Study Protocol

A Multicenter, Randomized, Double-Blind, Parallel-Controlled Clinical Study of Jianpi Lishi Jiedu Granules for Preventing Postoperative Recurrence of Advanced Colorectal Adenomas

Study Period: March 2026 – February 2028

Version: V1.0

Version Date: February 5, 2026

Ethics Approval No.: KY20260305-07

1. Study Design, Methods, and Procedures

1.1 Study Type

This is a prospective, multicenter, randomized, double-blind, parallel-controlled clinical study.

1.2 Study Centers

Departments of Gastroenterology, Nanjing First Hospital; Nanjing Hospital of Traditional Chinese Medicine; Changzhou Traditional Chinese Medicine Hospital; Kunshan Traditional Chinese Medicine Hospital; Jiangsu Integrated Traditional Chinese and Western Medicine Hospital.

1.3 Study Population

Patients with advanced colorectal adenomas with TCM syndrome of Spleen Qi deficiency and damp-heat toxin, who underwent endoscopic resection between March 1, 2026, and December 31, 2026, at the study centers.

1.4 Sample Size Calculation

According to literature, the 1-year overall recurrence rate after endoscopic resection of advanced colorectal adenomas is 26.9%. Our preliminary small-sample clinical data show a 1-year recurrence rate of approximately 12.3% in the Jianpi Lishi Jiedu granule treatment group.

This randomized controlled trial has a treatment group receiving Jianpi Lishi Jiedu granules and a placebo control group. With one-sided $\alpha=0.05$ and power 0.9, 150 patients per group are required. Considering 20% potential loss to follow-up, at least 188 patients per group are needed, totaling 376 patients.

Patients meeting the inclusion/exclusion criteria will be randomized 1:1 into the treatment and placebo groups. Adenoma recurrence will be assessed via 1-year endoscopic follow-up.

1.5 Diagnostic Criteria

1.5.1 Advanced Colorectal Adenomas

Colorectal adenomas are benign tumors arising from colorectal mucosal epithelium, classified as tubular (<25% villous component), villous (>75% villous), and tubulovillous (25%-75% villous). Criteria from relevant guidelines are adopted. Diagnosis requires one or more of the following: diameter >10mm; villous or tubulovillous structure; high-grade intraepithelial neoplasia.

1.5.2 TCM Spleen Qi Deficiency with Damp-Toxin

Primary symptoms: constipation, loose stools, abdominal pain or distension, stool with blood, dark purple blood; Secondary symptoms: shortness of breath, reduced appetite, fatigue, rectal urgency. Tongue: pale or light red; coating: white or yellow greasy; pulse: weak or slippery. Diagnosis requires ≥2 primary and ≥2 secondary symptoms.

1.6 Inclusion/Exclusion Criteria

1.6.1 Inclusion

a. Aged between 18 and 80 years, male or female

b. Have undergone endoscopic resection of colorectal adenoma within the past 2 weeks (cold snare resection or endoscopic mucosal resection for adenomas < 20 mm, or endoscopic submucosal dissection for adenomas ≥ 20 mm), and have been diagnosed with colorectal advanced adenoma by endoscopy and pathology

c. Traditional Chinese Medicine (TCM) syndrome type conforms to the Spleen Deficiency and Dampness Toxin syndrome

d. The patient is fully informed, voluntarily consents to data collection, and has signed the informed consent form

1.6.2 Exclusion

a. Patients with diseases such as familial polyposis, inflammatory bowel disease, serrated polyposis syndrome, Peutz-Jeghers syndrome, or other hereditary polyposis syndromes

b. Patients with long-term use of medications such as aspirin, metformin, intestinal flora regulators, folic acid, calcium preparations, and vitamin D

c. Patients with a bleeding tendency (referring to a pathological state where hemostasis is difficult after spontaneous bleeding or minor trauma due to coagulation dysfunction or vascular structural issues) or those currently using anticoagulant medications (e.g., aspirin, clopidogrel)

d. Patients in the active phase of inflammatory bowel disease (such as ulcerative colitis, Crohn's disease, etc.), with a history of intestinal bleeding, or a history of intestinal surgery

e. Patients with high-grade intraepithelial neoplasia involving the vascular system, lymphatic tumor thrombi, or small arteries; patients with biopsy pathology confirming suspected malignancy or established malignancy

f. Pregnant or lactating women, or patients planning a recent pregnancy

g. Patients with an allergic constitution or a history of allergies to multiple drugs

h. Patients with severe heart, brain, lung, liver, kidney diseases, or mental disorders who cannot tolerate colonoscopy, treatment, and clinical intervention

i. Patients currently participating in other clinical trials or those previously involved in trials related to this study.

1.7 Exclusion from Analysis (Withdrawal Criteria)

a. Violation of inclusion/exclusion criteria

After enrollment, it is found that the subject does not meet the originally defined inclusion criteria or meets any of the exclusion criteria.

b. Never received the assigned treatment

After randomization, the subject did not receive any of the allocated study drug interventions.

c. Major protocol violation

Including but not limited to:

(1) Extremely poor treatment compliance: The subject did not receive treatment according to the protocol requirements, and the actual medication intake was lower than the minimum compliance standard specified in the protocol.

(2) Incorrect intervention: The subject mistakenly received treatment assigned to the other study group.

(3) Missing key visits/examinations: Failure to complete the colonoscopy examination required at the study endpoint, resulting in the inability to evaluate the primary endpoint.

d. Withdrawal of informed consent with request for data deletion

The subject explicitly withdraws the informed consent during the study and clearly requests that all previously collected data not be used for research analysis.

e. Data falsification or inaccuracy

If the data provided by the subject are found to be false or inaccurate, affecting the scientific validity and reliability of the study.

f. Unexpected major unblinding

Before completion of the study, the subject or investigator inadvertently or unnecessarily becomes aware of the subject's group allocation, and the Study Steering Committee determines that this seriously compromises the blinding principle and credibility of the study results.

g. Lack of primary endpoint data

Due to complete loss to follow-up, refusal to provide key information, or loss of records, resulting in complete absence of primary efficacy or safety endpoint data.

1.8 Criteria for Study Termination

1.8.1 Criteria for Early Termination of the Study

a. Major errors are identified in the clinical trial protocol during the study, making evaluation of the study drug difficult.

b. Severe adverse events occur that are likely related to the investigational intervention, suggesting potential significant safety concerns of the study drug, and the trial should therefore be terminated in a timely manner.
c. External evidence (such as other high-quality studies or reliable evidence) demonstrates that the intervention is either ineffective or already proven effective, making continuation of the current clinical trial unnecessary.
d. The National Medical Products Administration (NMPA) or the Ethics Committee orders termination of the study for any reason.

1.8.2 Withdrawal of Subjects from the Study

Withdrawal of a subject refers to situations in which the subject is unable to continue completing the visits specified in the protocol due to early termination criteria, loss of contact, or other reasons. This includes but is not limited to:
a. The subject meets the criteria for early termination described in Section 1.8.1 and cannot complete subsequent visits.
b. The subject cannot be contacted at the scheduled visit time according to the study protocol and is confirmed to be unable to attend any further visits.
c. The subject permanently leaves the original place of residence and cannot be contacted.

Investigators must inform subjects that they have the right to withdraw from the study at any time. Subjects who withdraw from the study will no longer receive the investigational drug or follow-up visits. Investigators should make every effort to contact subjects who fail to return for scheduled visits.

All data collected before the subject's withdrawal may still be used for analysis. The original records and Case Report Forms (CRFs) must document the date of withdrawal and the possible reason for withdrawal, with detailed explanations of the specific circumstances, including: Serious adverse event; Adverse event (non-serious); Protocol violation; Voluntary withdrawal not related to adverse events; Travel, relocation, or moving away from the study site; Lost to follow-up; Death; Other reasons

Subjects who withdraw due to adverse events must be clearly distinguished from those who withdraw for other reasons. For subjects who have received the investigational drug and subsequently withdraw or terminate participation, investigators should provide necessary treatment for any study-related clinical conditions and follow up adverse events/serious adverse events until a clear diagnosis is established, the condition stabilizes, recovery occurs, or the subject returns to baseline status. Definition of withdrawal time: The time when the investigator decides to terminate the subject's participation or when the subject voluntarily withdraws from the study. Definition of completion of the clinical study: A subject is considered to have completed the clinical study when all protocol-specified visits have been completed.

1.8.3 Lost to Follow-up and Protocol Deviation/Violation

a. Handling of Lost to Follow-up Subjects

If a subject is physically capable of providing study data but refuses to cooperate or declines to provide data related to the study, investigators should make every effort to persuade the subject while fully respecting the subject's rights. At a minimum, investigators should attempt to determine the subject's health status and document all efforts made, as well as any efficacy or safety data that can be collected.

b. Protocol Deviation / Protocol Violation

Protocol deviation refers to any modification or failure to follow the design or procedures of the clinical trial protocol without approval from the Ethics Committee. If the deviation does not affect the subject's rights, safety, or benefits, nor the integrity, accuracy, or reliability of the study data, and does not influence safety assessment or evaluation of primary endpoints, it is classified as a protocol deviation. If the deviation affects the subject's rights, safety, or benefits, or compromises the integrity, accuracy, or reliability of the study data, or influences the assessment of safety or primary endpoints, it is considered a major protocol deviation (protocol violation). For any protocol violation or deviation, investigators must promptly report the event to the Principal Investigator and the Ethics Committee, closely monitor the

involved subjects, collect safety information, ensure subject safety, and maintain detailed documentation.

1.9 Treatment Plan

1.9.1 Study Drug

(1) Treatment Group: Jianpi Lishi Jiedu Granules

Composition and Specification: Codonopsis, stir-fried Atractylodes, Smilax, Forsythia, Lonicera Stem, Swertia, in the ratio 15:8.3:7.5:4.5:4.6:2.

Manufacturing and Quality Control: Prepared uniformly by Beijing Kangrentang Pharmaceutical Co., Ltd. All batches share the same batch number, and a quality inspection report is provided for each batch (including appearance, particle size, solubility, microbial limits, and assay of main active components).

(2) Control Group: Placebo Granules

Appearance, Color, Odor, and Packaging: Identical to the treatment group.

Composition: Starch, maltose, caramel coloring, dextrin, and other inactive excipients; contains no active TCM ingredients.

Manufacturing and Quality Control: Produced by the same company using the same batch process as the treatment group and verified by consistency testing (appearance, dissolution, taste in blind test).

1.9.2 Randomization and Blinding

a. Assignment of Subject Identification Number

After signing the informed consent, each subject is assigned a unique identification number used to track them throughout the study. This ID must remain unchanged during the trial. Each study site assigns numbers in the format PISTIAS-XX-YYY, where “XX” is the site number and “YYY” is the patient number at that site; this number may differ from the randomized number.

b. Randomization

Block randomization stratified by center is applied. Randomization is performed

via a central randomization system, assigning eligible participants 1:1 to the Jianpi Lishi Jiedu granules group or the placebo group.

c. Blinding

Unrelated staff will blind both the study drug and placebo according to the randomization numbers. Each clinical site will dispense drugs in the order of patient enrollment. Two sealed copies of the blinding code are kept at the sponsor and Principal Investigator's national clinical research center.

d. Emergency Unblinding

In the event of a serious adverse event, the investigator at the site will contact the lead center to perform emergency unblinding, obtain the subject's group allocation, and take necessary medical measures. The subject is considered a withdrawal case, but any adverse reactions will still be included in safety analysis.

e. Blinding Procedure

A two-stage unblinding method is applied. After blind verification and data lock, the first unblinding is performed by the Principal Investigator, medical statistician, data manager, and sponsor representative. Each random number is coded as A or B for statistical analysis. The second unblinding occurs after statistical analysis and summary report completion to reveal the actual group allocation.

1.9.3 Drug Dispensation and Management

a. Randomization and Packaging: Random codes are generated by an independent statistician. Drugs are packaged according to the codes, with each subject receiving an individually packaged dose.

b. Double-Blind Maintenance: Each package bears a unique random number without group information. Investigators, subjects, outcome assessors, and statisticians remain blinded.

c. Dispensation and Return: Drugs are dispensed at each 4-week (±7 days) visit for the next treatment period, and remaining drugs and empty packets are collected.

Medication Compliance Calculation:

Compliance (%)=Actual intake/Prescribed amount×100% (Based on sachet counts)

1.10 Intervention Protocol

a. Treatment Group: Oral Jianpi Lishi Jiedu Granules, 1 sachet per dose, twice daily (1 hour after breakfast and dinner), for 3 months.

b. Control Group: Oral placebo granules (same dosage, appearance, and administration as treatment), for 3 months.

Subjects start medication after resuming diet post-advanced colorectal adenoma resection (≥2 weeks).

1.11 Concomitant Medication and Prohibitions

a. Allowed Standard Treatment:

(1) Routine postoperative care (e.g., dietary guidance, fluid therapy).

(2) Standard therapy for comorbidities (e.g., antihypertensives, antidiabetic medications) should maintain stable type and dose. All relevant drug information (name, dose, frequency) must be recorded. If drugs are discontinued during study or follow-up, the date and reason must be documented.

b. Prohibited Medications:

(1) Other anti-tumor TCM preparations.

(2) Chemopreventive drugs for colorectal adenomas (e.g., aspirin, metformin, probiotics, folate, calcium, vitamin D).

1.12 Standardization of Base Treatment

a. Endoscopic Procedures:

Colonoscopy and endoscopic colorectal polyp resection are performed by trained senior physicians (Associate Chief or above). Qualified bowel preparation is required (Boston score ≥8), withdrawal time ≥6 minutes, with detailed documentation of number, size, location, and morphology of polyps. Ensure no missed polyps, complete removal of specimens, and fixation in 4% formaldehyde for pathological examination.

b. Postoperative Standard Management:

Lifestyle and dietary education: avoid spicy, greasy, and heavy foods, avoid staying up late, and encourage appropriate exercise.

1.13 Compliance Control

a. Minimum Compliance Standard:

b. For inclusion in Per-Protocol (PP) analysis, medication intake ≥80%.

Handling Low Compliance:

(1) 70–80% compliance: provide additional education and record as protocol deviation.

(2) <70% compliance: assess reason; if due to non-medical causes and persists, classify as major protocol violation.

1.14 Assessment Indicators and Time Points

Study Stage	Screening Period (-14 to 0 days)	Baseline (Day 0)	Treatment Period (3 months ±7 days)	Follow-up (6 months ±14 days)	Follow-up (1 year ±1 month)
Visit	1	2	3	4	5
General information (age, sex, ethnicity, occupation, height, weight)	✓				
Informed consent	✓				
Present medical history	✓				
Medication history (antiplatelet drugs such as aspirin, clopidogrel, indobufen, ticagrelor, cilostazol; anticoagulants such as warfarin, rivaroxaban, dabigatran; metformin; probiotics; folic acid; calcium; vitamin D)	✓				
Past medical history (inflammatory bowel disease, intestinal bleeding history, intestinal surgery history,	✓				

hypertension, diabetes, hyperlipidemia, coronary heart disease, smoking/drinking history, other diseases)						
Family history (hereditary polyposis syndromes such as familial adenomatous polyposis, inflammatory bowel disease, serrated polyposis syndrome, Peutz-Jeghers syndrome; family history of colorectal cancer)		✓				
Review of inclusion/exclusion criteria		✓				
Vital signs (respiration, heart rate, blood pressure)		✓	✓	✓	✓	✓
Randomization			✓			
Laboratory tests	Blood routine (WBC, RBC, HGB, PLT, NEUT%)	✓	✓	✓	✓	✓
	Liver and kidney function (ALT, AST, TBIL, ALP, GGT, Urea/BUN, Cr)	✓	✓	✓	✓	✓
	Fasting blood glucose	✓				
	Coagulation (PT, APTT, TT, FIB, INR)	✓	✓	✓	✓	✓
	Stool routine and occult blood	✓	✓	✓	✓	✓
	Urine routine and urine biochemistry	✓	✓	✓	✓	✓
	Urine pregnancy test	✓				
Colorectal adenoma characteristics (number, size, location, morphology, pathology type, intraepithelial neoplasia)		✓			✓	✓
Electrocardiogram		✓	✓	✓	✓	✓

TCM clinical symptom score		✓	✓	✓	✓
TCM syndrome efficacy evaluation		✓	✓	✓	✓
Karnofsky Performance Status (KPS) scale		✓	✓	✓	✓
Gastrointestinal Symptom Rating Scale (GSRS)		✓	✓	✓	✓
Medication compliance		✓	✓		
Concomitant medication	✓	✓	✓	✓	✓
Adverse events		✓	✓	✓	✓
Serum collection		✓	✓	✓	✓
Stool collection		✓	✓	✓	✓
Urine collection		✓	✓	✓	✓

2. Efficacy Indicators and Evaluation Criteria

2.1 Primary Outcome

The primary outcome is the recurrence rate of advanced colorectal adenomas. According to the Diagnostic Criteria and Therapeutic Effect Evaluation Standards of TCM Diseases, recurrence is defined as the detection of adenomas at any location during follow-up colonoscopy and pathological examination after complete adenoma removal at baseline. Follow-up colonoscopy is performed at: 6 months and 1 year. Data collected include: number of adenomas, size, location, morphology, pathological type, degree of intraepithelial neoplasia, Patient age and sex will also be recorded.

Recurrence rate calculation:

Recurrence Rate=Number of recurrence cases/Total number of cases×100%.

2.2 Secondary Outcomes

a. Malignant Transformation and Interval Cancer

Malignant transformation refers to the process in which a benign colorectal adenoma gradually transforms into colorectal adenocarcinoma under the influence of genetic mutations and environmental factors. Diagnosis relies on colonoscopic biopsy pathology showing invasive carcinoma within the adenoma.

Interval cancer refers to colorectal cancer detected during the recommended surveillance interval after a previous colonoscopy that did not detect cancer (or after complete removal of polyps). Evaluation includes: adenoma malignant transformation rate at 1 year, malignant transformation risk.

Malignant transformation rate=Total number of patients/Number of malignant trans formation cases×100%

b. TCM Syndrome Efficacy

Based on: Clinical Guidelines for TCM Diagnosis and Treatment, Guidelines for Clinical Research of New TCM Drugs. Symptoms are graded into four levels: none, mild, moderate, and severe. Scores: 0 = none, 1 = mild, 3 = moderate, 5 = severe (Table 1). Tongue and pulse signs are used for diagnosis but not included in scoring.

Efficacy index calculation:

Efficacy Index=Scorebefore-Scoreafter/Scorebefore×100%

Total effective rate:

Total Effective Rate=Clinical cure+Markedly effective+Effective/Total cases×100% (Table 2)

Table 1. TCM Clinical Symptom Scoring Standard

Symptom	None (0)	Mild (1)	Moderate (3)	Severe (5)
Abdominal distension/pain	None	<3 times/week	3–7 times/week	Daily
Constipation	None	<3 times/week	3–7 times/week	Daily
Diarrhea	None	unformed stool <3/day	Loose stool <3/day	Watery ≥6/day
Poor appetite	None	<3 times/week	3–7 times/week	Daily
Dry or sticky mouth	None	<3 times/week	3–7 times/week	Daily
Fatigue	None	<3 times/week	3–7 times/week	Daily
Heaviness and	None	<3 times/week	3–7 times/week	Daily

fatigue of body				
Nausea/vomiting	None	<3 times/week	3–7 times/week	Daily
Borborygmus	None	<3 times/week	3–7 times/week	Daily

Table 2. Evaluation Criteria for TCM Syndrome Efficacy

Outcome	Symptom Change	Efficacy Index
Clinical cure	Symptoms disappear or nearly disappear	≥95%
Markedly effective	Symptoms almost disappear	70-95%
Effective	Significant improvement	30-70%
Ineffective	No improvement or worsening	< 30%

c. Quality of Life Assessment

Quality of life will be evaluated using the Karnofsky Performance Status (KPS) scale. Higher scores indicate better quality of life. Improvement classification: Significant improvement: increase ⩾20 points, Improvement: increase 10 – 20 points, Stable: change <10 points, Deterioration: decrease ⩾10 points

Total improvement rate:

Improvement Rate=Significant improvement+Improvement/Total cases × 100%(Table 3)

Table 3. Karnofsky Performance Status (KPS) scale

Score	Description
100	Normal, no symptoms
90	Able to carry on normal activity with minor symptoms
80	Normal activity with effort
70	Self-care but unable to work
60	Occasional assistance required
50	Considerable assistance required
40	Disabled, requires special care
30	Severely disabled
20	Very ill
10	Moribund
0	Death

d. Intestinal Function Indicators

The Gastrointestinal Symptom Rating Scale (GSRS) will be used to evaluate gastrointestinal symptoms during the previous week. 15 items total, 5 symptom domains, Each item scored 0 – 3, Higher scores indicate more severe gastrointestinal symptoms (Table 4).

Table 4. Gastrointestinal Symptom Rating Scale (GSRS)

Item	Scoring Criteria
Abdominal pain	0: No pain or transient pain 1: Occasional pain affecting some social activities 2: Prolonged pain requiring treatment and affecting many social activities 3: Severe pain affecting all social activities
Heartburn	0: No or transient heartburn 1: Occasional short-lasting heartburn 2: Frequent prolonged discomfort requiring treatment 3: Persistent discomfort relieved only temporarily by antacids
Acid regurgitation	0: None or transient reflux 1: Occasional unpleasant reflux 2: Reflux once or twice daily requiring treatment 3: Reflux several times daily with only temporary relief from antacid therapy
Upper abdominal tightness	0: None or transient sensation of tightness 1: Occasional short discomfort without need for food or antacids between meals 2: Prolonged or frequent discomfort relieved by food or antacids between meals 3: Persistent discomfort requiring frequent food or antacids
Nausea and vomiting	0: No nausea 1: Occasional transient discomfort 2: Frequent prolonged nausea without vomiting 3: Persistent nausea with frequent vomiting
Borborygmus (abdominal rumbling)	0: None or transient rumbling 1: Occasional short discomfort 2: Frequent prolonged rumbling controllable with activity without affecting social activities 3: Persistent rumbling severely affecting social activities
Abdominal bloating	0: None or transient bloating 1: Occasional short bloating 2: Frequent prolonged bloating manageable by adjusting clothing 3: Persistent bloating significantly affecting social activities
Belching	0: None or transient belching 1: Occasional unpleasant belching

	2: Frequent belching affecting some social activities 3: Frequent belching severely affecting social activities
Increased flatulence	0: No increase in flatulence 1: Occasional short discomfort 2: Frequent prolonged discomfort affecting some social activities 3: Frequent episodes severely affecting social activities
Reduced defecation frequency	0: Once daily 1: Once every 3 days 2: Once every 5 days 3: Once every 7 days or less
Increased defecation frequency	0: Once daily 1: Three times daily 2: Five times daily 3: Seven times daily or more
Loose stool	0: Normal consistency 1: Slightly loose 2: Mushy stool 3: Watery stool
Hard stool	0: Normal consistency 1: Slightly hard 2: Hard stool 3: Hard segmented stool occasionally mixed with diarrhea
Urgency of defecation	0: Normal control 1: Occasional urgency 2: Frequent urgency affecting social activities 3: Fecal incontinence
Feeling of incomplete evacuation	0: No incomplete evacuation or straining 1: Occasional difficulty or incomplete evacuation 2: Definite difficulty with frequent feeling of incomplete evacuation 3: Severe difficulty with persistent sensation of incomplete evacuation

2.3 Safety Indicators and Other Data

a. Safety Indicators

(1) Drug-Induced Liver Injury (DILI). Evaluation will be conducted according to Hy's Law. Core criteria include: Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥ 3× the upper limit of normal (ULN). Total bilirubin ≥ 2× ULN. No evidence of biliary obstruction (e.g., alkaline phosphatase not significantly

elevated). Other causes of liver injury must be excluded, including: Viral hepatitis; Alcoholic liver disease; Autoimmune liver disease.

(2) Renal Function Impairment. Renal injury is defined as: Serum creatinine increased ≥50% compared with baseline

b. Other Collected Data

(1) General Information: Age, sex, ethnicity, occupation, height, weight.

(2) Factors Affecting Treatment Efficacy. Medication history, past medical history, family history, concomitant medication.

(3) Vital Signs: Respiration, heart rate, blood pressure.

(4) Adverse Event Causality. The relationship between adverse events and study drugs will be evaluated using the Naranjo Scale. Specific reactions related to traditional Chinese medicine (such as abnormal liver function or allergic reactions) must record: Time of onset, dose relationship, re-challenge results.

2.4 Exploratory Outcomes

2.4.1 Exploratory Indicators

(1) Gut Microbiota Indicators

Fecal samples will be collected for intestinal microbiome sequencing analysis.

(2) Intestinal Immune Microenvironment and Inflammatory Cytokines

Serum inflammatory factors will be detected by ELISA, including: TNF-α, IL-6, IL-10, IL-17A, IL-1β, IL-4, IFN-γ.

2.4.2 Sample Collection Requirements

The following biological samples will be collected: 5 mL fasting venous blood, 20 mL midstream morning urine, 5 g fresh stool. All samples must be delivered to the laboratory within 2 hours after collection for standardized testing.

(1) Blood Sample Processing

3 mL whole blood placed in EDTA anticoagulant tube and serum separation tube

Remaining 2 mL blood centrifuged at 3000 rpm for 10 minutes. Supernatant will be transferred into 1.5 mL EP tubes: ≤0.5 mL per tube, 3 backup aliquots stored. Storage condition: −80°C ultra-low temperature freezer. Sample storage management: All biological samples (blood, urine, stool) will have duplicate backups. Storage conditions: Primary samples processed immediately, backup samples stored at −80°C (±3°C). A sample electronic database will record: sample ID, collection time, storage location.

3. Observation, Recording, and Management of Adverse Events

3.1 Definition of Adverse Events and Serious Adverse Events

An Adverse Event (AE) is defined as any unfavorable or unintended medical occurrence in a subject during the clinical trial, regardless of whether it is causally related to the investigational drug. Adverse events may include symptoms, signs, abnormal laboratory findings, or worsening of pre-existing diseases.

A Serious Adverse Event (SAE) refers to any adverse event that meets one or more of the following criteria:

a. Fatal: directly results in death.

b. Life-threatening: the subject is at immediate risk of death at the time of the event.

c. Hospitalization: requires hospitalization or prolongation of existing hospitalization (excluding planned hospitalization or scheduled examinations).

d. Permanent or significant disability/incapacity: such as organ failure or paraplegia.

e. Congenital anomaly/birth defect: affecting a fetus or newborn.

f. Other medically significant events: events that may not meet the above criteria but may jeopardize the subject's safety or require medical intervention to prevent serious outcomes (e.g., anaphylactic shock, status epilepticus).

3.2 Detection, Collection, and Reporting of Adverse Events

3.2.1 Detection Methods

a. Active monitoring:

Investigators will inquire about symptoms during scheduled visits (e.g., months 1, 3, and 6 of treatment) and evaluate patient status through laboratory examinations and colonoscopy findings.

b. Passive reporting:

Participants or their family members may voluntarily report any discomfort or symptoms to the investigators.

3.2.2 Data Collection

Details of adverse events will be recorded using Case Report Forms (CRFs) or an Electronic Data Capture (EDC) system. Key information to be recorded includes: Time of onset (accurate to the hour), duration, severity, clinical description of symptoms, impact on treatment compliance, management measures (drug name, dosage, treatment duration), outcome (resolved / unresolved / sequelae).

3.2.3 Reporting Procedures

a. Reporting Pathway

(1) Non-serious adverse events: recorded in the CRF and summarized periodically.

(2) Serious adverse events: reported to the Ethics Committee within 24 hours, and reported to regulatory authorities if required.

b. Reporting Content

Reports must include: Subject identification number, protocol number, name of the serious adverse event, time of onset, severity classification, current patient status (ongoing / recovered / death), causality assessment, measures taken and follow-up plan.

3.3 Severity Grading of Adverse Events

Adverse events will be graded according to commonly used AE evaluation criteria into five levels.

Table 5. Adverse Event Severity Classification

Grade	Clinical Characteristics	Priority Management
Grade1	Asymptomatic or mild, no treatment required	Routine monitoring
Grade2	Moderate, affecting daily activities, requiring non-invasive treatment	Adjust study intervention
Grade3	Severe, unable to care for oneself, requiring hospitalization or invasive treatment	Suspend study intervention
Grade4	Life-threatening, requiring emergency intervention	Terminate trial
Grade5	Death	Initiate investigation of cause of death

3.4 Assessment of the Relationship Between Adverse Events and Study Drugs

The Naranjo Algorithm will be used to assess the causal relationship between adverse events and the investigational drug.

Table 6. Naranjo Algorithm Questions and Scoring

Question	Response Options	Score
1.Are there previous conclusive reports on this reaction?	Yes / No / Unknown	+1/0/0
2.Did the adverse event occur after the drug was administered?	Yes / No / Unknown	+2/-1/0
3.Did the reaction improve after discontinuation of the drug (dechallenge)?	Yes / No / Unknown	+1/0/0
4.Did the reaction reappear upon re-administration (rechallenge)?	Yes / No / Unknown	+2/-1/0
5.Are there alternative causes (such as disease or other drugs)?	Yes / No / Unknown	-1/+2/0
6.Did the reaction reappear when placebo was given?	Yes / No / Unknown	-1/+1/0
7.Was the drug detected in toxic concentrations in blood?	Yes / No / Unknown	+1/0/0
8.Was the reaction aggravated or alleviated by dose adjustment?	Yes / No / Unknown	+1/0/0
9.Did the patient have a similar reaction to this drug previously?	Yes / No / Unknown	+1/0/0
10.Was the adverse event confirmed by objective evidence (e.g., laboratory test, biopsy)?	Yes / No / Unknown	+1/0/0

Table 7. Naranjo Total Score and Causality Assessment

Total Score	Causality Category	Interpretation
≥9 分	Definite	Strong evidence that the drug

		caused the event
5-8 分	Probable	Evidence supports but does not fully confirm causality
1-4 分	Possible	Possible association
≤0 分	Unlikely	No evidence supporting association

3.5 Treatment and Management of Adverse Events

3.5.1 Treatment Principles

Subject safety is the highest priority. Study intervention should be immediately suspended and symptomatic treatment initiated. Management will be based on severity grading:

Grade 1–2: Observation and documentation; adjust dose if necessary; participation in the study may continue.

Grade 3: Suspend study intervention and provide hospitalization until recovery to ≤ Grade 1.

Grade 4–5: Permanent withdrawal from the study and initiation of emergency rescue measures and multidisciplinary consultation.

3.5.2 Special Management

a. Management of Serious Adverse Events

SAEs must be reported to the Ethics Committee and sponsor within 24 hours.

Participants will receive necessary medical treatment free of charge, including emergency treatment for non-trial-related injuries if necessary.

b. Ethical Review

The Ethics Committee will evaluate whether the event affects the risk-benefit ratio of the study and determine whether the protocol should be modified or the trial terminated.

4. Data Safety Monitoring

A Data Safety Monitoring Plan will be established according to the risk level of the clinical study. All adverse events will be: recorded in detail, appropriately managed, followed until resolution or stabilization. Serious adverse events and unexpected events will be reported promptly to: the Ethics Committee, regulatory authorities, the sponsor, the drug regulatory agency.

The Principal Investigator will periodically conduct cumulative reviews of all adverse events. If necessary, investigator meetings will be held to evaluate the balance between study risks and benefits.

Emergency unblinding may be performed when necessary in double-blind trials to ensure participant safety and protection of subject rights.

5. Statistical Analysis

Statistical analysis will be conducted according to the Statistical Analysis Plan (SAP). Before database lock, statisticians, the Principal Investigator, and the sponsor will finalize the analysis plan based on the characteristics of the collected data.
This protocol describes only the basic statistical analysis framework. The final analysis will follow the Statistical Analysis Plan.

5.1 Description of Study Population

5.1.1 Enrollment and Completion

The total number of enrolled and completed cases will be summarized. Reasons for study discontinuation will be analyzed, and detailed listings will be provided for: dataset classification of all subjects, study completion status, study withdrawal status.

5.1.2 General Information and Baseline Characteristics

According to the CONSORT statement, the numbers of screened, enrolled, and completed subjects will be reported. The numbers of subjects in each analysis dataset will be summarized. Reasons for subject dropout will be analyzed. Demographic characteristics, previous medication use, comorbidities, and other baseline

characteristics will be described statistically. Comparisons between groups will follow standard statistical principles.

5.1.3 Sample Size and Characteristics of Analysis Sets

The following characteristics will be described: demographic data (age, sex), clinical characteristics (disease stage, previous treatment), laboratory indicators. Baseline balance among the following datasets will be compared: Intention-to-Treat (ITT), Per-Protocol (PP), As-Treated (AT). Comparisons will be presented in tables using p-values or standardized differences.

5.2 Statistical Analysis Plan

All data (including efficacy outcomes, exploratory indicators, and safety indicators) will be entered and analyzed by professional statisticians using SAS 9.4 and SPSS 26.0. Both ITT and PP analyses will be conducted, with ITT as the primary analysis set. Different statistical methods will be applied depending on the type of data.

5.2.1 Continuous Variables

Examples: gut microbiota indicators, intestinal function indicators. Longitudinal data will be analyzed using the Mixed Model for Repeated Measures (MMRM) and Analysis of Covariance (ANCOVA) for baseline adjustment. If data follow a normal distribution with homogeneous variance: data will be expressed as mean ± standard deviation ($x \pm s$), paired t-tests will be used for within-group comparisons, independent sample t-tests for between-group comparisons. If data are non-normally distributed: data will be expressed as median (P25, P75), rank-sum tests will be used. The chi-square test will be used to compare incidence rates between groups. Statistical significance will be defined as $P < 0.05$.

5.2.2 Binary or Ordinal Variables

Examples: TCM syndrome efficacy, improvement in quality of life. Methods include: Logistic regression (binary variables), Ordinal logistic regression (ordered variables),

Chi-square test or Fisher's exact test. Logistic regression results will report: variable names and coding methods, odds ratio (OR) with 95% confidence intervals, Wald test or likelihood ratio test p-values, model fit indicators (AIC, Hosmer-Lemeshow test). Ordinal logistic regression will report: Brant test results, cumulative OR values, global test p-values, threshold parameter estimates. Chi-square or Fisher tests will report: contingency table frequencies, test statistics (χ^2 value or exact p-value), effect sizes (Phi coefficient or Cramer's V).

5.2.3 Survival Data

Examples: adenoma recurrence rate, malignant transformation rate, interval cancer incidence. Methods include: Kaplan–Meier survival curves, Log-rank test.

The cumulative incidence of the first event from randomization to study completion will be analyzed. Event rate will be expressed as events per 100 person-years, calculated as: Number of patients with ≥1 event/Total person-time at risk. Additionally, a Cox proportional hazards model will be used to estimate Hazard Ratios (HR) and 95% confidence intervals. The proportional hazards assumption will be evaluated using Schoenfeld residual plots and time-dependent covariates. If non-proportional hazards are detected, the Royston-Parmar method based on restricted mean survival time (RMST) will be used to estimate treatment effects.

5.3 Handling of Missing Data

5.3.1 Preventive Measures

Missing data will be minimized through the following measures:

a. Mandatory fields will be implemented in the Case Report Forms (CRFs) and Electronic Data Capture (EDC) system

b. Regular monitoring and data verification will be conducted throughout the study.

c. Participants will be reminded periodically via telephone calls or text messages to attend scheduled visits in order to reduce loss to follow-up.

5.3.2 Handling Methods

The causes of missing data will be clearly identified. If missing data are related to treatment (e.g., withdrawal due to adverse events), potential bias will be evaluated through sensitivity analyses. Handling of missing data for primary efficacy endpoints will be determined jointly by the Principal Investigator and the statistician. If imputation is required, appropriate imputation methods will be applied after database lock, based on the mechanism of missing data: Missing Completely at Random (MCAR), Missing at Random (MAR), Missing Not at Random (MNAR). No imputation will be performed for secondary efficacy endpoints or exploratory endpoints. If outliers are detected, the Principal Investigator and statistician will jointly determine the handling approach before database lock. When necessary, outliers may be excluded and sensitivity analyses will be conducted.

5.4 Considerations for Data Analysis

5.4.1 Intention-to-Treat Analysis Set (ITT)

The Intention-to-Treat (ITT) analysis includes all randomized subjects in the final statistical analysis, regardless of whether they: actually received the assigned treatment, completed the study, or complied with the study protocol. This approach preserves the integrity of randomization and avoids selection bias caused by excluding participants, thereby providing a more realistic estimate of the intervention effect under real-world conditions.

5.4.2 Per-Protocol Analysis Set (PP)

The Per-Protocol (PP) analysis includes only subjects who strictly followed the study protocol. Participants who violate the protocol design (e.g., incomplete treatment, insufficient compliance, or use of prohibited medications) will be excluded. This analysis evaluates the maximum theoretical efficacy of the intervention under ideal conditions. The following situations are considered major protocol deviations:

a. Patients in the control group receiving the investigational drug
b. Failure to meet inclusion criteria

c. Use of prohibited medications specified in the protocol

d. Incomplete treatment data at key time points

5.4.3 As-Treated Analysis Set (AT)

The As-Treated (AT) analysis evaluates subjects based on the treatment actually received rather than the treatment assigned during randomization. Regardless of the original randomization group, participants are analyzed according to the actual treatment administered, allowing the analysis to more closely reflect real clinical treatment conditions and provide a more realistic evaluation of treatment effects.

5.4.4 Safety Analysis Set

The Safety Population will include all subjects who received at least one dose of the investigational drug. Subjects who withdraw informed consent immediately after randomization and do not receive any treatment will be excluded from all analysis populations.

6. Quality Control and Quality Assurance of the Study

6.1 Investigators

a. Before the initiation of the study, the clinical research center will establish standard operating procedures (SOPs) and strictly implement them to ensure quality control of the clinical trial.

b. The research center will designate the following personnel before study initiation: Principal Investigator and Clinical Research Quality Control Officer.

c. All personnel participating in the clinical study must receive appropriate training and authorization from the Principal Investigator. Their responsibilities must be clearly defined before participating in study activities.

d. The research center will conduct periodic quality control activities to examine the performance of each study procedure, identify problems and risks in a timely manner, and follow up until issues are resolved to ensure compliance with GCP and the study protocol.

e. The following personnel will be designated: drug administrator, randomization code manager, dispensing nurse, drug administration nurse, unblinded study staff, blinded investigators.

These roles ensure that all evaluation data remain objective, accurate, and reliable throughout the study.

6.2 Clinical Monitors

The number of clinical monitors must be sufficient to meet study requirements. Monitors should possess educational backgrounds and professional experience in medicine, pharmacy, or related disciplines. Clinical monitors are responsible for supervising and inspecting the entire clinical trial process to ensure compliance with Good Clinical Practice (GCP) and the study protocol. The responsibilities of monitors include:

a. Confirming before study initiation that the research institution has appropriate conditions, including qualified personnel, adequate training, and fully functioning laboratory facilities.

b. Providing guidance and training to study personnel to ensure familiarity with the protocol, operational procedures, and regulatory requirements.

c. Verifying that investigators' qualifications and authorizations meet protocol requirements.

d. Monitoring investigators' compliance with the study protocol, SOPs, and relevant regulations during the study.

e. Verifying that informed consent has been obtained from all subjects prior to study participation and confirming subject eligibility.

f. Understanding the progress of the study and reporting it to the sponsor and research team.

g. Ensuring that all subject information is accurately, timely, and completely recorded in source documents and that CRFs are consistent with source data. All errors or discrepancies must be corrected or clarified.

h. Confirming that all adverse events are recorded and that serious adverse events are reported within the required timeframe.

i. Ensuring that all protocol deviations and violations are documented accurately and reported as required.

j. Verifying that investigational drugs are supplied, stored, distributed, used, and returned in accordance with regulatory requirements, with appropriate documentation.

k. Conducting regular monitoring visits to the study center and submitting monitoring reports to Nanjing First Hospital.

7. Ethical Principles and Requirements for Clinical Research

This clinical study will comply with: the Declaration of Helsinki of the World Medical Association, and the Ethical Review Measures for Biomedical Research Involving Human Subjects issued by the National Health and Family Planning Commission of the People’ s Republic of China.

The study will adhere to the following ethical principles: informed consent, protection of participant privacy, provision of free research participation and compensation where applicable, risk control, protection of vulnerable populations, compensation for research-related injury.

The clinical study may only be conducted after approval of the study protocol by the Ethics Committee. Before enrollment, investigators must provide subjects or their legal representatives with complete and comprehensive information about the study, including: study objectives, study procedures, potential risks. Participants must sign a written informed consent form before participation. Subjects must be informed that participation in the clinical study is entirely voluntary. They have the right to: refuse participation, or withdraw from the study at any time during the trial, without discrimination, retaliation, or any negative impact on their medical care or legal rights.

The informed consent form will be retained as part of the clinical trial documentation, and strict measures will be taken to protect participants' privacy and confidentiality of personal data