

Study Protocol

Clinical Efficacy and Safety of Jingfang Granules in Treating Diarrhea-Predominant Irritable Bowel Syndrome with Cold-Dampness Disturbing Spleen Syndrome

Research Site: Nanjing First Hospital

Responsible Department: Gastroenterology

Study Duration: March 2026 – February 2028

Version: V1.0

Version Date: March 1, 2026

Ethics Approval Number: KY20260402-10

Protocol Summary

Study Design (Select all that apply)	☐ Case-control study ☐ Cohort study ☐ Cross-sectional study ☒ Randomized controlled study ☐ Blinding ☐ Other:
Type of Study (Please tick based on the project type)	☒ Interventional study ☐ Observational study ☐ Other (to be determined by the investigator, please specify:)
Total number of cases	106
Risk/Benefit Analysis	Risk: Any study involves known or unknown risks. Some are mild and transient, while others are serious and permanent. Whether risks occur, which risks occur, and their severity vary from person to person. The study doctor will take all preventive measures and closely monitor the participants' health. Known adverse reactions of Jingfang Granules identified in previous studies and clinical use include: gastrointestinal symptoms (such as nausea, loss of appetite, abdominal distension, constipation, etc.), allergic reactions (such as rash, itching, etc.; severe cases may experience dyspnea or anaphylactic shock), and in isolated reports, dry mouth, dizziness, etc. Risks associated with study procedures may include: blood sample collection may cause local pain, bleeding, bruising, infection, or vasovagal reaction/fainting; questionnaires may cause temporary emotional or psychological discomfort. All examinations and procedures in this study strictly comply with clinical practice standards and do not add any unnecessary medical risks.

	Benefits: Participants' health condition may improve during this study. Participation in this study will provide free medication, along with related TCM syndrome evaluation and health status monitoring. Participants will also receive regular follow-up and attention from a professional medical team, which is beneficial for their health management.
Risk Assessment	☒ No greater than minimal risk ☐ Greater than minimal risk Minimal risk: The probability and magnitude of expected risks in the trial are not greater than those encountered in daily life, or during routine physical examinations or psychological tests.
Study Duration	From March 1, 2026 to February 28, 2028

1 Study Background

Irritable Bowel Syndrome with Diarrhoea (IBS-D) is a common functional bowel disorder of the digestive system and one of the important clinical subtypes of Irritable Bowel Syndrome (IBS). It is mainly manifested as abdominal pain and diarrhea, characterized by persistent or intermittent episodes. The Rome IV criteria define it as a disorder of abnormal brain-gut interaction, with pathophysiological factors including visceral hypersensitivity, abnormal intestinal motility, altered intestinal barrier function, immune activation, gut microbiota dysbiosis, and genetic factors. With social development and changes in modern lifestyles, the incidence of IBS has been rising year by year. Globally, the prevalence of IBS is 3.8%, among which IBS-D is the most

common subtype, accounting for 31.5%. In China, the overall prevalence of IBS in the general population is approximately 4 – 10.8%, accounting for about 30% – 50% of gastrointestinal diseases. It is more commonly seen in young and middle-aged adults aged 20 – 50 years, with a slightly higher prevalence in females than in males. Currently, Western medicine treatment for IBS-D mainly focuses on symptomatic intervention, with commonly used medications including antispasmodics, serotonin-related agents, antidiarrheals, intestinal microecological preparations, and antidepressants. In recent years, results from multiple meta-analyses have shown that TCM treatment for IBS-D is superior to Western medicine treatment alone in terms of overall response rate, cure rate, reducing recurrence rate, and improving intestinal microecology. Therefore, conducting research on TCM treatment for IBS-D is of great practical significance.

Based on the clinical characteristics of IBS-D, it can be categorized under traditional Chinese medicine (TCM) disorders such as "diarrhea" (xie xie), "large intestinal diarrhea" (da chang xie), "soggy diarrhea" (ru xie), "diarrhea with undigested food" (sun xie), and "abdominal pain" (fu tong). In cases with prominent mental symptoms such as anxiety and depression, it may also be classified under "depression pattern" (yu zheng) or "intestinal depression" (chang yu). The 2024 Expert Consensus on TCM Diagnosis and Treatment of Irritable Bowel Syndrome categorizes

it into liver depression and spleen deficiency pattern, spleen deficiency with dampness predominance pattern, spleen-kidney yang deficiency pattern, and large intestinal damp-heat pattern, which are treated with Tongxie Yaofang, Shen Ling Baizhu San, Fuzi Lizhong Wan combined with Sishen Wan, and Gegen Qinlian Tang with modifications, respectively. In clinical practice, IBS-D patients often develop the pattern of cold-dampness encumbering the spleen due to external contraction of cold-dampness or internal injury from raw and cold foods, which directly damages the spleen yang of the middle burner, leading to internal generation of cold-dampness that obstructs qi movement. This pattern is characterized by "spleen yang being encumbered as the root and qi stagnation with dampness obstruction as the branch," presenting with typical signs such as a pale, enlarged tongue with a white, greasy coating and a soggy, slow pulse. This pattern is highly correlated with the clinical features of IBS-D patients, such as aggravated diarrhea after consuming cold drinks and frequent mucous stools. It is noteworthy that the cold-dampness encumbering the spleen pattern has been incorporated into the IBS-D syndrome differentiation system by multiple clinical studies and TCM textbooks. Cold congeals and causes qi stagnation with contraction and tightness, while dampness turbidity is sticky and leads to lingering illness; when the two combine, the disease becomes protracted, recurrent, and difficult to cure, representing an advantageous entry point

for TCM intervention.

Jingfang Granules, as a traditional classical formula, are a modern TCM preparation of "Jingfang Baidu San," mainly composed of 11 herbal ingredients: Jingjie (*Schizonepetae Herba*), Fangfeng (*Saposhnikoviae Radix*), Qianghuo (*Notopterygii Rhizoma et Radix*), Duhuo (*Angelicae Pubescentis Radix*), Chaihu (*Bupleuri Radix*), Qianhu (*Peucedani Radix*), Chuanxiong (*Chuanxiong Rhizoma*), Zhiqiao (*Aurantii Fructus*), Fuling (*Poria*), Jiegeng (*Platycodonis Radix*), and Gancan (*Glycyrrhizae Radix et Rhizoma*). This formula is traditionally used for common cold caused by external contraction of wind-cold-dampness, with the effects of inducing sweating to release the exterior and dispelling wind and eliminating dampness. Yu Chang, a physician of the Qing Dynasty, stated that Baidu San embodies the method of "hauling the boat upstream" (*ni liu wan zhou*), which can raise clear yang, disperse pathogenic qi, and stop diarrhea. Ancient physicians were adept at using it to treat intestinal wind with hematochezia. In this formula, the sovereign herbs Jingjie (*Schizonepetae Herba*) and Fangfeng (*Saposhnikoviae Radix*), which are pungent and warm in nature, excel at dispelling wind and dissipating cold, transforming dampness and invigorating the spleen. They directly target the cause of cold-dampness encumbering the spleen and are particularly effective at relieving the dampness turbidity obstructing the middle burner. Qianghuo (*Notopterygii Rhizoma et Radix*) and Duhuo

(*Angelicae Pubescentis Radix*) serve as minister herbs, assisting the sovereign herbs in raising, dispersing, venting, and outthrusting, thereby both expelling external cold and transforming internal dampness. Chaihu (*Bupleuri Radix*) and Qianhu (*Peucedani Radix*) are combined, with one ascending and the other descending, to dredge and regulate the qi dynamic of the triple burner. Chuanxiong (*Chuanxiong Rhizoma*) invigorates the blood and moves qi, and together with Zhiqiao (*Aurantii Fructus*) breaks through stagnation and relieves distension. Furthermore, Fuling (*Poria*), as an assistant, fortifies the spleen and percolates dampness, directing water-dampness out through the urine. Jiegeng (*Platycodonis Radix*) diffuses the lung to "lift the lid of the teapot," assisting qi transformation and promoting the elimination of water-dampness. Gancào (*Glycyrrhizae Radix et Rhizoma*) harmonizes all the herbs while also protecting the middle burner. The entire formula collectively achieves the effects of relieving the exterior and venting pathogenic qi, diffusing and regulating qi dynamic, warming and transforming water-dampness, and raising the clear and descending the turbid. As a result, cold-dampness is dispersed, spleen yang is extended, the transportation and transformation of water-dampness return to normal, and the large intestine's conduction becomes orderly. Its composition is highly consistent with the core pathogenesis of the cold-dampness encumbering the spleen pattern in IBS-D, namely "internal invasion of

cold-dampness, encumbrance of spleen yang, and obstruction of qi dynamic."

Based on the TCM theory of "treating different diseases with the same principle" (yi bing tong zhi), by focusing on the core pathogenesis, it can be inferred that Jingfang Granules is a potentially effective drug for treating diarrhea-predominant irritable bowel syndrome with cold-dampness encumbering the spleen pattern. This inspires us to conduct an open-label, randomized controlled clinical study to evaluate the efficacy and safety of Jingfang Granules in treating IBS-D with cold-dampness encumbering the spleen pattern, to explore its potential mechanisms of action, and thereby provide new drug options and evidence-based medical evidence for the clinical treatment of diarrhea-predominant irritable bowel syndrome, as well as improve patients' quality of life.

2 Study Objectives

2.1 Primary objective: To evaluate the clinical efficacy of Jingfang Granules in treating IBS-D with cold-dampness encumbering the spleen pattern.

2.2 Secondary objectives:

(1) To evaluate the safety of Jingfang Granules in treating IBS-D with cold-dampness disturbing the spleen pattern;

(2) To explore the potential mechanisms of Jingfang Granules in

treating IBS-D with cold-dampness disturbing the spleen pattern.

3 Study Design, Methods, and Study Procedures

3.1 Study Design

This study is a prospective, open-label, randomized controlled clinical trial, planning to enroll 106 patients with diarrhea-predominant irritable bowel syndrome with cold-dampness encumbering the spleen pattern. Recruitment, intervention, follow-up, and assessment of all participants will be conducted at Nanjing First Hospital. A completely randomized grouping design will be used to assign patients equally to the experimental group and the control group, with 53 cases in each group. A positive drug parallel control will be adopted. The control group will receive Trimebutine Maleate Tablets orally (100 mg/tablet, 1 tablet each time, 3 times daily, for 4 weeks), and the experimental group will receive Jingfang Granules orally (15 g/sachet, 1 sachet each time, 3 times daily, for 4 weeks).

3.1.1 Sample Size Calculation Rationale

This study used G*Power 3.1 software (Faul et al., 2007) to calculate the required sample size. The effect size was set at 0.15, α at 0.05, and $1 - \beta$ at 0.8. The calculation results indicated that 90 participants were needed. With an estimated dropout rate of 15%, the total sample size was calculated to be approximately 106. Therefore, a total of 106 patients are required for enrollment in this trial, with 53 cases

each in the experimental group and the control group.

3.1.2 Rationale and Basis for the Indication

IBS-D is a common functional bowel disorder mainly characterized by abdominal pain and diarrhea, with persistent or intermittent episodes. The cold-dampness encumbering the spleen pattern is a common TCM syndrome of IBS-D, primarily manifested as diarrhea, abdominal pain, borborygmus, aggravation with cold, epigastric and abdominal stuffiness and oppression, bland taste in the mouth without thirst, pale tongue, white greasy coating, and soggy slow pulse, among others. Its core pathogenesis is "internal encumbrance of cold-dampness, suppression of spleen yang, stagnation of qi dynamic, and loss of the large intestine's conveyance function."

Jingfang Granules are a modern TCM preparation of the classical formula "Jingfang Baidu San," mainly composed of 11 herbal ingredients: Jingjie (*Schizonepetae Herba*), Fangfeng (*Saposhnikoviae Radix*), Qianghuo (*Notopterygii Rhizoma et Radix*), Duhuo (*Angelicae Pubescentis Radix*), Chaihu (*Bupleuri Radix*), Qianhu (*Peucedani Radix*), Chuanxiong (*Chuanxiong Rhizoma*), Zhiqiao (*Aurantii Fructus*), Fuling (*Poria*), Jiegeng (*Platycodonis Radix*), and Gancan (*Glycyrrhizae Radix et Rhizoma*). This formula embodies the method of "hauling the boat upstream" (*ni liu wan zhou*), which can raise clear yang, disperse pathogenic qi, and stop diarrhea. Ancient physicians were adept at using

it to treat intestinal wind with hematochezia. In this formula, the sovereign herbs Jingjie and Fangfeng, which are pungent and warm in nature, excel at dispelling wind and dissipating cold, transforming dampness and invigorating the spleen. They directly target the cause of cold-dampness encumbering the spleen and are particularly effective at relieving the dampness turbidity obstructing the middle burner. Qianghuo and Duhuo serve as minister herbs, assisting the sovereign herbs in raising, dispersing, venting, and outthrusting to dispel dampness, dissipate cold, and relieve pain. Chaihu and Qianhu are combined, with one ascending and the other descending, to dredge and regulate the qi dynamic of the triple burner. Chuanxiong invigorates the blood, moves qi, and alleviates pain, and together with Zhiqiao breaks through stagnation and relieves distension. Furthermore, Fuling, as an assistant, fortifies the spleen and percolates dampness, directing water-dampness out through the urine. Jiegeng diffuses the lung to "lift the lid of the teapot," assisting qi transformation and promoting the elimination of water-dampness. Gancào harmonizes all the herbs while also protecting the middle burner. The entire formula collectively achieves the effects of relieving the exterior and venting pathogenic qi, diffusing and regulating qi dynamic, warming and transforming water-dampness, and raising the clear and descending the turbid. It is highly consistent with the core pathogenesis of IBS-D with cold-dampness encumbering the spleen pattern, namely

"internal encumbrance of cold-dampness, suppression of spleen yang, stagnation of qi dynamic, and loss of the large intestine's conveyance function."

Based on the TCM theory of "treating different diseases with the same principle" (yi bing tong zhi), Jingfang Granules is a potentially effective drug for treating IBS-D with cold-dampness encumbering the spleen pattern. The use of Jingfang Granules in the treatment of IBS-D with cold-dampness encumbering the spleen pattern is well-justified and warrants scientific verification through well-designed randomized controlled trials.

3.2 Methods

3.2.1 Participant Grouping

This study employs a completely randomized design. The 106 patients who meet the inclusion criteria will be assigned using the random number generator in SPSS 26.0 statistical software: variables will be set, numbers 1 to 106 will be entered, a random seed will be set to generate random numbers, and the patients will then be assigned to either the experimental group or the control group.

3.2.2 Intervention Protocol

- ① Control group: Oral Trimebutine Maleate Tablets (100 mg/tablet, 1 tablet each time, 3 times daily, for 4 weeks).
- ② Experimental group: Oral Jingfang Granules (15 g/sachet, 1 sachet

each time, 3 times daily, for 4 weeks).

(Composed of Jingjie [Schizonepetae Herba], Fangfeng [Saposhnikoviae Radix], Qianghuo [Notopterygii Rhizoma et Radix], Duhuo [Angelicae Pubescentis Radix], Chaihu [Bupleuri Radix], Qianhu [Peucedani Radix], Chuanxiong [Chuanxiong Rhizoma], Zhiqiao [Aurantii Fructus], Fuling [Poria], Jiegeng [Platycodonis Radix], and Gancan [Glycyrrhizae Radix et Rhizoma]).

In addition, patients are advised to avoid raw, cold, high-fat, spicy, and irritating foods, as well as alcohol, coffee, and tea; to engage in appropriate exercise; and to maintain a cheerful mood.

3.2.3 Outcome Measures

This study will employ a series of validated standardized scales as the core assessment instruments to evaluate the primary and secondary efficacy endpoints, and adverse events will be monitored throughout the study. Blood samples will be collected from patients to explore the potential pharmacodynamic mechanisms of action of Jingfang Granules through quantitative analysis of biomarker data.

3.3 Study Procedures

The study consists of three phases: a washout period, during which eligible subjects who meet the screening criteria will discontinue relevant medications for 1 week and undergo safety assessments to eliminate confounding factors; a treatment period, during which patients meeting

the criteria will be randomly assigned to the experimental group or the control group and receive 4 weeks of drug treatment; and a follow-up period, during which symptoms, concomitant medications, and adverse events will be assessed 12 weeks after discontinuation of treatment. During the study, the primary and secondary efficacy endpoints will be evaluated during the treatment period, blood samples will be collected before the start of treatment and after the end of treatment for data analysis, and adverse events will be monitored throughout the entire process.

4. Case Selection

4.1 Diagnostic Criteria

4.1.1 Western Medicine Diagnostic Criteria

Diarrhea-predominant irritable bowel syndrome refers to the Rome IV criteria: recurrent abdominal pain, occurring on average at least 1 day per week in the last 3 months, associated with two or more of the following:

- (1) Related to defecation;
- (2) Associated with a change in frequency of stool;
- (3) Associated with a change in form (appearance) of stool.

Symptoms must have onset at least 6 months prior to diagnosis, and the above diagnostic criteria must be met for the last 3 months. Stool form criteria: More than 1/4 (25%) of bowel movements are Bristol stool

form types 6 or 7, and less than 1/4 (25%) of bowel movements are Bristol stool form types 1 or 2.

4.1.2 TCM Diagnostic Criteria

The diagnostic criteria for cold-dampness encumbering the spleen pattern are formulated with reference to the 2024 Expert Consensus on TCM Diagnosis and Treatment of Irritable Bowel Syndrome and the 2023 Expert Consensus on TCM Diagnosis and Treatment of Diarrhea:

Primary symptoms: Loose or watery stools, abdominal pain, borborygmus.

Secondary symptoms: Loss of appetite, abdominal distension and oppression, heavy sensation in the head and body, nausea and retching, sticky or bland taste in the mouth, aversion to cold.

Tongue and pulse: Pale, enlarged tongue with white greasy coating; soggy and slow pulse.

Diagnosis can be made by fulfilling 2 primary symptoms plus 2 secondary symptoms, or 1 primary symptom plus 3 secondary symptoms, in combination with tongue and pulse manifestations.

4.2 Inclusion Criteria

(1) Meets the Western medicine diagnostic criteria for IBS-D (IBS symptoms persisting for ≥ 6 months, with the Rome IV criteria met in the last 3 months) and the TCM diagnostic criteria for cold-dampness encumbering the spleen pattern.

- (2) Aged between 18 and 65 years, regardless of gender.
- (3) Baseline IBS-SSS score ≥ 75 .
- (4) No use of any medication for IBS for at least 1 week prior to entering treatment.
- (5) The subject is informed and voluntarily signs the informed consent form.

4.3 Exclusion Criteria

- (1) Patients with severe diseases of major organs such as the cardiovascular, respiratory, hepatic, or renal systems, diabetes, tumors, hematopoietic system disorders, neurological or psychiatric diseases, etc.
- (2) Patients with other organic diseases of the digestive system (such as peptic ulcer) or systemic diseases affecting gastrointestinal motility (such as hyperthyroidism, diabetes).
- (3) Patients who are currently using or need to continuously use medications that may affect gastrointestinal function (antidiarrheals, antidepressants, anxiolytics, intestinal microbiota modulators, antibiotics, etc.).
- (4) Patients who have taken antibiotics, steroids and other hormones, Chinese herbal preparations (including oral and intravenous administration), microecological preparations, or probiotics such as yogurt within the last 1 week.
- (5) Patients who have previously undergone abdominal or anorectal

surgery.

(6) Pregnant or lactating women.

(7) Patients with a history of allergy to relevant medications or severe food allergies.

(8) Patients who have participated in or are currently participating in other interventional clinical studies within 3 months.

(9) Patients who refuse enrollment for observation or have other unstable factors, such as failure to take medication as prescribed, or use of other drugs that affect efficacy evaluation, leading to inability to assess efficacy, or incomplete data, etc.

4.4 Study Termination Criteria

(1) Occurrence of severe changes in condition or complications during the trial.

(2) Occurrence of serious adverse events during the trial that render it inappropriate to continue the clinical trial.

5 Alternative Treatment Methods

In clinical practice, for patients with diarrhea-predominant irritable bowel syndrome with cold-dampness encumbering the spleen pattern, apart from Jingfang Granules investigated in this study, there are multiple recognized and alternative treatment options available. Modern medical treatment primarily includes antispasmodics, antidiarrheals, intestinal microecological modulators, as well as low-dose antidepressants or

psychological and behavioral interventions and dietary modifications for refractory patients.

6 Assessment Items and Time Points

Study Phases		Screening Period (Day -7 to 0)	Treatment Period (Day 0)	Treatment Period (Week 4)	Follow-up Period (Week 16)
Visit		1	2	3	4
General Information		✓			
Informed Consent		✓			
History of Present Illness		✓			
Past Medical History		✓			
Verification of Inclusion/Exclusion Criteria		✓			
Vital Signs		✓	✓	✓	✓
Randomization		✓			
Laboratory Tests	Complete Blood Count	✓		✓	
	Blood Biochemistry	✓		✓	
	Urinalysis	✓		✓	
	Stool Routine and Occult Blood	✓		✓	
Electrocardiogram		✓		✓	
Medication Compliance			✓	✓	
Concomitant Medications		✓	✓	✓	✓

Adverse Events		✓	✓	✓
IBS-SSS Scale		✓	✓	✓
Bristol Stool Form Scale		✓	✓	✓
TCM Syndrome Score		✓	✓	✓
IBS-QOL Scale		✓	✓	✓
PRO Scale		✓	✓	
Blood Collection		✓	✓	

7 Efficacy Evaluation Criteria

7.1 Efficacy Assessment

7.1.1 Primary Efficacy Endpoint

(1) Irritable Bowel Syndrome Symptom Severity Scale (IBS-SSS): This scale is an internationally recognized assessment tool for IBS symptom severity, with a total score ranging from 0 to 500. Lower scores indicate milder symptoms.

7.1.2 Secondary Efficacy Endpoints

(1) TCM Syndrome Score: A quantitative scale formulated based on the Guiding Principles for Clinical Research of New Chinese Medicines and the diagnostic criteria for cold-dampness encumbering the spleen pattern, with graded scoring for primary and secondary symptoms. Lower scores indicate milder syndromes.

(2) Bristol Stool Form Scale (BSFS): Assesses stool form, classified into types 1 to 7. The mean daily BSFS score recorded on diary cards during the treatment period, and the typical BSFS type at each visit time point will be recorded.

(3) Patient-Reported Outcome (PRO) Scale: Assesses patients' self-perceived severity of core symptoms such as abdominal discomfort and bloating.

(4) Irritable Bowel Syndrome Quality of Life (IBS-QOL) Scale: Assesses the quality of life of patients with IBS.

(5) Clinical Recurrence Rate: $\text{Recurrence rate} = \text{number of recurrence cases} / \text{number of follow-up cases} \times 100\%$.

7.1.3 Efficacy Evaluation Criteria

With reference to the Guiding Principles for Clinical Research of New Chinese Medicines, the overall response rate = (clinical cure + marked improvement + improvement) $\div$ total number of cases $\times$ 100%. The symptom improvement rate (n) = (total symptom score before treatment - total symptom score after treatment) / total symptom score before treatment $\times$ 100%.

Criteria for overall symptom evaluation:

(1) Clinical cure: Symptoms disappear, $n \geq 95\%$;

(2) Marked improvement: Symptoms are markedly improved, $70\% \leq n < 95\%$;

(3) Improvement: Symptoms are improved, $30\% \leq n < 70\%$;

(4) No improvement: Symptoms show no improvement, $n < 30\%$, or symptoms worsen.

7.2 Safety Evaluation

Safety indicators including general condition, vital signs (body temperature, respiration, heart rate, blood pressure), complete blood count, urinalysis, stool routine, hepatic and renal function, and electrocardiogram.

8 Observation, Recording, and Management of Adverse Events

8.1 Definition of Adverse Events

The safety parameter for this study is defined as the occurrence of adverse events during the study.

Serious adverse events (SAEs), such as death or life-threatening events, hospitalization or prolongation of hospitalization, severe disability, or severe impairment of organ function, etc. Adverse reaction symptoms are recorded according to three grades of severity — mild, moderate, and severe — and causality assessments of possibly related, possibly unrelated, definitely related, and definitely unrelated. Mild adverse reaction: Symptoms are mild and can only be recalled upon inquiry by the physician; Severe adverse reaction: Symptoms are subjectively intense, affecting the patient's daily life or the continuation of the study; Moderate adverse reaction: The severity is between mild and severe.

Criteria for determining the relationship between adverse events and the investigational drug are based on the following five-level classification criteria: ① Definitely related to the investigational drug: There is evidence of taking the investigational drug; the temporal

sequence between the occurrence of the adverse event and drug administration is plausible; the adverse event is more reasonably explained by the investigational drug than by other causes; there is a positive dechallenge response; there is a positive rechallenge response; the pattern of the adverse event is consistent with prior knowledge of this drug or class of drugs. ② Probably related to the investigational drug: There is evidence of taking the investigational drug; the temporal sequence is plausible; the adverse event is more reasonably explained by the investigational drug than by other causes; there is a positive dechallenge response. ③ Possibly related to the investigational drug: There is evidence of taking the investigational drug; the temporal sequence is plausible; the adverse event may have been caused by the investigational drug or by other causes; there is a positive dechallenge response. ④ Possibly unrelated to the investigational drug: There is evidence of taking the investigational drug; the adverse event is more likely caused by other reasons; dechallenge response is negative or equivocal; rechallenge response is negative or equivocal. ⑤ Definitely unrelated to the investigational drug: The investigational drug was not taken; or the temporal sequence between the occurrence of the adverse event and drug administration is not plausible; other significant causes can explain the adverse event.

Adverse reactions include any newly occurring complaints of

discomfort during the study period. If there is only worsening of existing symptoms during the study, it will not be considered an adverse event. Any adverse events should be actively managed.

8.2 Recording

Adverse events suspected to be related to the investigational drug and occurring during the study observation period, as identified by the investigators, will be recorded on the adverse event page of the CRF. The investigators will also assess the relationship between the adverse events and the product. The investigator will use the following definitions to evaluate the relationship between adverse events and the investigational product:

Probably related: There is a strong temporal relationship between the adverse event and the investigational product, or the adverse event recurs upon re-administration, and other etiologies are unlikely or significantly less likely.

Possibly related: There is a strong temporal relationship between the adverse event and the investigational product, while another etiology may have a similar or weaker association with the adverse event.

Possibly unrelated: There is little or no temporal relationship between the adverse event and the investigational product, and/or another more probable cause exists.

Unrelated: The adverse event is caused by underlying or concomitant

diseases or drug effects and is unrelated to the investigational product (e.g., no temporal relationship with the investigational product or another more probable cause exists).

If a subject dies or sustains a serious injury, it is the investigator's responsibility to immediately notify the Ethics Committee. The investigator shall archive all correspondence with the Ethics Committee regarding the assessment of the event.

9 Quality Control and Quality Assurance of the Study

9.1 Investigator

(1) The clinical research center shall develop unified standard operating procedures (SOPs) before the start of the study and strictly implement them to ensure quality control of the clinical trial.

(2) The clinical research center shall designate the following personnel before the start of the study: principal investigator and clinical research quality control officer.

(3) All personnel participating in this clinical study must undergo appropriate training and obtain authorization from the principal investigator, and clearly understand their respective responsibilities before they can take part in the actual conduct of the clinical study.

(4) The clinical research center shall conduct quality control activities at appropriate times, inspect the work at each stage, promptly identify problems and risks, and follow up on identified issues until they are

resolved, to ensure that the clinical study complies with the requirements of GCP and the protocol.

(5) A drug administrator, randomization envelope manager, dispensing nurse, administering nurse, unblinded study members, and blinded investigators shall be designated to ensure that the evaluation data throughout the entire process are objective, authentic, and reliable.

9.2 Monitor

The number of monitors should be adequate to meet the needs of the work, and monitors should possess an educational background and work experience in medicine, pharmacy, or related fields.

The monitor is responsible for supervising and inspecting the entire clinical study process to ensure that it complies with GCP and the clinical study protocol. The monitor shall undertake the following responsibilities:

(1) Prior to the study, confirm that the research site has appropriate conditions, including staffing and training, complete and well-functioning laboratory equipment, and all conditions relevant to the study.

(2) Provide corresponding guidance and training to the participating research personnel, guiding them to become familiar with the study protocol, the operational procedures of each stage, and the requirements of relevant regulations.

(3) Verify that the qualifications and authorizations of the researchers

in the study meet the requirements.

(4) During the study, supervise the researchers' compliance with the study protocol, SOPs, and relevant regulations.

(5) Verify and confirm that informed consent has been obtained from all subjects prior to the study, and confirm that the enrolled subjects are eligible.

(6) Keep track of the study progress and report to the sponsor and the investigator.

(7) Ensure that all study information of the subjects is accurately, timely, completely, and standardizedly recorded in the source documents, and that the CRFs are consistent with the source data. All errors or queries have been corrected or clarified.

(8) Confirm that all adverse events are recorded, and that serious adverse events are reported and recorded within the specified timeframe.

(9) Ensure that all events such as protocol violations and deviations are timely and accurately recorded and reported as required.

(10) Verify that the investigational product is supplied, stored, dispensed, used, and retrieved in accordance with relevant regulations, and that corresponding records are maintained.

(11) Conduct regular monitoring visits to the research center and submit monitoring reports to Nanjing First Hospital.

9.3 Subjects

If a subject completes 4 weeks of treatment and 12 weeks of follow-up, has blood samples collected before and after treatment, and completes all scale assessments during treatment and follow-up, the subject is considered to have completed the study. Subjects who discontinue study treatment early for any reason during the treatment phase are not considered to have completed the study.

If a subject discontinues treatment before the end of treatment, all subjects who withdraw early from the trial must undergo evaluation at the end of treatment and at the post-treatment follow-up.

In the event that a subject is lost to follow-up, the study center staff must make every effort to contact the subject and determine the reason for treatment discontinuation/withdrawal from the trial. All measures taken must be documented.

If a subject withdraws before completing the trial, the reason for withdrawal must be recorded in the CRF and source data. Withdrawn subjects cannot be replaced by other patients.

10 Data Safety Monitoring

A data safety monitoring plan will be developed for the clinical study based on the level of risk. All adverse events will be documented in detail, appropriately managed, and followed up until properly resolved or the condition is stable. Serious adverse events and unexpected events will be promptly reported to the ethics committee, regulatory authorities, the

sponsor, and the drug regulatory department in accordance with regulations. The principal investigator will periodically conduct cumulative reviews of all adverse events and, when necessary, convene investigator meetings to assess the risks and benefits of the study. For double-blind trials, emergency unblinding may be performed if necessary to ensure the safety and welfare of the subjects.

11 Statistical Analysis

Continuous data will be presented as mean $\pm$ standard deviation, and categorical data as percentages or frequencies. Comparison of continuous data between two groups will be performed using the two independent samples t-test, comparison among groups using one-way ANOVA, and categorical data using the chi-square test. A P value < 0.05 will be considered statistically significant. Statistical analysis will be conducted using SPSS 26.0 and performed by the investigator team.

12 Ethical Principles and Requirements for the Clinical Study

The clinical study will follow the World Medical Association Declaration of Helsinki, the Measures for Ethical Review of Life Sciences and Medical Research Involving Human Subjects issued by the National Health and Family Planning Commission of the People's Republic of China, and other relevant regulations. It will specifically implement the principles and requirements regarding informed consent, privacy protection, free-of-charge participation and compensation, risk

control, protection of vulnerable subjects, and compensation for research-related injuries. The clinical study will only be initiated after the study protocol has been approved by the ethics committee. Before each subject is enrolled in this study, the investigator is responsible for fully and comprehensively explaining the purpose, procedures, and potential risks of the study to the subject and/or their legally authorized representative, and for obtaining the signed written informed consent form. Subjects should be made aware that their participation in the clinical study is entirely voluntary, and that they may refuse to participate or withdraw from the study at any time without facing discrimination or retaliation, and their medical care and rights will not be affected. The informed consent form shall be retained as a clinical study document for future reference, and the subjects' personal privacy and data confidentiality shall be effectively protected.

13 Study Progress

Time Period	Tasks
March 2026 – August 2026	1. Study initiation and ethical review 2. Research center initiation training 3. CRF construction
September 2026 – August 2027	1. Patient recruitment 2. Intervention implementation 3. Follow-up period management 4. Completion of outcome measurement and

	data collection
September 2027 – February 2028	1. Data cleaning and analysis 2. Result interpretation and manuscript writing

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