

**Targeting the gut–brain axis with Ganoderma lucidum
spores ameliorates depression in thyroid cancer patients: a
randomized, double-blind, placebo-controlled trial**

Study Site : Zhejiang Cancer Hospital

Principal Investigator:

Protocol Amendment Date: March 12, 2026

Version: 3.0

Protocol Signature Page

I have read this protocol. I understand and agree with its contents and consent to conduct the clinical study in accordance with this protocol. I agree to maintain confidentiality regarding this protocol and related information.

Study Site : Zhejiang Cancer Hospital

Principal Investigator:

Date: March 12, 2026

Research Protocol Summary

Title	Targeting the gut-brain axis with Ganoderma lucidum spores ameliorates depression in thyroid cancer patients: a randomized, double-blind, placebo-controlled trial
Intervention Group	Sporoderm-removed Ganoderma lucidum Spore powder (RGLS)
Control Group	placebo control
Trial Center	Zhejiang Cancer Hospital
Trial Objectives	To clarify the clinical efficacy of RGLS intervention on depressive symptoms accompanying post-operative papillary thyroid carcinoma.To elucidate the antidepressant mechanism of RGLS.
Trial Design	Randomized, Double-Blind, Placebo Parallel-Controlled Trial
Study Population	Patients with post-operative papillary thyroid carcinoma accompanied by depressive symptoms
Sample Size	300 cases; RGLS group: 200 cases, placebo group: 100 cases
Treatment Regimen	From the first day after enrollment, subjects take the trial drug (RGLS or placebo) orally at 4g/day for 90 days.
Efficacy Endpoints	1.Primary Endpoint:Hamilton Depression Rating Scale-24 (HAMD-24) score. 2.Secondary Endpoints: (1) Pittsburgh Sleep Quality Index (PSQI) score. (2) Changes in gut microbial diversity and metabolites. 3.Exploratory Endpoint: Thyroid Function.
Safety Endpoints	Incidence of Adverse Events
Statistical Analysis	Efficacy analyses will be based on the Full Analysis Set (FAS) and the Per-Protocol Set (PPS), while safety analyses will be based on the Safety Analysis Set (SAS). Appropriate statistical methods will be applied to compare both primary and secondary endpoints between groups. All tests will be two-sided, with a $P < 0.05$ considered statistically significant.

Flowchart

Process	baseline	Visit 1	Final Visit
	baseline	Month 1	Month 3
Informed Consent	√		
Collection of Demographic Data	√		
Collection of Medical History (Current, Past, Medication)	√		
Psychiatric Examination	√		
HAMD-24 Assessment	√	√	√
Inclusion/Exclusion Criteria Check	√		
Randomization	√		
Stool Sample Collection	√		√
PSQI Assessment	√	√	√
Residual Blood Collection	√		√
Concomitant Medication Recording	√	√	√
Adverse Event Recording		√	√
Trial Drug Dispensing	√	√	
Trial Drug Retrieval	√	√	√

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1. Trial Background

Depression, as a major global public health issue, has become a significant component of the global disease burden due to its high rates of disability and mortality. With socioeconomic development and epidemiological transition, non-communicable diseases have replaced infectious diseases as the primary health threat, among which the incidence and mortality of cancer show a sustained upward trend. The immense psychological stress associated with the cancer diagnosis and treatment process leads to comorbid depression in approximately 50% of patients, significantly increasing suicide risk, severely impacting quality of life, and creating a heavy socioeconomic burden. The pathological mechanism of cancer comorbid with depression involves the interaction of multiple factors, including cancer-related fatigue, nutritional and metabolic disorders, chronic stress, systemic inflammation, immune dysfunction, and neurometabolic imbalance, collectively leading to impaired neuroprotective mechanisms and accumulation of neurotoxic substances. Existing evidence indicates that poor prognosis and decreased quality of life are significantly correlated in cancer patients with comorbid depression.¹ Papillary thyroid carcinoma, as the most common pathological type of thyroid cancer, although associated with good postoperative prognosis and long-term survival rates, is marked by significantly reduced quality of life, often accompanied by depressive symptoms and sleep disturbances. Studies show that thyroid hormones are crucial for brain development and functional maintenance, and dysfunction of the hypothalamic-pituitary-thyroid (HPT) axis can lead to neuropsychiatric abnormalities.² In clinical management, to prevent recurrence and metastasis, patients require long-term levothyroxine sodium administration to maintain a subclinical hyperthyroid state. This iatrogenic dysregulation of the thyroid axis further increases the risk of psychiatric disorders such as depression, anxiety, and insomnia. Notably, existing evidence indicates a significant association between thyroid dysfunction and gut microbiota dysbiosis.³

Notably, epidemiological data clearly indicate that women represent a high-risk population for both thyroid cancer and cancer-related depression.⁴ This susceptibility stems from a complex interplay of multiple factors: the unique biological foundation involving interactions between sex hormones (e.g., estrogen) and the thyroid axis; the distinct threat and anxiety posed by cancer diagnosis and treatments (such as radioactive iodine therapy and TSH suppression therapy) to women's reproductive function and pregnancy planning; and the specific psychosocial pressures

arising from the multiple roles women often undertake within society and family.⁵ These intertwined factors collectively render female post-operative thyroid cancer patients a high-risk group for depressive symptoms. To focus on this vulnerable and mechanistically distinct population, this study will restrict participants to women, aiming to obtain more homogeneous and in-depth scientific insights and to provide more targeted interventional evidence for this group.

The gut microbiota plays a key role in neuropsychiatric disorders through the bidirectional regulatory mechanisms of the gut-brain axis, influencing central nervous system function via the following pathways: 1) Microbial metabolites (e.g., SCFAs) directly act on vagal nerve endings, transmitting signals via the nucleus tractus solitarius-paraventricular thalamic pathway; 2) Regulating neurotransmitter balance and neurotrophic factor expression; 3) Modulating systemic inflammatory responses and oxidative stress levels; 4) Affecting blood-brain barrier integrity. When gut microbiota is disrupted, this regulatory network becomes imbalanced, leading to the development of neuropsychiatric diseases such as depression through multiple interactions within the neuro-immune-endocrine system.⁶ There are 56 ethnic subgroups in China, and their intestinal flora differs.⁷ Therefore, in this study, we will only select the Han Chinese population.

Current societal awareness and acceptance of mental illnesses like depression remain inadequate. Influenced by stigma and social prejudice, coupled with a lack of public mental health knowledge, a large number of patients fail to seek professional diagnosis and treatment in a timely manner. This delay in seeking medical care causes most patients with depression to miss the golden window for early intervention, severely affecting disease prognosis and outcomes.

The pathological basis of depression involves dysfunction of central neural networks, and its pathogenesis exhibits characteristics of multisystem interaction. Major hypotheses include: neurotransmitter imbalance (monoamine hypothesis), neurotrophic dysfunction, hypothalamic-pituitary-adrenal (HPA) axis dysregulation, neuroinflammation, immune dysregulation, and the microbiota-gut-brain axis disruption hypothesis. Clinical treatment adopts a comprehensive intervention strategy combining pharmacotherapy, psychotherapy, and physical therapy. Among these, first-line antidepressants such as SSRIs (Selective Serotonin Reuptake Inhibitors) and SNRIs (Serotonin-Norepinephrine Reuptake Inhibitors), although widely used, have significant limitations: delayed onset of action (2-4 weeks), unstable maintenance of efficacy, non-response in some patients, and drug side effects such as sexual dysfunction, central nervous

system effects (headache, drowsiness), and gastrointestinal adverse reactions, leading to reduced long-term treatment adherence.⁸

Ganoderma lucidum,⁹ a rare and precious variety among traditional Chinese medicinal herbs, has long been revered as the "elixir herb," with its medicinal value recorded in ancient texts such as Shennong's Classic of Materia Medica. Modern research indicates that *Ganoderma* spores, the reproductive cells of the fungus, are rich in bioactive components, primarily including: 1) *Ganoderma* polysaccharides (crude polysaccharide content $\geq 8.0\text{g}/100\text{g}$) with immunomodulatory effects; and 2) Triterpenoids (total triterpenoids $\geq 4.0\text{g}/100\text{g}$) exhibiting antitumor activity. Sporoderm-removed *Ganoderma lucidum* Spore powder (RGLS) significantly improves bioavailability and is now widely used in the field of adjuvant cancer therapy. Preliminary clinical observations suggest that RGLS can significantly improve depressive symptoms in patients with colorectal adenoma (HAMD-24 score reduction $\geq 30\%$) and enhance immune function (CD4+/CD8+ ratio increased by 1.5-fold).

2. Research Objectives

This study employs microbial metagenomics combined with metabolomics technology to systematically investigate the interventional effects and mechanisms of RGLS on depressive symptoms in patients after papillary thyroid carcinoma surgery. The focus is to elucidate: 1) Its clinical efficacy (improvement in HAMD-24¹⁰/PSQI¹¹ scores); 2) The molecular mechanisms through which it regulates depressive states via the microbiota-gut-brain axis; and 3) The regulatory role of key microbial metabolites in the development of depression. The research findings will provide evidence-based medical support for the clinical application of *Ganoderma* spore powder.

3. Research Significance

Based on the multi-component, multi-target characteristics of traditional Chinese medicine, this study innovatively integrates multi-omics technologies such as microbial metagenomics and metabolomics to systematically analyze the molecular mechanisms underlying the antidepressant effects of RGLS. The research is grounded on two major scientific findings: 1) Characteristic gut microbiota dysbiosis exists in patients with depression; and 2) *Ganoderma* spore powder exhibits potential antidepressant activity. By constructing a multidimensional regulatory network of "microbiota-metabolites-gut-brain axis," the study aims to elucidate: ① The clinical improvement

effect of RGLS on depression after thyroid cancer surgery; ② The regulatory role of key functional microbiota and their metabolites; and ③ The mediating mechanism of the microbiota-gut-brain axis in the development of depression. This research strategy provides an innovative methodological paradigm for studying the mechanisms of complex traditional Chinese medicine systems.

4. Trial Design

This study adopts a randomized, double-blind, placebo parallel-controlled clinical trial design.

5. Technical Roadmap

(1) Integrating microbiomics and metabolomics technologies to construct a multidimensional analysis system of "Microbiota-Metabolites-Gut-Brain Axis":

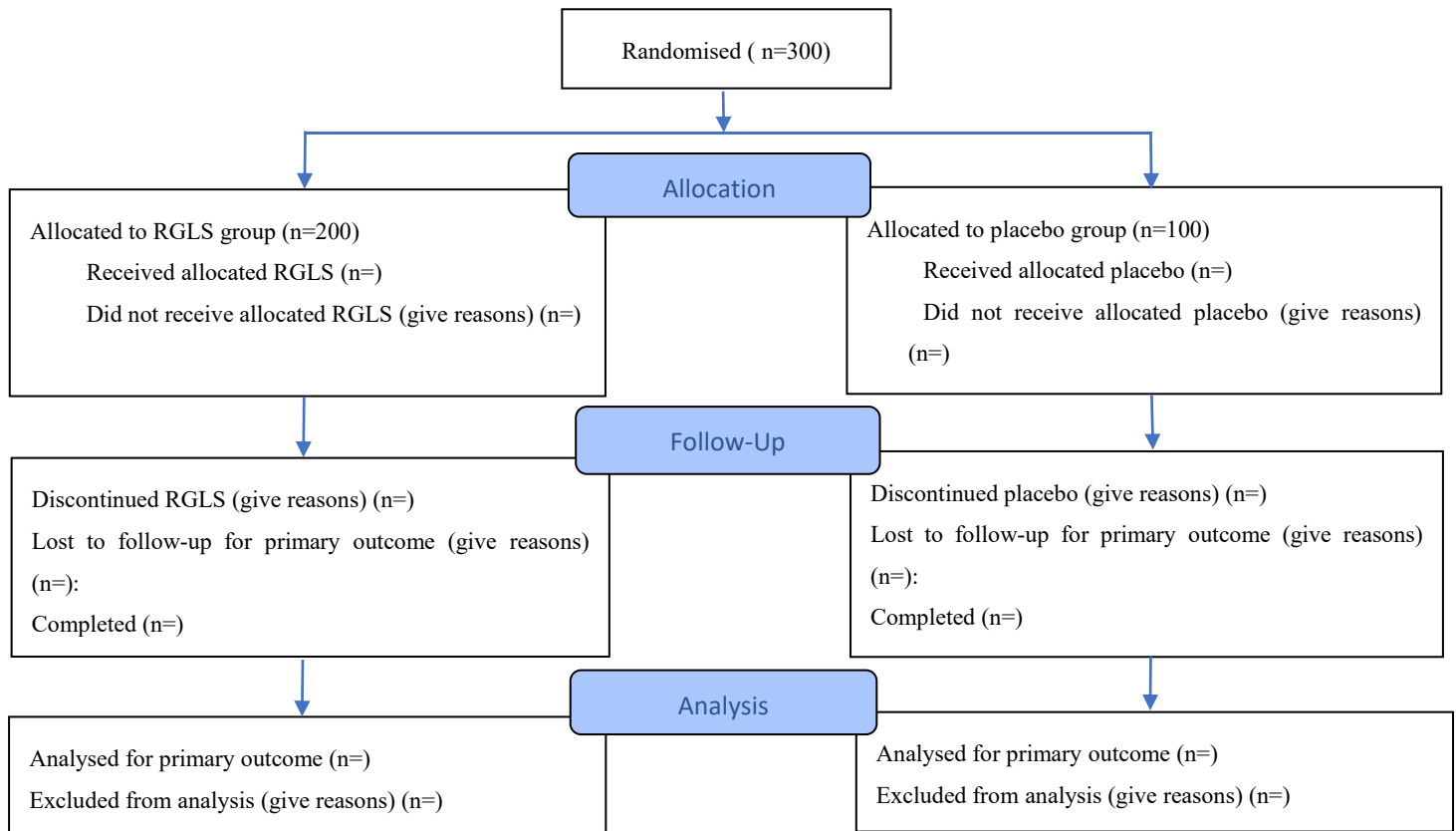
(2) Pre-intervention: Baseline clinical assessment (HAMD-24/PSQI) and biological sample collection.

(3) Intervention process: Strictly quality-controlled standardized drug administration protocol.

(4) Post-intervention: Multi-omics data collection and analysis.

(5) Integrated Analysis: Correlation modeling of microbiota, metabolites, and clinical indicators.

6. Technical Route



7. Subject Selection

7.1 Inclusion Criteria

(1) Outpatients at Zhejiang Cancer Hospital with histologically confirmed papillary thyroid carcinoma (post-surgery) and depressive symptoms (HAMD-24 score ≥ 8 , confirmed by a clinical psychologist).

(2) Han Chinese ethnicity.

(3) No history of depression or other psychiatric disorders.

(4) Age 18-80 years.

(5) Female.

7.2 Exclusion Criteria

(1) Suffering from other gastrointestinal system diseases.

(2) History of gastrointestinal surgery prior to intervention.

(3) Including those with other concurrent malignancies requiring chemotherapy, radiotherapy, biological therapy, or traditional Chinese medicine treatment.

(4) Received antibiotic treatment or microecological modulators within 3 months prior to intervention.

(5) Acute intestinal obstruction.

(6) Patients who are currently taking antidepressant medication, or those whom the investigators judge require immediate initiation of antidepressant medication treatment.

(7) Organic brain diseases, brain trauma.

(8) History of psychiatric disorders, use of psychoactive substances (e.g., drugs).

(9) Severe liver or kidney dysfunction.

(10) Pregnancy or lactation.

7.3 Exclusion Criteria (Post-enrollment)

(1) Subjects found not to meet inclusion criteria after enrollment.

(2) Subjects who did not take RGLS according to the study protocol or did not complete the intervention course.

(3) Poor compliance, voluntary withdrawal mid-study, or inability to assess efficacy or safety due to missing primary endpoint data or incomplete records.

7.4 Criteria for Subject Withdrawal from the Trial

(1) The investigator deems it medically or ethically necessary to stop the trial.

(2) Occurrence of adverse events, complications, or special physiological changes making continued participation inadvisable.

(3) Pregnancy during the treatment period.

8. Trial Protocol

8.1 Sample Size and Allocation

Sample size calculation was performed using G*Power 3.1.9.7 software. Parameters were set as follows: independent samples t-test, two-tailed, significance level $\alpha=0.05$, power $(1-\beta)=0.90$, experimental to control group ratio of 2:1. Based on previous study,¹² the effect size (Cohen's d) was set to 0.5 (mean difference between groups equals 0.5 times the standard deviation), assuming equal variances. The calculated required sample size was 128 for the RGLS group and 64 for the control group. The final sample of 300 (200 RGLS / 100 control) was determined considering:

(1) Statistical power assurance.

(2) Ethical optimization: Using a 2:1 allocation reduces placebo use.

(3) Quality control: Presetting a 15% dropout rate buffer.

8.2 Study Drug Information

(1) Experimental Drug

Generic Name: Sporoderm-removed *Ganoderma lucidum* Spore powder (RGLS)

Specification: 2g/sachet

Quality Control: Complies with relevant standards of the Chinese Pharmacopoeia 2025 Edition.

(2) Control Drug

Composition: placebo control.

Specification: 2g/sachet

Simulant Characteristics: Appearance/packaging identical to the experimental drug.

Quality Control Standard: Same as the experimental drug.

(3) Drug Source

Manufactured on the GMP-certified production line of Jinhua Shouxian Valley Pharmaceutical Co., Ltd., with full batch quality inspection reports provided.

8.3 Intervention Protocol Design

(1) Grouping Design

This is a single-center, randomized, double-blind, placebo parallel-controlled trial. A total of 300 patients with post-operative papillary thyroid carcinoma accompanied by depressive symptoms (HAMD-24 score ≥ 8) will be enrolled. Subjects will be randomized in a 2:1 ratio (RGLS group to control group). To maintain strict double-blinding, a three-code (A, B, C) operational grouping system will be implemented:

Two of the code groups receive the same active intervention (RGLS), while the third code group receives a placebo identical in appearance, packaging, and administration. The correspondence between codes A, B, C and the assigned intervention is sealed and concealed from both investigators and participants. All statistical analyses will be performed after unblinding, comparing the combined RGLS group against the placebo group.

(2) Administration Regimen

Dosage and Frequency: Twice daily, 2 g (one sachet) each time.

Intervention Duration: Continuous intervention for 90 days.

Administration Method: Dissolve in 50 ml of warm water to form a homogeneous suspension for oral intake.

(3) Ethical Oversight:

Approved by the Ethics Committee of Zhejiang Cancer Hospital (IRB-2026-288, IIT). All subjects will provide written informed consent. The study will be conducted in accordance with GCP guidelines and the Declaration of Helsinki. Case data will be de-identified, and biological samples will be anonymized. Pre-intervention samples will be labeled with codes 001-300, and post-intervention samples with codes 001(2)-300(2).

(4) Drug Packaging Specifications

A three-level packaging system will be used:

Primary Packaging Unit: Aluminum foil sachet (2g/sachet, containing RGLS or placebo).

Secondary Packaging: 60 sachets/box (equivalent to a 30-day supply).

Total Supply: 3 boxes/subject (covering the 90-day course).

Quality Control:

All packaging materials comply with the YBB00132002 standard for pharmaceutical aluminum foil. Full batch traceability is implemented.

8.4 Drug Management and Retrieval

(1) Storage Conditions:

Environmental requirements: Cool, ventilated area (temperature $\leq 25^{\circ}\text{C}$, relative humidity $\leq 60\%$).

Security measures: Dedicated drug cabinet with dual locks.

Special precautions: Stored in an area inaccessible to children.

(2) Dispensing and Supervision:

Authorized research personnel dispense drugs according to the visit schedule.

Dual signature for medication records (investigator-subject).

Real-time registration of drug batch number/expiry date.

(3) Retrieval Process:

Used sachets are collected and counted at each follow-up visit.

Final verification of total drug amount at trial end.

Discrepancy recording: Missing quantities and reasons annotated in the CRF.

9. Concomitant Medication Restrictions

This study strictly prohibits the following concomitant medications that may affect efficacy

evaluation:

(1) Immunomodulators: Including biological agents, traditional Chinese medicine compounds, and functional health products.

(2) Psychotropic drugs: Antidepressants, sedative-hypnotics, and other central nervous system drugs.

(3) Microecological agents: Antibiotics, probiotic/prebiotic preparations.

(4) Others: Dietary supplements with neuroregulatory or immunomodulatory activity.

10. Study Procedures

10.1 Screening/Baseline Period (Baseline)

- (1) Signing of informed consent.
- (2) Verification of inclusion/exclusion criteria.
- (3) Collection of demographic and medical history data.
- (4) Psychiatric examination and HAM-D-24 assessment.
- (5) PSQI assessment.
- (6) Stool sample collection.
- (7) Residual blood collection for thyroid function tests.
- (8) Urine pregnancy test for women of childbearing age.
- (9) Recording of concomitant medications.
- (10) Drug dispensing.

10.2 Visit 1 (Month 1)

- (1) HAM-D-24 assessment.
- (2) PSQI assessment.
- (3) Recording of concomitant medications and adverse events.
- (4) Drug retrieval and dispensing.

10.3 Visit 2 / Final Visit (Month 3)

- (1) HAM-D-24 assessment.
- (2) PSQI assessment.
- (3) Stool sample collection.
- (4) Residual blood collection for thyroid function tests.
- (5) Recording of concomitant medications and adverse events.

(6) Drug retrieval.

10.4 Principles of Indicator Collection

(1) Psychological Scale Assessment

HAMD-24 Scoring: Independently completed by two senior psychiatrists (Kappa value ≥ 0.75).

PSQI Scoring: Self-administered by the subject under the supervision of a psychologist.

Assessment Time Points: Baseline, Mid-intervention (Month 1), End of intervention (Month 3).

(2) Stool Sample Collection Method:

Place a stool collector in the toilet bowl. Defecate into the collector. Use a long-handled scoop to thoroughly mix the fresh stool sample (avoid urine contamination).

Aliquot the mixed stool sample into two pre-labeled 5-10 mL sterile tubes or pre-sterilized collection tubes, filling each to approximately 2-3 mL.

Use a wooden stick to transfer about 1 mL of the sample from the 5-10 mL collection tube to a 1.5 mL centrifuge tube; or directly transfer about 1 mL from the mixed stool sample in step ① to a 1.5 mL centrifuge tube.

Cap the centrifuge tube tightly, check the seal, place in a sample box in order of code, and store in a -80°C freezer.

If collecting stool at home, place the collection tube in a ziplock bag and deliver to the hospital sample reception point as soon as possible (within 2 hours).

Sample Collection and Storage Notes:

Samples should not be exposed to room temperature for more than 2 hours.

Collection should be performed at the same time of day to avoid circadian rhythm effects on metabolism.

Samples should be mixed immediately after collection, aliquoted, and frozen.

Prepare duplicate samples to avoid repeated freeze-thaw cycles.

(3) Blood Sample Collection:

Residual serum from routine clinical thyroid function tests was used. Approximately 500 μL to 1 mL of supernatant was transferred into 1.5 mL centrifuge tubes (multiple aliquots recommended), placed in a cryobox, and stored at -80°C . Sample labeling followed the same

numbering system as the corresponding fecal samples: Pre-intervention: 001–300.
Post-intervention: 001(2)–300(2).

(4) Stool and Blood Sample Collection Timing, Preservation, and Transport:

Collection Time Points: Baseline, End of intervention (Month 3).

Preservation: Flash-freeze in liquid nitrogen for 15 minutes before transferring to a -80°C freezer.

Ensure continuous freezer operation without power interruption, as power failure will cause sample thawing.

11. Efficacy Evaluation Criteria

11.1 Primary Efficacy Endpoint

Change in Hamilton Depression Rating Scale-24 (HAMD-24) score:

(1) Scale Characteristics: A 24-item standardized tool for assessing depressive symptoms, covering emotional, cognitive, and somatic dimensions.

(2) Clinical Severity Grading:

No depression (0-7 points)

Subclinical depression (8-20 points)

Mild to moderate depression (21-35 points)

Severe depression (≥ 36 points)

(3) Application Value: Widely used as a gold standard for evaluating antidepressant treatment efficacy.

(4) Definition of Clinical Remission: After 90 days of intervention (Month 3), meeting both criteria:

HAMD-24 score decreases to the non-depressed range (< 8 points) or improves by ≥ 1 severity level.

Inter-group comparison shows statistical significance ($P < 0.05$, two-tailed test).

11.2 Secondary Efficacy Endpoints

(1) Sleep Quality Assessment (PSQI)

Scale Structure: 7-dimension self-assessment scale (Cronbach's $\alpha = 0.83$).

Cut-off Value:

Normal sleep (< 7 points)

Sleep disturbance (≥ 7 points)

Effective Criteria:

Individual level: PSQI score decreases to the non-sleep disturbance range (< 7 points).

Inter-group comparison: Difference is statistically significant ($P < 0.05$).

(2) Gut microbiota Indicators

After 90 days of intervention (Month 3):

① Alpha Diversity Analysis

Indices: Shannon, Simpson, and Richness indices.

Method: The differences in alpha diversity indices between the two groups post-intervention will be compared using an independent samples t-test (if normality and homogeneity of variance assumptions are met) or the Mann-Whitney U test (nonparametric).

② Beta Diversity Analysis

Metric: Based on Bray–Curtis dissimilarity.

Method: Dimensionality reduction and visualization will be performed using Principal Coordinate Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS).

③ Differential Species Analysis

Taxonomic Level: Analysis will be conducted at the genus and species levels.

Methods:

LEfSe (Linear Discriminant Analysis Effect Size) will be applied to identify biomarker species, with a threshold of LDA score > 2.5 and $P < 0.05$.

DESeq2 will be used for differential abundance testing. Significant differential species will be defined as those with an FDR-adjusted $P < 0.05$ and $|\log_2 \text{fold change}| > 0$.

(3) Serum metabolite Indicators

Method: An Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) model will be constructed based on untargeted metabolomic data.

Screening Criteria: Metabolites satisfying both Variable Importance in Projection (VIP) > 1.0 and Student's t-test $P < 0.05$ (or the corresponding nonparametric test) will be defined as differential metabolites between groups. KEGG pathway enrichment analysis will be performed on the identified differential metabolites. Post-intervention differences in these indicators between the RGLS and placebo groups will be compared. Statistical significance is defined as $P < 0.05$ (or

FDR < 0.05).

11.3 Exploratory Endpoint

To compare changes in thyroid function parameters (TSH,T3,T4,FT3,FT4,TPOAb,Tg) from baseline to Month 3 between the RGLS and placebo groups. For between-group comparisons, continuous variables will be analyzed using a t-test if normally distributed, or the Mann-Whitney U test if not normally distributed.

12. Adverse Event Evaluation

12.1 Definition of Adverse Event (AE)

Any unfavorable or unintended occurrence during the clinical trial period, including:

- (1) New clinical symptoms/signs.
- (2) Laboratory abnormalities (worsening by ≥ 1 grade compared to baseline).
- (3) Exacerbation of pre-existing conditions (according to NCI-CTCAE v5.0 standards).

Regardless of its relationship to the trial drug.

12.2 Causality Assessment

Using the WHO-UMC Causality Assessment System.

Required Tests: Blood routine, high-sensitivity C-reactive protein (hs-CRP), liver and kidney function (ALT/AST/Scr).

Supportive Tests: Based on clinical presentation, may include ECG, thyroid function tests, etc.

12.3 Monitoring Process

- (1) Recording Standards:

Complete documentation on a dedicated CRF page: Time of onset, severity (Grade 1-5), management measures, outcome.

Investigator signs the "Adverse Event Assessment Form."

- (2) Reporting Mechanism:

Serious Adverse Events (SAE): Investigators must report to the principal investigator and the institutional ethics committee within 24 hours of becoming aware of the event, and subsequently submit a detailed follow-up report within 7 days. For fatal or life-threatening SAEs, an additional report must be submitted to the Center for Drug Evaluation of the National Medical Products Administration within 24 hours.

(3) Safety Dataset: Based on the Full Analysis Set (FAS) principle, including all exposed subjects.

13. Data Management

13.1 Case Report Form (CRF) Management

(1) Use standardized CRF forms.

(2) Implement three-level quality control: Investigator completion → Monitor verification → Data manager review.

13.2 Data Entry Standards

(1) Software System: EpiData 3.1 Professional.

(2) Entry Process:

Double data entry by independent personnel (Kappa consistency ≥ 0.9).

Logical checks (pre-set value ranges/skip logic).

Query resolution mechanism.

13.3 Database Locking Criteria

(1) Prerequisites: Completion of blind data review.

(2) Locking Procedure:

Joint signature by statistician, investigator, and sponsor.

Generation of a read-only database (with audit trail enabled).

Backup of the original database (encrypted with SHA-256).

14. Clinical Trial Management and Quality Control

14.1 Ethical Compliance Management

(1) Any protocol amendments must receive written approval from the Ethics Committee (Approval document archive number: IRB-2026-288, IIT).

(2) Implement a dual-signature system for informed consent (investigator - subject/guardian).

(3) Informed Consent Process: Prior to the screening visit, a trained investigator will provide a detailed explanation to potential subjects regarding the study purpose, procedures, potential risks and benefits, alternative treatments, confidentiality provisions, and the principle of voluntary participation. Subjects will be given adequate time to ask questions and will sign the written informed consent form in a pressure-free environment. A copy of the signed consent form will be provided to the subject for their retention.

14.2 Standardized Operating Procedures (SOPs)

(1) Personnel Training:

Unified training for GCP-certified investigators (training records archived).

Consistency assessment for scale assessors ($ICC \geq 0.8$).

(2) Data Collection:

Source Data Verification (SDV) ratio $\geq 20\%$.

Outlier determination: Refer to standard reference ranges of the laboratory LIS system.

14.3 Document Management Standards

(1) Recording Requirements:

CRF completion follows ALCOA principles (Attributable, Legible, Contemporaneous, Original, Accurate).

Modification standards: Preserve original entries; modifications signed and dated by the person making the change.

(2) Retention Period:

Original documents (including HAMD-24/PSQI scales) retained for 5 years after trial completion.

Electronic data backed up to the National Clinical Research Data Platform.

14.4 Quality Monitoring Mechanism

(1) Implement three-level quality control: Investigator self-check → Institutional QC → Sponsor audit.

(2) Key document list management (Essential Document List).

15. Statistical Analysis of Trial Data

Statistical work will be undertaken by Professor Yi-Min Zhu, an expert from the Division of Biostatistics, Department of Public Health at Zhejiang University. He will participate in trial design and protocol implementation, be responsible for data management and statistical analysis, and complete the statistical summary report.

15.1 Full Analysis Set (FAS)

(1) Inclusion Criteria:

Randomized patients with post-operative papillary thyroid carcinoma (≥ 30 days post-operation).

Received at least one dose of the trial drug.

(2) Data Handling: Missing values addressed using Multiple Imputation.

(3) Analysis Purpose: Intention-to-Treat (ITT) analysis for primary efficacy endpoints.

15.2 Per-Protocol Set (PPS)

(1) Criteria:

Fully adhered to the trial protocol.

Medication compliance $\geq 80\%$.

No major protocol deviations.

(2) Analysis Purpose: Sensitivity analysis.

15.3 Safety Analysis Set (SAS)

(1) Composition Criteria:

Received at least one dose of the trial drug.

Has post-baseline safety assessment data.

(2) Analysis Purpose:

Calculation of adverse event incidence rates.

Analysis of laboratory abnormalities.

16. Statistical Analysis Plan

Statistical analysis will be performed using SPSS 25.0, GraphPad Prism 9 and R 4.3.1.

(1) Subject Distribution: Comparison of dropout and exclusion rates between groups using χ^2 test or Fisher's exact test.

(2) Comparability Analysis: Comparison of demographic data and other baseline indicators to assess group comparability. Use χ^2 test, Fisher's exact test, CMH test, t-test, ANOVA, or non-parametric tests based on data type and distribution.

(3) Compliance Analysis: Comparison of whether subjects in both groups used the trial drug on time and in the correct dosage and avoided prohibited medications. Primarily use χ^2 test or Fisher's exact test.

(4) Efficacy Analysis: Statistical methods for two independent samples to compare differences between the experimental and control groups. If normality and homogeneity of variance assumptions are met, use t-test; otherwise, use Mann-Whitney U test (Wilcoxon rank-sum test). Furthermore, perform repeated measures mixed linear model analysis

incorporating group (experimental/control), time point (baseline, end), and group-by-time interaction as fixed effects.

(5) Safety Analysis: Descriptive statistics for adverse reactions. Comparison of adverse reaction rates between groups using χ^2 test or Fisher's exact test.

(6) All statistical tests should report the test statistic and corresponding p -value. For Fisher's exact test, report the p -value directly. A p -value ≤ 0.05 indicates statistical significance.

17. Blinding, Unblinding, and Emergency Unblinding

17.1 Blinding Design

(1) Randomization Coding System:

Use block randomization method (block size=6).

Generate random allocation sequence using SAS 9.4.

Establish three treatment groups: A/B/C.

(2) Drug Blinding Specifications:

Double-blind design: Both subjects and research team remain unaware of group assignment.

Emergency envelopes: Sealed and stored in the institutional pharmacy (temperature monitored).

Blind code custody: Three copies (statistics team/sponsor/institutional office).

17.2 Formal Unblinding Process

(1) Prerequisites for Unblinding:

Database locked (confirmed by three parties).

Final version of the Statistical Analysis Plan (SAP) completed.

(2) Unblinding Procedure:

First-level unblinding: Reveal the attribute of groups A/B/C (e.g., which is active/placebo).

Second-level unblinding: Disclose the specific intervention scheme for each group.

Opening of the blind code requires witnessing by the statistician, investigator, and monitor.

17.3 Emergency Unblinding Criteria

(1) Indications:

Serious Adverse Event (SAE, \geq CTCAE Grade 4).

Life-threatening complications.

(2) Execution Process:

Three-level approval: Investigator → Institutional Office → Independent Data Monitoring Committee (IDMC).

Submit the "Unblinding Record Form" within 24 hours after unblinding.

(3) Handling Principles:

The unblinded case is immediately withdrawn from the study.

Initiate the alternate randomization number mechanism.

18. Trial Schedule

Clinical trial period: March 2026 - July 2026.

Completion of final report: August 2026.

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Appendix 1: Hamilton Depression Rating Scale (HAMD)

Hamilton Depression Rating Scale (HAMD)

Name _____ Age _____ Gender _____ ID _____

Outpatient/Hospital No. _____ Diagnosis _____

Date _____

Home Address _____ Contact Phone _____ Rater _____

Circle the score that best fits the patient's condition.

- | | |
|---|-----------|
| 1. Depressed Mood | 0 1 2 3 4 |
| 2. Feelings of Guilt | 0 1 2 3 |
| 3. Suicide | 0 1 2 3 4 |
| 4. Insomnia (Early) | 0 1 2 |
| 5. Insomnia (Middle) | 0 1 2 |
| 6. Insomnia (Late) | 0 1 2 |
| 7. Work and Activities | 0 1 2 3 4 |
| 8. Retardation | 0 1 2 3 4 |
| 9. Agitation | 0 1 2 3 4 |
| 10. Anxiety (Psychic) | 0 1 2 3 4 |
| 11. Anxiety (Somatic) | 0 1 2 3 4 |
| 12. Somatic Symptoms (Gastrointestinal) | 0 1 2 |
| 13. Somatic Symptoms (General) | 0 1 2 |
| 14. Genital Symptoms | 0 1 2 |
| 15. Hypochondriasis | 0 1 2 3 4 |
| 16. Loss of Weight | 0 1 2 |
| 17. Insight | 0 2 |
| 18. Diurnal Variation: A. Morning | 0 1 2 |
| B. Evening | 0 1 2 |
| 19. Depersonalization & Derealization | 0 1 2 3 4 |
| 20. Paranoid Symptoms | 0 1 2 3 4 |
| 21. Obsessional Symptoms | 0 1 2 |
| 22. Helplessness | 0 1 2 3 4 |
| 23. Hopelessness | 0 1 2 3 4 |
| 24. Worthlessness | 0 1 2 3 4 |

Note:

Total Score:

Appendix 2: Pittsburgh Sleep Quality Index (PSQI)

Pittsburgh Sleep Quality Index (PSQI)

Name _____ Gender ____ Age ____ Education Level _____

Instructions: The following questions relate to your usual sleep habits during the past month only.

Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night? (Please use 24-hour format)

Bedtime: ____ Hour ____ Minute

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

① Less than 15 minutes ② 16-30 minutes ③ 31-60 minutes ④ More than 60 minutes

3. During the past month, what time have you usually gotten up in the morning? (Please use 24-hour format)

Wake-up time: ____ Hour ____ Minute

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

Actual sleep time: ____ Hours ____ Minutes

For each of the remaining questions, check the one best response. Please answer all questions.

During the past month, how often have you had trouble sleeping because you.

5. Cannot get to sleep within 30 minutes

① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

6. Wake up in the middle of the night or early morning

① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

7. Have to get up to use the bathroom

① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

8. Cannot breathe comfortably

① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

9. Cough or snore loudly

① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

10. Feeling too cold

- ① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

11. Feeling too hot

- ① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

12. Having bad dreams

- ① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

13. Experiencing pain

- ① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

14. Other reasons (please describe)

During the past month, how often have you had trouble sleeping because of the reasons listed above?

- ① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

15. How would you rate your overall sleep quality during the past month?

- ① Very good ② Fairly good ③ Fairly bad ④ Very bad

16. During the past month, how often have you taken medication (prescribed by a doctor or "over the counter") to help you sleep?

- ① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

17. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activities?

- ① Not during the past month ② Less than once a week
③ Once or twice a week ④ Three or more times a week

18. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?

- ① No problem at all ② Only a very slight problem
③ Somewhat of a problem ④ A very big problem

19. Do you have a bed partner or roommate?

- ① No bed partner or roommate ② Partner/roommate in other room
③ Partner in same room, but not same bed ④ Partner in same bed