

Neoadjuvant chemoradiotherapy combined with PD-1 monoclonal antibody (tislelizumab) and Probio-M9 for the treatment of pMMR/MSS in locally advanced mid-low rectal cancer: A single-center, prospective, randomized controlled study

Research Plan

I. Research Background

Rectal cancer is one of the most common digestive tract tumors in China, with 253,000 new cases in China each year, accounting for about 18.6% of the world's total. With changes in people's lifestyles and the improvement of living standards, the incidence of rectal cancer is still gradually increasing ^[1]. According to the 2017 edition of the Chinese Expert Consensus on the Diagnosis and Treatment of Locally Advanced Rectal Cancer, locally advanced rectal cancer (LARC) is currently defined as: rectal cancer in which the primary tumor, as discovered by pathological examination or imaging, invades the muscular layer of the intestinal wall and extends to the surrounding tissues (c/p T3-4) or lymph node metastasis occurs in the mesentery and true pelvis (c/p N1-2) without distant metastasis (M0) ^[2]. Rectal cancer has an insidious onset, and early clinical symptoms are not obvious. Moreover, most people lack knowledge about the diagnosis and treatment of rectal cancer. Therefore, about 75% of the clinically diagnosed rectal cancer cases in China are already in the middle and late stages, and some patients have even lost the opportunity for surgery when they seek medical attention ^[3]. Among

them, mid-to-low rectal cancer (the distance between the tumor and the anus is $\leq 10\text{cm}$) accounts for the majority of rectal cancer patients diagnosed in my country. Due to its proximity to the anus, the rate of anal preservation is low, and patients have a poor quality of life after surgery.

For patients with locally advanced rectal cancer, the current standard treatment regimen is neoadjuvant chemoradiotherapy (nCRT) combined with total mesenteric resection (TME), including long-course chemoradiotherapy (LCRT, 50 Gy/25 Fx, concurrent 5-FU or capecitabine) and short-course radiotherapy (SCRT, 25 Gy/5 Fx). However, the pathologic complete response (pCR) rate for patients receiving neoadjuvant chemoradiotherapy before surgery is currently about 11%-15%, which still needs to be improved ^[4-5]. Meanwhile, some literature reports that the overall survival (OS) of neoadjuvant chemoradiotherapy before surgery is not significantly improved compared to postoperative adjuvant chemoradiotherapy ^[6-7]. In recent years, immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway (PD-1/PD-L1 monoclonal antibodies) have provided a new treatment option for malignant tumors. The Keynote-177 trial showed that PD-1 monoclonal antibodies have good efficacy and safety in patients with advanced colorectal cancer (CRC) with high microsatellite instability (MSI-H) or loss of mismatch repair (dMMR) ^[8]. The NICHE trial was the first study to evaluate the efficacy and safety of immunotherapy in

neoadjuvant therapy for colorectal cancer patients. The results showed that neoadjuvant immunotherapy was safe and showed good tumor response ^[9]. However, dMMR/MSI-H patients account for only 5%-20%, and the vast majority of CRC patients are patients with no loss of mismatch repair (pMMR) or microsatellite stable (MSS). Improving the sensitivity of pMMR/MSS patients to immunotherapy remains a challenge.

A neoadjuvant study initiated by Zhang Zhongtao's team in China (a single-arm, multicenter, phase II clinical trial of neoadjuvant chemoradiotherapy combined with tislelizumab for locally advanced mid-to-low rectal cancer, ClinicalTrials.cn registration number: NCT04911517) enrolled patients with initially resectable, solitary, mid-to-low rectal adenocarcinoma. Patients received neoadjuvant chemoradiotherapy combined with tislelizumab. From April 2021 to June 2022, 46 patients with pMMR/MSS locally advanced rectal cancer underwent surgical treatment. The median age was 63 years, and the median distance of the tumor from the anal verge was 5.1 cm. Efficacy analysis showed that 20 patients achieved pathological complete response (pCR) after neoadjuvant chemoradiotherapy with tislelizumab (pCR rate 43.5%). The objective response rate was 76.1%, and the radical resection rate was 100%. The incidence of grade 3 or higher treatment-related adverse events (irAEs) after treatment was 4.3% (2/46), indicating that this

treatment strategy has good safety ^[10-11].

Professor Zhang Heping of China isolated a probiotic strain, *Lactobacillus rhamnosus* Probio-M9, from human colostrum ^[12] and verified its role in tumor prevention and synergistic therapy ^[13-14]. The team demonstrated that intestinal microbiota dysbiosis caused by inflammation or antibiotics can be effectively restored after treatment with Probio-M9, thereby improving the anti-tumor efficacy of colorectal cancer treatment. Further studies have shown that Probio-M9 promotes the growth of beneficial intestinal microorganisms (such as *Lactobacillus* and *Bifidobacterium animalis*), produces beneficial metabolites including butyrate in the intestine, and accumulates α -ketoglutarate, N-acetyl-L-glutamate and pyridoxine from the blood, thereby promoting the infiltration and activation of cytotoxic T lymphocytes (CTLs) and inhibiting the function of regulatory T cells (Tregs) in the tumor microenvironment (TME), thereby enhancing the immunotherapy response ^[15].

In recent years, the gut microbiota, as an important immune regulatory hub, has been recognized as a key factor influencing the tumor immune microenvironment and the efficacy of immunotherapy, and has gradually become an important research direction for overcoming the bottlenecks in immunotherapy. Numerous basic and translational studies have shown that the gut microbiota can influence the local immune status

of tumors through its metabolites and immunomodulatory effects, thereby regulating the intensity and persistence of anti-tumor immune responses.

Probio-M9 (*Lactobacillus rhamnosus*), a marketed food-grade probiotic strain with clear immunomodulatory potential (Production License No.: SC10633070200513, Standard: Q/JYH0006S) , has been shown in previous studies to participate in regulating the tumor microenvironment through its metabolites, improving immunosuppression, and to some extent enhancing the anti-tumor effect of immunotherapy . However, its clinical application value in patients with pMMR/MSS locally advanced rectal cancer, especially its synergistic effect in the context of neoadjuvant therapy, still lacks a systematic review of prospective randomized controlled trials.

The necessity of this study lies in exploring whether Probio-M9 can synergistically modulate the tumor immune microenvironment through the introduction of gut microbiota intervention, thereby promoting the transformation from immunosuppressive "cold tumors" to immune-activated "hot tumors," and ultimately improving the pathological complete response rate (pCR) and sphincter-preserving rate in pMMR/MSS rectal cancer patients. This research not only holds promise for providing a new supplementary strategy to existing neoadjuvant therapy modalities but also offers new insights and clinical evidence for the exploration of precision treatment for refractory MSS rectal cancer.

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II. Research Objectives and Significance

(1) By selecting suitable enrolled patients during the clinical diagnosis and treatment process and completing the treatment according to the study plan, we will evaluate the moderating effect of neoadjuvant chemoradiotherapy combined with PD-1 monoclonal antibody (tislelizumab) and Probio-M9 treatment on the efficacy of pMMR/MSS locally advanced mid-low rectal cancer .

(2) To explore the feasibility of organ-sparing surgery (non-TME surgery) or a wait-and-see strategy for patients who have achieved clinical complete remission or are expected to achieve pathological complete remission after the above treatments, and the impact on patients' functional and quality of life. This will provide new options for the treatment of locally advanced rectal cancer.

III. Research Content

This study aims to include patients with locally advanced mid-to-low rectal cancer and evaluate the moderating effects of neoadjuvant chemoradiotherapy combined with PD-1 monoclonal antibody (tislelizumab) and the probiotic Probio-M9 as a special food intervention on the efficacy of locally advanced rectal cancer . Given that Probio-M9 is already manufactured and marketed as a common food/dietary supplement by a relevant company (Production License No.: SC10633070200513, Standard: Q/JYH0006S), this study will explore its synergistic value in standardized clinical treatment regimens.

IV. Research Design

1. Research Types and Overall Design

This study was a **prospective, randomized, controlled clinical trial** . All subjects who met the inclusion criteria were randomly assigned to the experimental or control group in a 1 :1 ratio after completing the assessment during the screening period.

2. Randomized controlled trial method

A simple randomization method (without stratification) is used, where a random assignment system generates random sequences, and patients are divided into groups at a 1 :1 ratio:

- **Experimental group (Group A)** : Received neoadjuvant

chemoradiotherapy + PD-1 monoclonal antibody (tislelizumab) + Probio-M9 (this product is a marketed ready-to-eat probiotic food, classified as a general food/dietary supplement, taken orally once daily, 2g each time) ;

- **Control group (Group B)** : Received neoadjuvant chemoradiotherapy + PD-1 monoclonal antibody (tislelizumab) + placebo (oral, once daily).

3. Sample size and calculation basis

Currently, there are no relevant clinical studies. The studies available on clinical trials are shown in the following figure:

ClinicalTrials.gov. ID	研究发起中心	研究开始时间	预计入组人数	疾病	治疗方案	研究设计
NCT05122546	美国希望之城医疗中心	2021/11/1	30	转移性肾细胞	nivolumab + cabozantinib S-malate ± CBM588	RCT研究
NCT06865521	四川华西医院	2025/2/21	22	晚期结直肠癌	anti-PD-1 monoclonal antibody + TKI treatment/anti-PD-1 monoclonal antibody ± Chemotherapy ± Bevacizumab treatment + Akkermansia probiotics	单臂队列研究
NCT05094167	江西省肿瘤医院	2021/10/19	46	非小细胞肺癌	Carilizumab+Platinum ± Lactobacillus Bifidobacterim V9(Kex02)	RCT研究

This project employs a 1 :1 randomized controlled trial (dr.:control) allocation, with an expected complete remission rate of 30% in the control group and 66% in the experimental group, and a dropout rate of 8%. The final required sample size is 50 cases, with 25 cases in the experimental group and 25 cases in the control group.

Tests for Two Proportions**Numeric Results for Testing Two Proportions using the Likelihood Ratio Test**Hypotheses: $H_0: P_1 - P_2 \geq 0$ vs. $H_1: P_1 - P_2 < 0$

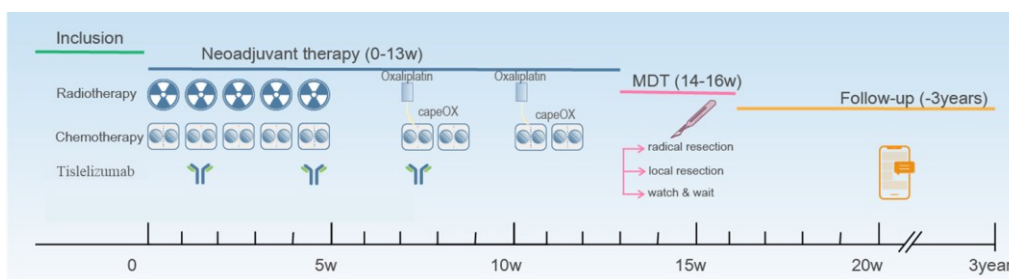
Target Power	Actual Power*	N1	N2	N	P1	P2	Diff D1	Alpha
0.8	0.80407	23	23	46	0.3	0.66	-0.36	0.05

4. Test subjects

The target population of this study is patients with pathologically confirmed locally advanced mid-to-low rectal cancer who meet the indications for neoadjuvant chemoradiotherapy, are expected to receive neoadjuvant therapy based on chemoradiotherapy combined with PD-1 monoclonal antibody therapy, and are deemed to have surgical or organ-sparing potential after evaluation by a multidisciplinary team (MDT). The study focuses on evaluating changes in gut microbiota, efficacy, and safety in these patients after receiving adjuvant therapy with the probiotic Probio-M9.

5 Intervention measures

- **Experimental group (Group A)** : Neoadjuvant chemoradiotherapy + PD-1 monoclonal antibody (tislelizumab) + Probio-M9 (oral, once daily);
- **Control group (Group B)** : Neoadjuvant chemoradiotherapy + PD-1 monoclonal antibody (tislelizumab) + placebo (oral, once daily).



Experimental group: Neoadjuvant chemoradiotherapy (50 Gy/25 fractions, capecitabine 850-1000 mg/m², BID, PO, D1-D5, QW) was administered from week 1 to week 5. After radiotherapy, the patient rested for two weeks and then received two cycles of Capeox treatment (q3w; Day 1: Oxaliplatin, 130 mg/m² · IV.gtt; Day 1-Day 14: Capecitabine, 850-1000 mg/m² · BID, PO). Tislelizumab (200 mg, IV.gtt, Q3W) was administered on week 2, week 5, and week 8, respectively. Probio-M9 (2 g, QD) was administered once daily starting from week 1 .

Control group: Neoadjuvant chemoradiotherapy (50 Gy/25 fractions, capecitabine 850-1000 mg/m² · BID, PO, D1-D5, QW) was administered from week 1 to week 5. After radiotherapy, a two-week rest period was followed by two cycles of Capex therapy (q3w; Day 1: Oxaliplatin, 130 mg/m² · IV.gtt; Day 1-Day 14: Capecitabine, 850-1000 mg/m² · BID, PO). Tislelizumab (200 mg, IV.gtt, Q3w) was administered on week 2, week 5, and week 8, respectively. A placebo (2 g, QD) was administered once daily starting from week 1.

6. Primary and Secondary Observation Indicators

Primary endpoint :

- 1) Major complete response rate (including clinical complete response

rate cCR and pathological complete response rate pCR)

Secondary endpoint :

Table 1. AJCC 8th Edition Tumor Regression Criteria

Modified Ryan Scheme for Tumor Regression Score ²	
Description	Tumor Regression Score
No viable cancer cells (complete response)	0
Single cells or rare small groups of cancer cells (near complete response)	1
Residual cancer with evident tumor regression, but more than single cells or rare small groups of cancer cells (partial response)	2
Extensive residual cancer with no evident tumor regression (poor or no response)	3

Table 2 MSKCC Tumor Regression Criteria

表2 MSKCC肿瘤退缩标准			
	完全退缩	接近完全退缩	不完全退缩
内镜标准	<ul style="list-style-type: none"> 肠壁光滑或有白色斑痕 毛细血管扩张 无溃疡 无结节样改变 	<ul style="list-style-type: none"> 黏膜不平整 小黏膜结节或局灶黏膜异形改变 表浅的溃疡 红斑样瘢痕 	<ul style="list-style-type: none"> 肉眼可见肿瘤残留
肛门指检	<ul style="list-style-type: none"> 正常 	<ul style="list-style-type: none"> 光滑的硬结或轻微黏膜异常 	<ul style="list-style-type: none"> 可触及肿瘤结节
MRI-T2加权	<ul style="list-style-type: none"> 仅见黑色连续的T2信号 并且 无可见淋巴结 	<ul style="list-style-type: none"> 大部分为黑色的T2信号，部分区域T2信号不连续 和（或） 淋巴结部分退缩 	<ul style="list-style-type: none"> 中等信号为主，无T2疤痕 和（或） 无淋巴结退缩
MRI-DWI	<ul style="list-style-type: none"> B800-B1000信号无可见肿瘤 和（或） ADC为缺失或低信号 肿瘤上方的肠壁可出现一致的线形信号 	<ul style="list-style-type: none"> B800-B1000信号见肿瘤明显退缩 和（或） ADC极少量残留信号 	<ul style="list-style-type: none"> B800-B1000信号未见肿瘤明显退缩 和（或） ADC明显低信号

注：MSKCC：纪念斯隆-凯特琳癌症中心；ADC：表面扩散系数

1) Validity

Pathological response rate (MPR); objective response rate (ORR, based on RECIST 1.1 criteria); disease-free survival (DFS); overall survival (OS); organ preservation rate (OPR); neoadjuvant therapy score for rectal cancer (NAR); quality of life score (QoL).

2) Security

3.1 Adverse events (AEs), including type, incidence, grade (judged according to NCI-CTCAE V5.0 criteria), severity, and relevance to the investigational drug;

3.2 Abnormal values of laboratory indicators, type, incidence, and grade (judged according to NCI-CTCAE V5.0 standards); vital signs, including blood pressure, pulse, respiratory rate, body temperature, and PS scores of ECG and ECOG.

3) Changes in fecal intestinal flora (lactobacters, bifidobacteria, etc.).

7. Safety and effectiveness evaluation

- **Safety assessment** : Collect data on adverse events (AEs), serious adverse events (SAEs), abnormal laboratory indicators, and changes in vital signs;
- **Efficacy evaluation** : The efficacy was comprehensively assessed by imaging, endoscopy, and surgical pathology. All pathological results were independently interpreted by two experienced rectal cancer pathologists, back to back. If the results differed significantly, a third expert was invited to review them. The pathological evaluation adopted the AJCC 8th edition of the diagnostic criteria for rectal cancer: tumor size, histological classification, depth of invasion, lymph node retrieval and number of metastases, whether the surgical margin was positive, tumor regression grade TRG, ypTNM staging, etc.

8. Statistical Analysis Methods

This study primarily employs intention-to-treat (ITT) analysis, with per-protocol (PP) used for sensitivity analysis.

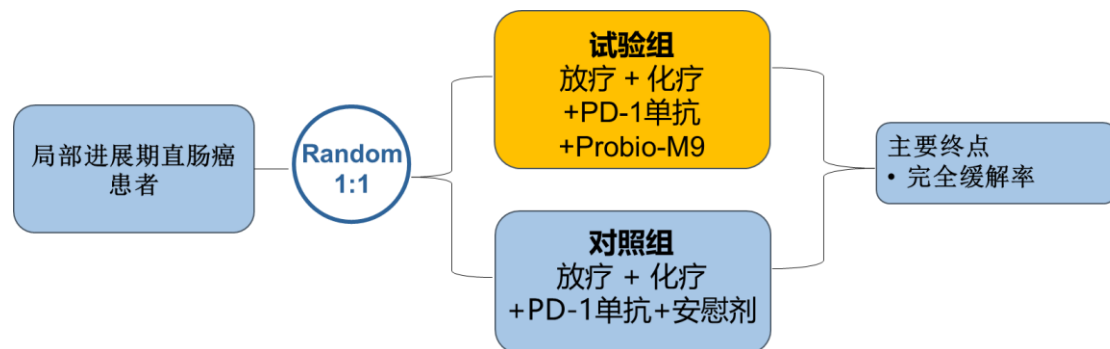
Primary endpoint: To compare changes in gut microbiota abundance between the experimental and control groups. Initially, an independent samples t-test or analysis of covariance (ANCOVA, adjusted baseline) was used to select an appropriate parametric or non-parametric method based on the distribution.

Secondary endpoints included radiological response rate, pathological response rate, organ preservation rate, and disease-free survival rate. Response rates were compared using the χ^2 test or Fisher's exact test; survival data were estimated using the Kaplan-Meier method with parallel log-rank tests, and multivariate analysis was performed using a Cox proportional hazards model when necessary.

Safety endpoint: Descriptive statistical methods were used to calculate the frequency and percentage of adverse events and to compare differences between groups.

Significance level: All tests were two-tailed, with a significance level of $\alpha = 0.05$.

V. Technical Approach



VI. Selection/Exclusion Criteria and Withdrawal/Termination Criteria for

Trial Subjects

Inclusion criteria:

1. Sign a written informed consent form and voluntarily participate in this study;
2. Age 18-75 years old, gender not limited;
3. Histopathologically confirmed rectal adenocarcinoma;
4. Clinical stage II-III as assessed by MRI (according to AJCC 8th edition);
5. The lower edge of the tumor is $\leq 10\text{cm}$ from the anal verge;
6. Surgically resectable;
7. Able to swallow pills normally;
8. ECOG PS 0-1;
9. Has not previously received any anti-tumor treatment for rectal cancer, including radiotherapy, chemotherapy, surgery, etc.;
10. Surgical treatment is planned after neoadjuvant therapy is completed;
11. No contraindications to surgery;
12. Major organ functions are normal, including:
 - a) Complete blood count (no blood transfusions or blood products administered within 14 days prior to the first treatment, and no G-CSF or other hematopoietic stimulating factors used for correction):
 - Neutrophil count $\geq 1.5 \times 10^9 / \text{L}$

- Platelet count $\geq 100 \times 10^9 /L$

- Hemoglobin ≥ 90 g/L

b) Blood biochemistry tests:

Total bilirubin $\leq 1.5 \times \text{ULN}$

ALT $\leq 2.5 \times \text{ULN}$, AST $\leq 2.5 \times \text{ULN}$,

- Serum creatinine $\leq 1.5 \times \text{ULN}$, or creatinine clearance ≥ 50 mL/min

(Cochcroft-Gault formula)

c) Coagulation function:

- International Normalized Ratio (INR) $\leq 1.5 \times \text{ULN}$

- Activated partial thromboplastin time (APTT) $\leq 1.5 \times \text{ULN}$

Female subjects of childbearing age must undergo a serum pregnancy test within 72 hours prior to the start of administration of the study drug, and the result must be negative;

Exclusion criteria:

1. History of allergy to monoclonal antibodies, any component of PD-1 monoclonal antibodies, capecitabine, or oxaliplatin;

2. Have previously received or are currently receiving any of the following treatments:

a) Any surgical, radiotherapy, chemotherapy, targeted therapy, immunotherapy, etc. treatment for tumors;

b) The patient has been using immunosuppressive drugs or systemic hormones for immunosuppression (dose > 10 mg/day prednisone or

equivalent) within 2 weeks prior to the first use of the study drug; in the absence of active autoimmune disease, inhaled or topical steroids and corticosteroids at doses > 10 mg/day prednisone or equivalent may be used as replacements.

c) Received a live attenuated vaccine within 4 weeks prior to the first use of the investigational drug;

d) Underwent major surgery or suffered severe trauma within 4 weeks prior to the first use of the investigational drug;

3. Patients with any active autoimmune disease or a history of autoimmune disease, including but not limited to: interstitial pneumonia, enteritis, hepatitis, hypophysitis, vasculitis, nephritis, hyperthyroidism, hypothyroidism (can be considered after hormone replacement therapy); patients with psoriasis or childhood asthma/allergy that has been completely remitted and requires no intervention in adulthood can be considered, but patients requiring medical intervention with bronchodilators should not be included;

4. History of immunodeficiency, including HIV test positive, or other acquired or congenital immunodeficiency diseases, or history of organ transplantation or allogeneic bone marrow transplantation;

5. The presence of uncontrolled cardiac clinical symptoms or diseases, including but not limited to: (1) NYHA class II or above heart failure, (2) unstable angina, (3) myocardial infarction within 1 year, (4) clinically

significant supraventricular or ventricular arrhythmias that are not controlled by clinical intervention or are still poorly controlled after clinical intervention.

6. Severe infection (CTCAE > 2) that occurred within 4 weeks prior to the first use of the study drug, such as severe pneumonia, bacteremia, or infectious complications requiring hospitalization; baseline chest imaging showing active lung inflammation; symptoms and signs of infection within 14 days prior to the first use of the study drug; or patients requiring oral or intravenous antibiotic treatment, excluding those who used prophylactic antibiotics.

7. Those who have been found to have active pulmonary tuberculosis infection through medical history or CT examination, or who have a history of active pulmonary tuberculosis infection within 1 year prior to enrollment, or who have a history of active pulmonary tuberculosis infection more than 1 year ago but have not received regular treatment;

8. Possesses active hepatitis B (HBV DNA \geq 2000 IU/mL or 104 copies/mL) or hepatitis C (positive hepatitis C antibody, and HCV RNA above the detection limit of the analytical method);

9. Patients who have been diagnosed with other malignancies within 5 years prior to their first use of the investigational drug may be considered for enrollment unless they have a low risk of metastasis or death (5-year survival rate >90%), such as well-treated basal cell carcinoma of the skin,

squamous cell carcinoma of the skin, or cervical carcinoma in situ.

10. Pregnant or breastfeeding women;

11. The researchers determined that there were other factors that might force the study to be terminated midway, such as other serious illnesses (including mental illnesses) requiring combined treatment, alcoholism, substance abuse, family or social factors that may affect the safety or compliance of the participants.

Exit Criteria

1. The following withdrawals from the study were due to patient non-compliance and reasons not related to the treatment itself.

2. Subjects were found to be ineligible for inclusion/exclusion criteria after enrollment;

3. Serious violation of test protocol requirements;

4. The simultaneous use of other treatments affected the evaluation;

5. Poor patient compliance;

6. Patients who require emergency surgical resection due to intestinal obstruction, intestinal perforation, intestinal bleeding, or other reasons after enrollment in the study;

7. Patients who, after being enrolled in the study, experience severe allergic reactions or intolerance to chemotherapy, radiotherapy, or immunotherapy;

8. Patients who become pregnant during the trial period or at least 3

months after the last dose after enrollment in the study ;

9. Patients who request to withdraw from the study cohort for various reasons after being enrolled;

10. Patients who have other non-tumor conditions that prevent them from continuing this treatment regimen;

11. Patients who, after being enrolled in the study , took other immunosuppressants and other probiotics for various reasons ;

1.2 . Patients who are unable to complete the research plan after being selected for the study for various reasons (if a patient withdraws from the trial, the reason and time of withdrawal should be recorded in the CRF form).

VII. Contingency Plan for Handling Common Adverse Reactions/Serious Adverse Reactions

Adverse event classification is detailed in Appendix 1.

Contingency Plan

1. Adverse Event Handling

- All subjects were required to be closely monitored for adverse events (AEs) and serious adverse events (SAEs) during the trial.
- For immune-related adverse reactions (such as immune colitis, hepatitis, thyroid dysfunction, etc.), they should be graded and managed in accordance with the Society for Immunotherapy of Cancer (SITC) guidelines for the management of adverse reactions to

immunotherapy; for immune-related adverse reactions of grade ≥ 3 , PD-1 monoclonal antibody should be suspended and immunosuppressive therapy such as glucocorticoids should be given.

- The toxicities associated with radiotherapy and chemotherapy (such as bone marrow suppression, intestinal reactions, and skin damage) should be assessed according to the CTCAE v5.0 standard, and the dosage should be adjusted or treatment delayed if necessary.
- For probiotic-related adverse reactions (such as bloating, diarrhea, infection), discontinue probiotic use according to the severity and provide symptomatic and supportive treatment.

2. Contingency Plan for Trial Termination/Withdrawal

- If a patient experiences intolerable adverse reactions, significant disease progression, or if the investigator assesses that the risks of continuing the trial outweigh the benefits, the trial treatment should be terminated immediately.
- When a patient requests to withdraw from the trial, their wishes should be respected, and efforts should be made to complete the final efficacy and safety assessment, i.e., the primary and secondary endpoints.
- The reasons for exiting or terminating must be recorded in detail and included in the security analysis set.

3. Intervention interruption or alternative solutions

- If intervention measures are interrupted temporarily due to force majeure (such as an epidemic or insufficient drug supply), the intervention measures can be resumed according to the patient's specific condition , and a risk assessment record should be made.
- If necessary, surgery or other standard treatment procedures can be initiated earlier after MDT assessment, and intention-to-treat (ITT) analysis can be included in the outcome analysis.

4. Abnormalities in drug/probiotic supply and management

- If the investigational drug or probiotic has quality problems or supply disruptions, the intervention should be immediately suspended and reported to the ethics committee and the sponsor, while a temporary alternative supply plan should be developed.

5. Safety Emergency Handling

- In the event of a life-threatening serious adverse event, standard first aid measures should be administered immediately, and the incident should be reported to the ethics committee and the national adverse drug reaction monitoring system.
- Emergency unblinding may be performed if necessary to allow for targeted medical intervention (if the design is double-blind).

VIII. Quality Control

(1) Surgical standardization: The surgery shall be performed by an experienced surgeon, and all key steps of the surgery shall be documented

and traceable. Surgeons performing open surgery shall have at least 30 cases of open surgery experience. Surgeons performing laparoscopic surgery shall have at least 30 cases of open surgery and 30 cases of laparoscopic surgery experience respectively.

(2) Enrolled patients shall undergo the required examinations, treatments, and evaluations, and these shall be recorded in the CRF form. All forms must be completed completely and signed and dated by the person completing them. The study supervisor shall collect the enrollment case information and CRF forms from each center each month, urge each center to complete the CRF forms, and review and sign them. If the forms are not completed, the reason must be stated.

(3) Clinical data and follow-up quality control: Follow-up is carried out by experienced professional follow-up personnel; clinical data is improved by gastrointestinal surgeons.

Nine Data Statistical Methods

Public health statisticians and principal investigators developed and documented a statistical analysis plan based on the research protocol. Statistical analyses were then conducted according to the plan. Efficacy analyses employed both intention-to-treat (ITT) principles and evaluable case analysis methods. The analysis was performed using computer software (IBM SPSS 25.0 or Prism 7.0). For descriptive statistics, rates were used for categorical data, and means and 95% confidence intervals

were used for continuous data. For comparisons between two groups, t-tests were used for continuous data, and χ^2 tests were used for categorical data. Survival analyses employed Kaplan-Meier and Log Rank methods, and prognostic analyses used a Cox proportional hazards model. At all stages, probability p-values were used for statistical inference, and a statistical significance threshold of 0.05 was used.

Appendix 1: CTCAE-based grading of immune-related adverse events caused by immune checkpoint inhibitor therapy and timing of discontinuation, and the Clinical Practice Guidelines of the Society for Cancer Immunotherapy (SITC) regarding adverse reactions.

I. General Principles of the Clinical Practice Guidelines on Adverse Reactions from the Society for Cancer Immunotherapy (SITC):

Patients should be encouraged to use contraception while receiving immunotherapy. Fertility issues should be discussed before treatment.

Before initiating ICI treatment, the following tests should be performed: complete blood count (CBC) and differential, comprehensive metabolic assessment (CMP), thyroid-stimulating hormone (TSH), and free thyroxine (fT4). Urinalysis should be considered to assess underlying kidney disease.

Baseline electrocardiogram (EKG) should be performed in patients at high risk of myocarditis (e.g., those with coexisting heart disease, diabetes, or those using combined anti-PD-(L)1 and anti-CTLA-4 ICI therapy). Baseline troponin measurement can also provide information for assessing potential future cardiotoxicity.

When starting hormone therapy, patients should be specifically asked about potential toxic reactions, including hyperglycemia, mood disorders, insomnia, gastritis, weight gain, and opportunistic infections (such as *Pneumocystis carinii* pneumonia).

During ICI treatment, complete blood cell count, CMP, TSH, and fT4 should be monitored intermittently.

Patients should consult with specialists for diagnosis and treatment advice in the following situations: when the toxicity reaction is grade ≥ 3 ; when any level of toxicity reaction is unresponsive to hormone therapy; when hospitalization or treatment with less toxic drugs is required due to toxicity reaction, such as the occurrence of neurotoxic or rheumatic toxicity reactions (e.g., arthritis that does not affect daily life, mild pain with erythema or joint swelling).

The diagnosis of all irAEs requires exclusion of other possible causes (such as diarrhea/colitis associated with *Clostridium difficile*), and treatment for irAEs should be initiated when clinically deemed appropriate.

For patients with life-threatening autoimmune diseases, ICI treatment should only be initiated after a thorough discussion of the risks and benefits of ICI therapy between the patient and healthcare provider, and after consideration of alternative therapies. The risks of autoimmune disease flare-ups should be weighed against the benefits of ICI therapy, especially in cancer patients with a high expected incidence of adverse reactions.

Patients with a pre-existing non-life-threatening autoimmune disease should be consulted about the possibility of its onset or worsening before

receiving ICI treatment. These patients should be closely monitored for any exacerbation of their existing autoimmune disease while receiving ICIs, and consideration should be given to having appropriate professionals conduct concurrent monitoring and treatment.

Unless there are special circumstances, patients with irAEs grade 1 should continue ICI treatment under close monitoring for symptom worsening.

Unless there are special circumstances, patients who develop grade 2 irAEs should temporarily discontinue ICI treatment and be given hormone therapy according to the toxicity.

The decision to discontinue ICI treatment in patients can be complex. Factors that may lead clinicians to decide against ICI therapy include serious or life-threatening irreversible adverse events (irAEs), the need for long-term use or the use of multiple immunosuppressants, long-term use of ICI therapy, and/or patients who have achieved complete remission or demonstrated significant clinical benefit.

Unless there are special circumstances, patients who have experienced irAEs grade 2 may resume ICI treatment only when their symptoms and signs have subsided or their symptoms are controlled with ≤ 10 mg of prednisone/day (or equivalent dose).

Whether patients who have experienced grade 3 or 4 irAEs should undergo ICI treatment again requires a risk assessment based on the expected benefits of the treatment and the potential toxicity risks.

Patients with any level of myositis, myocarditis, or neurological symptoms (such as myasthenia gravis):

1. Should consult a specialist.
2. Should undergo a series of diagnostic tests, as overlapping symptoms may occur, putting the patient at higher risk.
3. Should undergo a series of laboratory tests, including: erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), creatine kinase (CK), antibody testing (acetylcholine, specific kinase (MusK), striational), aldolase, troponin, EKG, nerve conduction studies, and electromyography (EMG).
4. In addition to receiving classic irAE treatment (e.g., hormones), frequent lung evaluations are necessary.

CTCAE-based grading of immune-related adverse events and timing of discontinuation induced by immune checkpoint inhibitor therapy

1. Skin diseases

1) Maculopapular rash/dermatitis

grade	describe
1	Macules/papules covering less than 10% of the body surface area, with or without symptoms (such as itching, burning, or tightness).
2	Macules/papules cover 10%-30% of the body surface area, with or without symptoms (such as itching, burning, tightness); mild limitation of daily living abilities.
3	Macules/papules covering more than 30% of the body surface area, with or without related symptoms; limiting the ability to perform daily activities.
4	Grade 4 maculopapular rash/dermatitis is not included in CTCAE.
Timing for discontinuing medication: Level 3: Discontinue medication	

2) Skin itching

grade	describe
1	Mild or localized; localized areas require intervention.
2	Intense or widespread; intermittent attacks; skin changes caused by scratching (e.g., edema, papules, abrasions, lichenification, exudation/scabs); oral medication intervention; mild limitation of daily living abilities.
3	Intense or widespread; persistent; limiting self-care ability or

sleep restriction; requiring oral corticosteroids or immunosuppressive therapy.

4 Level 4 is not included in CTCAE

Timing for discontinuing medication: None

2. Gastrointestinal diseases

1) Colitis

grade	describe
1	Asymptomatic; clinical or diagnostic observation only; no intervention measures. [Grade 1 diarrhea frequency ≤ 4 times/day]
2	Abdominal pain; mucus or blood in stool [Grade 2 diarrhea frequency: 4–6 times/day]
3 or 4	Severe abdominal pain; changes in bowel habits; need for medical intervention; presence of peritoneal signs. [Grade 3 diarrhea frequency ≥ 7 times/day] Level 4: Life-threatening consequences; urgent intervention required.

Timing of medication discontinuation: Level 2 medication suspension

2) Hepatitis

grade	describe
1	ULN < AST, ALT < 3 × ULN; ULN < total cholesterol < 1.5 × ULN
2	3×ULN < AST, ALT $\leq 5 \times$ ULN; 1.5×ULN < Total cholesterol < 3 × ULN
3 and 4	AST, ALT > 5 × ULN; Total cholesterol > 3 × ULN

Timing of drug discontinuation: If AST/ALT increases by $\geq 50\%$ for ≥ 1 week,

discontinued.

3. Endocrine

1) Hypophysitis

grade	describe
1	Asymptomatic or mild symptoms; requiring only clinical or
2	Moderate symptoms; requiring minimal, localized, or no independently.
3	Severe symptoms or, although medically significant, no extension of existing hospitalization; completely contraindicated
4	It could have life-threatening consequences; urgent interven

Timing of discontinuation: If there is a grade ≥ 2 irAE, discontinue ICI until symptoms started.

2) Hypothyroidism

grade	describe
1	Asymptomatic; requires only clinical or diagnostic ob
2	Symptoms present; requires thyroid hormone replacem
3	Severe symptoms; requiring hospitalization; limiting a
4	It could have life-threatening consequences; urgent in

Timing of discontinuation: If there is a grade 3 or higher irAE, ICI should be suspended better.

3) Hyperthyroidism

grade	describe
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1	Asymptomatic; requires only clinical or diagnostic observation; no intervention is needed.
2	Symptoms present; requires thyroid hormone suppression therapy; mildly affects daily living abilities.
3	Severe symptoms; requiring hospitalization; limiting ability to care for oneself
4	It could have life-threatening consequences; urgent intervention is required.

Timing of drug discontinuation: \geq Grade 3 irAE

4) Type 1 diabetes (hyperglycemia)

grade	describe
1	ULN < fasting blood glucose < 8.9 mmol/L
2	8.9 < fasting blood glucose < 13.9 mmol/L
3	13.9 < fasting blood glucose < 27.8 mmol/L; hospitalization
4	Fasting blood glucose > 27.8 mmol/L; life-threatening complication

Timing of medication discontinuation: ICI should be discontinued in type 1 diabetes with diabetic ketoacidosis.

Type 1 diabetes without diabetic ketoacidosis, grade ≥ 3 , ICI should be discontinued.

4. Lungs

1) Pneumonia

grade	describe
1	Asymptomatic; only clinical or diagnostic observation required
2	Symptoms present; mildly affecting the ability to care for oneself

3	Severe symptoms; limited ability to care for oneself; require
4	Life-threatening respiratory damage; requiring emergency in
Timing for discontinuation: Level 1: Consider suspending ICI; Level 2: Susp discontinue ICI.	

2) Pulmonary sarcoidosis

grade	describe
1	Undefined in CTCAE
≥2 levels	To date, there have been no studies on the side effects of using cheo to treat sarcoidosis.
Timing for discontinuation: Consider suspending ICI for Level 1 cases; suspend ICI above.	

5. Rheumatism

1) Inflammatory arthritis

grade	describe
1	Mild pain accompanied by inflammatory symptoms (morning stiffness), skin redness, and joint swelling (which may include soft tissue swelling, joint effusion, or synovitis).
2	Moderate pain associated with inflammatory symptoms, skin erythema, joint swelling, and limited ability to perform daily activities using tools.
3	Severe pain associated with inflammatory symptoms, skin erythema, joint swelling, irreversible joint damage, disability, and limited ability to perform daily activities of self-care.

Timing for discontinuing medication: None

6. Infusion reaction

grade	describe
1	Mild, transient reaction; no intervention required.
2	Treatment or infusion interruption is required, but the patient responds rapidly to symptomatic treatment (such as antihistamines, nonsteroidal anti-inflammatory drugs, anesthetics, and intravenous infusions); prophylactic medication: ≤ 24 hours.
3	Symptoms that persist for a long time (e.g., no rapid response to symptomatic medications or discontinuation of intravenous fluids), or symptoms that recur after initial improvement, require hospitalization to prevent sequelae.
4	Life-threatening, emergency intervention

Timing of drug discontinuation: Permanent discontinuation for grade 3/4.

7. Cardiovascular diseases

grade	describe
1	Abnormalities in cardiac function-related biomarkers, including electrocardiogram (ECG).
2	Abnormal screening test results, accompanied by mild symptoms
3	Symptoms appear even with moderate abnormality detection or mild activity.

4 Moderate to severe decompensation requires intravenous medication or interventional treatment and is life-threatening.

Timing of drug discontinuation: Level 4: Permanent discontinuation of medication

8. Blood diseases

1) Anemia

grade	describe
1	Hgb < lower limit of normal -10.0 g/dL, lower limit of normal - 6.2 mmol/L; lower limit of normal -100 g/L
2	Hgb<10-8g/dl; 6.2-4.9mmol/l; 100-80g/l
3	Hgb < 8.0 g/dL; 4.9 mmol/L; 80 g/L, requires blood transfusion.
4	Life-threatening, emergency intervention

Timing of drug discontinuation: Level 4 - permanent discontinuation of medication

2) Decreased platelet count

grade	describe
1	Lower limit of normal value -75000mm ³ ; <Lower limit of normal - 75.0x10 ^{e9} /l
2	< 75000-50000mm ³ ; < 75.0-50.0x10 ^{e9} /l
3	<50000-25000mm ³ ; <50.0-25.0x10 ^{e9} /l
4	<25000mm ³ ; <25.0x10 ^{e9} /l

Timing of drug discontinuation: Significant clinical symptoms, steroid-

thrombocytopenia

9. Kidney disease

1) Nephritis

grade	describe
1	An increase in creatinine levels >0.3 mg/dL, or creatinine levels 1.5–2.0 times higher than baseline.
2	Creatinine 2-3 times higher than baseline
3	A creatinine level more than 3 times the baseline or >4.0 mg/dL requires hospitalization.
4	Life-threatening, requires dialysis

Timing of drug discontinuation: Level 4: Permanent discontinuation of medication

10. Nervous system

1) Encephalopathy/leukoencephalopathy/reversible posterior leukoencephalopathy syndrome

grade	describe
1	Mild symptoms
2	Moderate symptoms; limited ability to perform daily activities using tools.
3	Severe symptoms; limited ability to perform daily living activities and care for oneself.
4	Life-threatening, requiring emergency intervention.

Timing of drug discontinuation: Permanent discontinuation for grade 3/4.

2) Peripheral motor and sensory neuropathy

grade	describe
1	Refer to the level definitions of each disease
2	
3	
4	

Timing of drug discontinuation: Permanent discontinuation for grade 3/4.

11. Nervous system

1) Encephalopathy / leukoencephalopathy / reversible posterior leukoencephalopathy syndrome

1 2. Ophthalmology

1) Uveitis

grade	describe
1	Asymptomatic, discovered only through clinical examination
2	Anterior uveitis requires drug intervention.
3	Posterior uveitis or panuveitis
4	Blindness in the affected eye (equal to or worse than 20/200)

Timing for discontinuing medication: Permanent discontinuation for levels 3 and 4.

2) Episcleritis

grade	describe
1	Asymptomatic, discovered only through clinical examination
2	Symptoms include moderate visual impairment (equivalent to or better than 20/40), limiting daily living activities with the use of tools.
3	Symptoms include significant visual impairment (worse than 20/40), limiting the ability to perform daily living activities and self-care.
4	Blindness in the affected eye (equal to or worse than 20/200)
Timing for discontinuing medication: Permanent discontinuation for levels 3 and 4.	

3) Blepharitis

grade	describe
CTCAE	does not define a classification.
Timing for discontinuing medication: None	