xu Y, et al. Xihuang pill promotes apoptosis of Treg cells in the tumor microenvironment in 4T1 mouse breast cancer by upregulating MEKK1/SEK1/JNK1/AP-1 pathway. *Biomed Pharmacother*. 2018;102:1111–1119. doi:10.1016/j.biopha.2018.03.063