

Young Fecal Microbiota Transplantation (yFMT) Combined with Immunotherapy and Chemotherapy in Microsatellite Stable Metastatic Colorectal Cancer (MSS mCRC): An Exploratory Study

Study Site: The First Affiliated Hospital of Xiamen University

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PROTOCOL SUMMARY

Study Title	Young Fecal Microbiota Transplantation (yFMT) Combined with Immunotherapy and Chemotherapy in Microsatellite Stable Metastatic Colorectal Cancer (MSS mCRC): An Exploratory Study
Study Objectives	<p>Primary Objective: To evaluate the safety of young fecal microbiota transplantation (yFMT) combined with immunotherapy and chemotherapy in patients with microsatellite stable metastatic colorectal cancer (MSS mCRC).</p> <p>Secondary Objectives: To explore the efficacy of yFMT combined with immunotherapy and chemotherapy in MSS mCRC patients, its effects on the immune microenvironment, and the synergistic mechanisms with immunotherapy and chemotherapy.</p>
Study Design	<p>(1) Study Type</p> <p>Design: Single-center, single-arm study.</p> <p>Group: yFMT combination therapy group (10 subjects): receiving yFMT combined with immunotherapy and chemotherapy.</p> <p>(2) Study Flow</p> <p>Screening Period (2 weeks): Baseline assessment to confirm eligibility.</p> <p>Treatment Period (3 months): Subjects receive corresponding</p>

	<p>treatment.</p> <p>Follow-up Period (9 months): Regular assessment of treatment efficacy and safety.</p> <p>(3) Study Endpoints</p> <p>Primary Endpoints: Incidence of serious adverse events (SAEs), incidence of treatment-related adverse events (TRAEs), and rate of intervention modification due to adverse events.</p> <p>Secondary Endpoints: Progression-free survival (PFS), objective response rate (ORR), overall survival (OS).</p> <p>(4) Sample Size</p> <p>This study plans to enroll 10 patients. The sample size is not based on statistical power calculations but on the exploratory nature of the study and ethical risk control principles, aiming to evaluate technical feasibility, obtain preliminary safety data, and provide basis for subsequent study design.</p> <p>(5) Intervention</p>
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	<p>yFMT combination therapy group: yFMT treatment every 2 weeks for a total of 6 times; concurrent immunotherapy (PD-1 inhibitor) and chemotherapy (FOLFIRI).</p> <p>(6) Study Schedule</p> <p>Visit Schedule:</p> <p>Screening Period: Complete baseline assessment.</p> <p>Treatment Period: Follow-up at each visit.</p> <p>Follow-up Period: Follow-up at 1, 3, 6, and 9 months after yFMT.</p> <p>Discontinuation Criteria: Serious adverse events, disease progression, or poor compliance.</p>
Study Population	<p>Sample size of 10 subjects, aged 18-75 years, both genders, all with microsatellite stable metastatic colorectal cancer, progressed after first-line chemotherapy and targeted therapy, from Xiamen and surrounding areas.</p>
Eligibility Criteria	<p>Inclusion Criteria:</p> <p>1) Age ≥ 18 years and ≤ 75 years, male or non-pregnant female;</p>

	<p>2) ECOG performance status score 0-1;</p> <p>3) Pathologically confirmed microsatellite stable metastatic colorectal cancer;</p> <p>4) Progression after failure of first-line chemotherapy and targeted therapy;</p> <p>5) Women of childbearing potential must have negative urine or serum pregnancy test within 7 days before enrollment and agree to use highly effective contraception during the study and for at least 120 days after the last dose;</p> <p>6) Non-sterilized males must agree to use highly effective contraception during the study and for at least 120 days after the last dose;</p> <p>7) Patient or family member able to understand the purpose of the trial, voluntarily participate, and sign informed consent;</p> <p>8) Subjects must have adequate organ function to tolerate immunotherapy combined with chemotherapy regimen.</p>
	<p>Exclusion Criteria:</p> <p>1) Previous fecal microbiota transplantation treatment;</p> <p>2) Severe cardiac, pulmonary, hepatic, or renal dysfunction;</p> <p>3) History of other malignant tumors;</p> <p>4) Psychiatric disorders or cognitive impairment unable to cooperate with the study;</p> <p>5) Complicated with intestinal obstruction, perforation, or bleeding requiring emergency surgery;</p> <p>6) Pregnant or lactating women;</p> <p>7) Patient or family member unable to understand the conditions and objectives of this study, unwilling to sign informed consent.</p>
Study Intervention	Intervention: yFMT combined with immunotherapy and standard chemotherapy regimen (FOLFIRI).

	Assessment methods: Efficacy evaluated through imaging examinations (CT/MRI), tumor marker detection, and immune microenvironment assessment.
Efficacy Evaluation	<p>Primary Efficacy Indicators: Incidence of serious adverse events (SAEs), incidence of treatment-related adverse events (TRAEs), and rate of intervention modification due to adverse events.</p> <p>Secondary Efficacy Indicators: Progression-free survival (PFS), objective response rate (ORR), overall survival (OS).</p>
Methods	<p>Primary Endpoint Analysis</p> <p>SAE incidence, TRAE incidence, intervention modification rate: Descriptive statistical methods used, calculating incidence rates and 95% confidence intervals (binomial distribution method); stratified description by system classification or intervention-related category.</p> <p>Correlation analysis: If sample size permits, Fisher's exact test used to analyze association between baseline characteristics (age, gender, tumor burden) and SAE/\geqGrade 3 TRAE occurrence.</p> <p>Secondary Endpoint Analysis</p> <p>Progression-free survival (PFS), overall survival (OS): Kaplan-Meier method used to plot survival curves, calculating median survival time and 95% confidence interval; if exploring influencing factors, Cox proportional hazards regression model used for univariate and multivariate analysis (including baseline age, gender, ECOG score as potential covariates).</p> <p>Objective response rate (ORR): Calculate ORR and 95% confidence interval (binomial distribution method); stratified analysis by baseline characteristics, describing efficacy differences among different subgroups.</p>

Study Duration	From study initiation to data analysis completion is estimated to be 2 years.
Subject Participation Duration	Each subject is expected to complete all follow-ups in approximately 12 months.
Study Site/Location	Department of Gastrointestinal Oncology, The First Affiliated Hospital of Xiamen University

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1. Background

(I) Current Research Status Domestically and Internationally

In recent years, tumor immunotherapy has made significant progress, particularly immune checkpoint inhibitors (ICIs, represented by anti-PD-1/PD-L1 antibodies) showing good efficacy in various advanced solid tumors. In microsatellite instability-high (MSI-H) colorectal cancer, immunotherapy has demonstrated significant clinical benefits, with objective response rate (ORR) and long-term survival data significantly superior to traditional chemotherapy regimens. However, microsatellite stable (MSS) patients, accounting for approximately 95% of mCRC, show primary resistance to ICIs with ORR less than 5%. Current first-line standards remain FOLFOX/FOLFIRI combined with anti-EGFR (cetuximab or panitumumab) or anti-VEGF (bevacizumab). Once first-line treatment fails, patients enter the post-line treatment phase with median overall survival (mOS) of only 5-8 months, urgently requiring clinical solutions. Increasing evidence indicates that intestinal microecology composition is closely related to ICIs efficacy.

For example, Davar et al. (2021) demonstrated that fecal microbiota transplantation (FMT) can significantly improve response rates to immunotherapy in patients with advanced solid tumors. Additionally, Zackular et al. (2020) Phase I clinical trial found that FMT combined with anti-PD-1 therapy showed good safety and efficacy in patients with advanced solid tumors. Zhao et al. (2023) Phase II clinical trial (RENMIN-215) systematically evaluated the efficacy and safety of FMT combined with tislelizumab (anti-PD-1) and fruquintinib (anti-angiogenic TKI) in refractory MSS mCRC population. Results showed that FMT plus tislelizumab and fruquintinib as third-line and above treatment improved survival in refractory MSS mCRC with manageable safety, providing a valuable new treatment option for this patient population. This study also found that treatment responders had specific microbiota characteristics (such as higher abundance of Proteobacteria and Lachnospiraceae), further confirming the great potential of FMT combination regimens in refractory MSS mCRC. Furthermore, Yu et al. (2023) established a microbiota dysbiosis

CRC mouse model and evaluated the effect of fecal microbiota transplantation (FMT) on CRC progression, ultimately proving that FMT inhibits colorectal cancer development by reversing intestinal microbial disease, alleviating excessive intestinal inflammation, and cooperating with anti-cancer immune responses. These studies provide important evidence for the application of FMT in tumor immunotherapy.

(II) Preliminary Research Foundation

Scientific Literature Summary:

Multiple studies have shown that specific beneficial bacteria (such as Bifidobacterium, Firmicutes) can enhance immune cell activity and promote anti-tumor immune responses. For example, Sivan et al. (2015) found that Bifidobacterium can significantly enhance anti-PD-L1 treatment efficacy.

Intestinal microbiota composition is closely related to immunotherapy efficacy. Routy et al. (2018) found that the presence of specific microbiota is associated with better immunotherapy responses. Gopalakrishnan et al. (2018) further confirmed the relationship between intestinal microbiota and PD-1 immunotherapy efficacy. Davar et al. (2022) found that intestinal microbiota is an "adjustable switch" for immune checkpoint inhibitor efficacy, providing immediately actionable new combination strategies for cancer immunotherapy. De et al. (2021) showed that microbial communities from the gut, oral cavity, and even tumor microenvironment can shape carcinogenic pathways, modulate immune activity, and alter chemotherapy and immunotherapy efficacy. Additionally, Zhu et al. (2025) found that butyrate-producing bacteria and high levels of SCFAs are "universal gain factors" for ICB efficacy across cancer types, while microbial metabolite PAGln is a newly discovered resistance mediator. Targeting PAGln reduction and SCFA enhancement can become a precise microecological strategy to improve immunotherapy response rates.

Laboratory Work:

Our research team has verified the regulatory effects of yFMT on the immune microenvironment through in vitro experiments and animal models in preliminary laboratory

work. Experimental results showed that yFMT can significantly alter intestinal microbiota composition and enhance immune cell activity.

Animal Experiment Results:

In animal models, we found that yFMT can significantly improve immunotherapy efficacy. Through flow cytometry and immunohistochemistry, we observed that yFMT can increase CD8⁺ T cell infiltration in tumor tissue and reduce the proportion of regulatory T cells.

Preclinical Summary:

Preliminary preclinical research results indicate that yFMT combined with immunotherapy shows good anti-tumor effects in animal models. These studies provide a theoretical basis for yFMT clinical application.

(III) Current Clinical Application Status

Currently, FMT in clinical application is mainly used for treating *Clostridioides difficile* infection (CDI) and has achieved significant efficacy. In recent years, FMT application in tumor treatment has gradually gained attention. For example, Davar et al. (2021) showed that FMT can significantly improve response rates to immunotherapy in patients with advanced solid tumors. Additionally, Zackular et al. (2020) Phase I clinical trial also confirmed the safety and efficacy of FMT combined with anti-PD-1 therapy in patients with advanced solid tumors.

(IV) Value and Significance of the Topic

Clinical Need:

MSS mCRC patients show poor response to immunotherapy with poor prognosis. Exploring new treatment methods to improve immunotherapy efficacy has important clinical significance.

Scientific Value:

Intestinal microbiota is closely related to immunotherapy efficacy. Regulating intestinal microbiota through FMT is expected to provide new strategies for immunotherapy.

Social Significance:

Improving treatment outcomes and quality of life for MSS mCRC patients has important social significance.

In summary, this study aims to explore the application value of yFMT combined with immunotherapy and chemotherapy in MSS mCRC patients, providing new ideas and methods for clinical treatment.

2. Study Objectives

1. Primary Objective: To evaluate the safety of young fecal microbiota transplantation (yFMT) combined with immunotherapy and chemotherapy in patients with microsatellite stable metastatic colorectal cancer (MSS mCRC).

2. Secondary Objectives: To explore the efficacy of yFMT combined with immunotherapy and chemotherapy in microsatellite stable metastatic colorectal cancer (MSS mCRC) and its effects on patient immune microenvironment, as well as its synergistic mechanisms with immunotherapy and chemotherapy.

3. Study Design Type, Principles, and Procedures

3.1 Overall Design

Hypothesis: yFMT combined with immunotherapy and chemotherapy can significantly improve progression-free survival (PFS) in MSS mCRC patients while ensuring safety (adverse event incidence), and increase objective response rate (ORR) and overall survival (OS).

Study Phases: Screening period (2 weeks), Treatment period (3 months), Follow-up period (9 months).

Specific Study Design Description: Single-center, prospective, single-arm study.

Bias Reduction Methods: Reduce bias through strict inclusion and exclusion criteria.

Study Intervention Duration: yFMT combination therapy study intervention duration is 3 months.

Study Intervention Method: yFMT combination therapy group, receiving yFMT combined with immunotherapy and chemotherapy.

3.2 Definition of Study Endpoints

If subjects complete all phases of the study or follow-up according to the protocol, or withdraw informed consent, they reach the study endpoint.

3.3 Sample Size Determination

Sample size is not based on statistical hypothesis testing but determined according to the following scientific and ethical principles:

Exploratory Study Positioning: As this center's first introduction of FMT combined with systemic anti-tumor treatment, this study's primary goal is to establish standardized operation procedures, evaluate intervention feasibility, and identify potential safety signals. Early phases typically adopt small sample designs.

Ethics and Risk Control: FMT in tumor treatment remains experimental. Limiting enrollment to 10 subjects can obtain key preliminary data while minimizing exposure to uncertain risks, consistent with the principle of minimal risk.

Therefore, this study plans to enroll 10 patients. This sample size is scientifically sufficient to support feasibility assessment and preliminary safety analysis, and provide basis for subsequent formal clinical trial design and sample size estimation.

3.4 Study Procedures



4.Study Subjects

Inclusion Criteria:

Age ≥ 18 years and ≤ 75 years, male or non-pregnant female;

ECOG performance status score 0-1;

Pathologically confirmed microsatellite stable metastatic colorectal cancer;

Progression after failure of first-line chemotherapy and targeted therapy;

Women of childbearing potential must have negative urine or serum pregnancy test within 7 days before enrollment and agree to use highly effective contraception during the study and for at least 120 days after the last dose;

Non-sterilized males must agree to use highly effective contraception during the study and for at least 120 days after the last dose;

Patient or family member able to understand the purpose of the trial, voluntarily participate, and sign informed consent;

Subjects must have adequate organ function to tolerate immunotherapy combined with chemotherapy regimen.

Exclusion Criteria:

Previous fecal microbiota transplantation treatment;

Severe cardiac, pulmonary, hepatic, or renal dysfunction;

History of other malignant tumors;

Psychiatric disorders or cognitive impairment unable to cooperate with the study;

Complicated with intestinal obstruction, perforation, or bleeding requiring emergency surgery;

Pregnant or lactating women;

Patient or family member unable to understand the conditions and objectives of this study, unwilling to sign informed consent.

Withdrawal Criteria:

Found not meeting inclusion criteria or meeting exclusion criteria before treatment;

Serious adverse events or disease progression during treatment, unable to continue study participation;

Voluntary withdrawal from study or loss to follow-up.

Study Termination Criteria:

Subject experiences serious adverse events affecting safety;

Disease progression, unable to benefit from study intervention;

Poor subject compliance, unable to complete treatment or follow-up according to study protocol.

Subject Recruitment

Considerations:

Subject Source: Inpatients, outpatients.

Recruitment Location: Department of Gastrointestinal Oncology, The First Affiliated Hospital of Xiamen University.

Identification and Acquisition of Potential Subjects: Screen eligible patients through hospital information system, preliminary assessment by research team.

Planned Recruitment Methods: Hospital internal promotion, department promotion, patient referral, etc.

Measures to Enhance Subject Compliance: Regular follow-up reminders, transportation subsidies, establishment of good doctor-patient communication, etc.

Vulnerable Population Inclusion Strategy: This study does not involve pregnant women, children, or other vulnerable populations.

Economic Compensation or Incentive Measures: No economic compensation, but necessary medical support and follow-up services provided.

Subject Allocation Method

This study is single-arm design without control group. All subjects meeting inclusion criteria will be assigned to the yFMT combined immunotherapy group. Each enrolled subject will be assigned a unique study number, coded according to consecutive enrollment sequence, used

for identification and data management.

5. Study Intervention

Study Intervention Description

yFMT combination therapy group: yFMT treatment every 2 weeks for a total of 6 times; concurrent immunotherapy and chemotherapy.

Dosage and Administration Method

- yFMT: Single dose of 50 mL fecal bacteria solution via nasogastric tube infusion followed by oral FMT capsules, 12 capsules single dose. Every 2 weeks for a total of 6 times.
- Immunotherapy: PD-1 inhibitor (sintilimab provided as donation), every 2 weeks, dose calculated according to patient weight.
- Chemotherapy: FOLFIRI regimen, every 2 weeks per cycle.

Clinical and Laboratory Examination Items and Frequency

Required examinations at each follow-up: Physical examination, complete blood count, liver and kidney function, tumor markers, intestinal microbiota composition analysis, immune microenvironment analysis.

Responsibility

Provision Method: Study intervention-related expenses provided by research team.

Research team will ensure quality and safety of all drugs and devices.

Disposal Plan: Expired or unused drugs will be disposed according to hospital drug management regulations, ensuring compliance with relevant regulations and ethical requirements.

Composition, Appearance, Packaging, and Labeling

Study Intervention: yFMT bacterial solution prepared by professional laboratory, packaged in sterile containers, labels indicating dosage, preparation date, expiration date, etc.

Immunotherapy drugs and chemotherapy drugs provided by relevant drug suppliers, packaging and labeling compliant with drug management regulations.

Manufacturer: yFMT bacterial solution provided by Meiyitian Biopharmaceutical (Wuhan) Co., Ltd.; immunotherapy drugs and chemotherapy drugs provided by relevant drug suppliers.

Product Storage and Stability

yFMT Bacterial Solution: Must be stored at -80°C, avoid repeated freeze-thaw cycles, ensure stability and activity of bacterial solution.

Immunotherapy Drugs: According to drug instructions, usually need to be stored at 2-8°C, protected from light.

Chemotherapy Drugs: According to drug instructions, usually need to be stored at room temperature, protected from light and high temperature.

Preparation

yFMT Bacterial Solution: Needs to be thawed under sterile conditions before use, diluted and mixed according to standard operating procedures.

Immunotherapy Drugs: Dissolved and diluted according to drug instructions, ensure drug concentration meets treatment requirements.

Chemotherapy Drugs: Prepared according to drug instructions, ensure drug concentration and dosage accuracy.

Follow-up Schedule:

Screening Period: Complete baseline assessment.

Treatment Period: Follow-up at each visit.

Follow-up Period: Follow-up at 1, 3, 6, and 9 months after yFMT.

Measures to Improve Compliance:

Regular Follow-up Reminders: Remind subjects to attend visits on time through phone calls, text messages, etc.

Transportation Subsidies: Reduce economic burden on subjects.

Establish Good Doctor-Patient Communication: Timely answer subjects' questions and concerns, enhance subjects' sense of participation and trust.

Study Intervention Commitment

Research Team: Will strictly implement study intervention measures according to study protocol, ensure accuracy and traceability of the study.

Subjects: Need to complete treatment process as required and record relevant conditions.

Research team will use electronic monitoring devices to record subjects' treatment status and require subjects to complete medication diaries and other documents.

Source Documents and Records: All source documents and records will be used for study intervention evaluation to ensure authenticity and integrity of study data.

Study Schedule

Visit	Screening Period	Treatment Period	Follow-up Period
	Informed consent, inclusion/exclusion criteria assessment, baseline examination	yFMT treatment, immunotherapy, and chemotherapy	Follow-up at 1, 3, 6, 9 months after yFMT (imaging examination, laboratory examination)

6. Study Intervention Discontinuation and Subject Withdrawal

6.1 Study Intervention Discontinuation

Monitoring Tests and Clinical Signs: ‘

Subject experiences serious adverse events or toxic reactions, such as Grade 3 or 4 treatment-related adverse events (TRAEs) that have not resolved after standard treatment.

Subject's tumor shows obvious progression, and research team judges that continued treatment cannot bring clinical benefit.

Subject experiences intolerable toxic reactions affecting quality of life or safety.

Subject experiences serious clinical adverse reactions, such as immune-related adverse events (irAEs), including but not limited to severe skin reactions, immune hepatitis, immune pneumonia, immune enteritis, etc.

Abnormal laboratory test results, such as blood routine, liver and kidney function, tumor markers, etc., that may threaten subject's health.

Subject experiences other clinical conditions making continued participation no longer in the subject's best interest.

Reasons and Timeframe for Temporary Study Intervention Discontinuation:

Reasons: Such as mild or moderate adverse reactions requiring further assessment and management; or subject needs treatment regimen adjustment, etc.

Timeframe: According to subject's specific condition and severity of adverse reactions, discontinuation time may range from several days to several weeks. During discontinuation, subject will receive close monitoring and necessary treatment.

Data Collection During Study Intervention Discontinuation Period:

Detailed records of adverse events, including occurrence time, symptoms, severity, management measures, and outcome.

Laboratory examination results, including blood routine, liver and kidney function, tumor markers, etc.

Imaging examination results, such as CT, MRI, etc., to assess tumor changes.

Subject's symptom and sign changes, including vital signs such as temperature, blood pressure, heart rate.

Subject's quality of life and compliance assessment.

Follow-up Measures for Subjects After Study Intervention Discontinuation:

Safety Follow-up: Research team will continue safety follow-up for subjects, monitor health status, record any adverse events, serious adverse events, and unexpected problems.

Subsequent Treatment Recommendations: Provide appropriate subsequent treatment recommendations and support according to subject's condition.

Communication and Documentation: Maintain communication with subjects, timely understand their physical condition and quality of life, and document follow-up status in detail.

Completion of Remaining Study Procedures:

Study intervention discontinuation does not mean study termination. Remaining study procedures should still be completed according to the study protocol, including data organization and analysis of subjects, and summary and reporting of study results.

6.2 Subject Discontinuation/Withdrawal

Reasons for Subject Discontinuation:

Voluntary Withdrawal: Subjects have the right to voluntarily withdraw from the study at any time without providing any reason.

Non-compliance with Study Intervention: Subject obviously non-compliant with study intervention, such as frequent missed doses, not receiving treatment on time, or self-adjusting drug dosage.

Clinical Adverse Reactions or Abnormal Laboratory Tests: Subject experiences serious clinical adverse reactions or abnormal laboratory tests making continued participation no longer in the subject's best interest.

Disease Progression: Subject's tumor shows obvious progression, and research team judges that continued treatment cannot bring clinical benefit.

Meeting Exclusion Criteria: Subject develops new or confirmed exclusion criteria situations.

Unable to Receive Study Intervention: Subject unable to receive study intervention for certain period, such as unable to attend visits on time due to personal reasons.

Other Reasons: Such as subject loss to follow-up, study early termination, etc.

Documentation and Reporting:

Reasons for subject discontinuation/withdrawal should be documented in detail on the case report form and reported according to study protocol and ethical requirements.

6.3 Loss to Follow-up

Description of Study Follow-up Period:

Study follow-up period is 9 months. Subjects need to complete all follow-ups according to study protocol.

Definition of Loss to Follow-up:

When study subjects stop scheduled study follow-up, cannot complete study-required procedures, or researcher cannot contact study subjects, it can be considered loss to follow-up.

Plan to Reduce Loss to Follow-up and Missing Data:

Establish Contact Mechanism: Research team will maintain contact with subjects through phone calls, text messages, emails, etc., reminding them to attend follow-ups on time.

Provide Convenience: Provide transportation subsidies, flexibility in appointment scheduling, etc., to reduce burden on subjects.

Strengthen Communication: Regular communication with subjects to understand their physical condition and quality of life, enhance subjects' sense of participation and trust.

Documentation and Tracking: Detailed recording of subjects' contact information and follow-up status. For subjects lost to follow-up, research team will try to contact them through various means and record reasons for loss to follow-up.

Data Integrity: For subjects lost to follow-up, research team will try to collect all data before loss to follow-up and conduct appropriate processing in data analysis to reduce data missing and bias.

7. Observation Items and Assessment Time Points

This study's observation items cover three categories: safety assessment, efficacy assessment, and exploratory assessment. Assessment time points strictly correspond to study phases to ensure data integrity and timeliness.

7.1 Screening/Baseline Period (within 2 weeks before enrollment)

Purpose: Assess whether subjects meet inclusion criteria, establish baseline data, provide reference for subsequent efficacy and safety comparison.

Assessment Items:

History Collection and Physical Examination: Record past medical history, treatment history, allergy history, vital signs, and systemic examination results;

Laboratory Tests: Complete blood count, blood biochemistry (ALT, AST, creatinine, electrolytes, etc.), coagulation function, tumor markers (CEA, CA19-9, CA125);

Imaging Examinations: Abdominal/pelvic enhanced CT, electronic total colonoscopy (assess tumor baseline status);

Other Examinations: Electrocardiogram (ECG), fecal sample collection (intestinal microbiota baseline analysis), peripheral blood sample collection (immune microenvironment baseline assessment);

Quality of Life Assessment: Complete EORTC QLQ-C30 and QLQ-CR38 scale completion.

7.2 Intervention/Treatment Period (3 months total, 2 weeks per treatment cycle)

Purpose: Monitor safety and preliminary efficacy during treatment, timely detect and manage adverse events, adjust treatment regimen.

Assessment Items (every 2 weeks, synchronized with treatment):

Physical Examination: Monitor vital signs such as temperature, blood pressure, heart rate, assess general status;

Laboratory Tests: Complete blood count, blood biochemistry, tumor markers (dynamic monitoring of efficacy trends);

Safety Monitoring: Record adverse event occurrence, increase targeted examinations if necessary (such as liver ultrasound, renal function imaging when liver and kidney function abnormalities occur);

Exploratory Examinations: Intestinal microbiome analysis (fecal sample collection for high-throughput testing), immune microenvironment analysis (peripheral blood sample collection);

Imaging Examinations: Every 6 months, abdominal/pelvic enhanced CT/MRI, assess tumor efficacy according to RECIST 1.1 criteria;

Quality of Life Assessment: Complete EORTC QLQ-C30 and QLQ-CR38 scales at Week 4, Week 8, and Week 12 (end of treatment) of treatment period.

7.3 Follow-up Period (9 months after last yFMT dose)

Purpose: Assess long-term efficacy and safety, monitor disease recurrence or progression, collect survival status data.

Assessment Items:

Month 1 Follow-up: Physical examination, complete blood count, blood biochemistry, tumor markers;

Month 3 Follow-up: Physical examination, complete blood count, blood biochemistry, tumor markers, fecal sample collection (intestinal microbiota analysis), quality of life assessment;

Month 6 Follow-up: Physical examination, complete blood count, blood biochemistry, tumor markers, abdominal/pelvic enhanced CT, gastroscopy/colonoscopy, quality of life

assessment;

Month 9 Follow-up (final follow-up): Physical examination, complete blood count, blood biochemistry, tumor markers, immune microenvironment assessment (peripheral blood sample), quality of life assessment;

Survival Status Monitoring: Every 3 months confirm subject survival status through outpatient or phone follow-up until study end or subject death.

8. Efficacy Evaluation Criteria

This study's efficacy evaluation criteria revolve around preset primary endpoints (safety and tolerability) and secondary endpoints (anti-tumor efficacy). All indicators follow Good Clinical Practice (GCP), Common Terminology Criteria for Adverse Events (CTCAE) v5.0, and Response Evaluation Criteria in Solid Tumors (RECIST 1.1), ensuring standardized assessment procedures and traceable data.

8.1 Primary Endpoints: Safety and Tolerability Related Indicators

(1) Serious Adverse Event (SAE) Incidence

Definition: Adverse events meeting any of the following criteria: requiring hospitalization or prolonging hospitalization, causing permanent disability or dysfunction, life-threatening, causing death, or other medical events judged as "serious" by researchers (such as important organ failure, severe infection, etc.).

Assessment Criteria: Graded according to CTCAE v5.0 (Grades 1-5), only count Grade 3-5 events meeting SAE definition; researchers judge causality with study intervention (yFMT, immunotherapy, chemotherapy) based on event occurrence time, intervention exposure history, symptom characteristics, and outcome, divided into 5 categories: "definitely related, probably related, possibly related, probably not related, cannot be assessed."

Observation Time: From first receipt of any study intervention (yFMT/immunotherapy/chemotherapy) to end of final follow-up (9 months after last yFMT dose).

Documentation and Analysis: Detailed recording of each SAE occurrence time, symptom

presentation, diagnostic basis, management measures (such as drug discontinuation, symptomatic treatment, rescue measures), outcome time, and final outcome; calculate total SAE incidence and 95% confidence interval, stratified statistics by intervention-related category (yFMT-related, immune-related, chemotherapy-related, mixed-related), clarifying safety risk characteristics of different interventions.

(2) Treatment-Related Adverse Event (TRAE) Incidence

Definition: Adverse events "definitely related, probably related, possibly related" to study intervention, excluding events unrelated to intervention such as underlying disease recurrence, incidental diseases, or externally caused events.

Assessment Criteria: Graded according to CTCAE v5.0 (Grades 1-5), respectively count incidence of all-grade TRAEs and \geq Grade 3 TRAEs (toxicity requiring medical intervention, possibly affecting quality of life or safety).

Observation Time: Same as SAE observation time.

Documentation and Analysis: Record specific adverse event types by system organ classification (gastrointestinal system, hematologic system, immune system, liver and kidney function, skin and subcutaneous tissue, etc.) (such as nausea, leukopenia, immune rash, etc.); calculate total incidence, incidence by grade, and 95% confidence interval, focusing on describing distribution and outcome of \geq Grade 3 TRAEs.

(3) Intervention Modification Rate Due to Adverse Events

Definition: Proportion of patients requiring suspension, dose reduction, or permanent discontinuation of study intervention (yFMT, immunotherapy, chemotherapy) due to adverse events, including single intervention modification (such as only suspending chemotherapy) and combined intervention modification (such as simultaneously suspending yFMT and immunotherapy).

Assessment Criteria: Clarify direct cause of intervention modification (corresponding specific adverse event name and grade), modification method (suspension/reduction/discontinuation), modification duration (in case of suspension), and

recovery status (such as whether treatment restarted after suspension).

Observation Time: Same as SAE observation time.

Documentation and Analysis: Respectively calculate intervention suspension rate, dose adjustment rate, permanent discontinuation rate, and 95% confidence interval; describe types and severity of adverse events related to modification, analyze impact of modification on treatment completion rate, assess clinical tolerability of combination regimen.

8.2 Secondary Endpoints: Anti-tumor Efficacy Related Indicators

(1) Progression-Free Survival (PFS)

Definition: Time from subject enrollment to first radiologically confirmed disease progression (according to RECIST 1.1 criteria) or death from any cause. If subject has not experienced disease progression or death at last follow-up, last follow-up date is censoring time; if subject permanently discontinued treatment due to adverse events but has not shown disease progression, continue follow-up until disease progression or death.

Assessment Criteria:

Disease Progression (PD): Sum of longest diameters of target lesions increased $\geq 20\%$ from baseline, with absolute increase $\geq 5\text{mm}$; or appearance of new metastatic lesions; or definite progression of non-target lesions.

Assessment Process: Evaluated by two independent reviewers; third-party review when opinions differ.

Observation Time: From enrollment until disease progression, subject death, or study end (data analysis cutoff date).

Documentation and Analysis: Detailed recording of disease progression time, basis (imaging/clinical symptoms/tumor markers), and death time; use Kaplan-Meier method to plot PFS survival curve, calculate median PFS and 95% confidence interval.

(2) Objective Response Rate (ORR)

Definition: Proportion of patients with confirmed complete response (CR) and partial response (PR) among all enrolled patients who completed at least one post-baseline efficacy

assessment (according to RECIST 1.1 criteria). Patients who did not complete at least one post-baseline efficacy assessment (such as withdrawing before receiving treatment after enrollment) are not included in statistics.

Assessment Criteria:

Complete Response (CR): All target lesions completely disappeared, no new lesions appeared, and tumor markers (CEA, CA19-9) returned to normal reference range, sustained for at least 4 weeks.

Partial Response (PR): Sum of longest diameters of target lesions decreased $\geq 30\%$ from baseline, no new lesions appeared, sustained for at least 4 weeks.

Stable Disease (SD): Changes in sum of longest diameters of target lesions did not meet PR or PD criteria.

Disease Progression (PD): Meets PD definition in PFS above.

Observation Time: From enrollment until disease progression or study end.

Documentation and Analysis: Count number of patients with CR, PR, calculate ORR and 95% confidence interval; present patient distribution by response status (CR, PR, SD, PD), describe duration of response (DoR, time from first confirmed CR/PR to disease progression or death).

(3) Overall Survival (OS)

Definition: Time from subject enrollment to death from any cause. If subject still alive at last follow-up, last follow-up date is censoring time; if subject lost to follow-up, last valid follow-up date is censoring time.

Assessment Method: Confirm subject survival status through outpatient follow-up, phone follow-up, inpatient medical record review, etc. Death date is based on medical death certificate, written confirmation from family, or inpatient records.

Observation Time: From enrollment until patient death or study end (data analysis cutoff date).

Documentation and Analysis: Use Kaplan-Meier method to plot OS survival curve, calculate

median OS and 95% confidence interval, count 12-month survival rate and 95% confidence interval.

8.3 Supplementary Instructions

All efficacy assessment indicators must be documented in detail in the case report form (CRF), with original data (such as imaging reports, laboratory test sheets, follow-up records) archived for future reference.

Adverse event coding adopts the latest version of Medical Dictionary for Regulatory Activities (MedDRA) to ensure terminology standardization.

If subjects withdraw from the study during treatment, still try to complete subsequent efficacy and survival status follow-up to reduce data missing.

9. Adverse Event Observation

9.1 Definition of Adverse Events

Adverse Event (AE): Any unfavorable medical event occurring in subjects after receiving trial treatment, not necessarily having a causal relationship with trial drugs or interventions. These events may include but are not limited to symptoms, signs, diseases, or laboratory abnormalities.

9.2 Expected Adverse Events in This Study

Based on known adverse reactions of young fecal microbiota transplantation (yFMT), immunotherapy (PD-1 inhibitor), and chemotherapy (FOLFIRI regimen), expected possible adverse events include:

Gastrointestinal Reactions: Nausea, vomiting, diarrhea, abdominal pain, constipation, intestinal infection, etc. This study combines immunotherapy and chemotherapy; subjects have risk of bacteremia or sepsis due to decreased immunity or intestinal mucosal barrier damage causing intestinal bacterial translocation. Once subjects show warning signs such as temperature $\geq 38.3^{\circ}\text{C}$ or significantly elevated inflammatory indicators, research team will immediately activate emergency plan. Primary measures include suspending FMT and immunotherapy, simultaneously conducting fecal pathogen and blood culture tests, while

testing complete blood count, CRP, PCT, and intestinal mucosal barrier markers to clarify infection status. On this basis, immediately invite infectious disease specialists for consultation, initiate anti-infective treatment to ensure effective infection control before pathogen identification results return, ensuring subject life safety. Immediately conduct 定向培养 of pathogenic bacteria and metagenomic retrospective testing on retained samples of the same batch of microecological preparations to exclude preparation contamination risk and track homologous pathogens.

Immune-Related Adverse Events (irAEs): Immune hepatitis, immune pneumonia, immune enteritis, immune nephritis, skin reactions (such as rash, pruritus), etc.

Hematologic Toxicity: Leukopenia, thrombocytopenia, anemia, etc.

Cardiovascular System: Hypertension, arrhythmia, etc.

Nervous System: Headache, dizziness, peripheral neuropathy, etc.

Metabolic/Endocrine System: Blood glucose abnormalities, thyroid function abnormalities, etc.

Others: Fatigue, fever, injection site reactions, etc.

9.3 Causality Assessment Between Adverse Events and Drugs

Researchers will judge causality between adverse events and study intervention according to the following criteria:

Definitely Related: Temporal sequence reasonable, symptoms consistent with known drug adverse reactions, symptoms relieved after drug discontinuation, symptoms reappear after rechallenge.

Probably Related: Temporal sequence reasonable, symptoms consistent with known drug adverse reactions, symptoms relieved after drug discontinuation.

Possibly Related: Temporal sequence reasonable, symptoms partially consistent with known drug adverse reactions.

Probably Not Related: Temporal sequence unreasonable, or symptoms inconsistent with known drug adverse reactions.

To Be Assessed: Causality not yet clear, needs further assessment.

Cannot Be Assessed: Insufficient information to judge causality.

9.4 Adverse Event Recording

Recording Content:

Adverse event name, occurrence time, duration, severity (mild, moderate, severe), management measures, outcome (disappeared, persistent, worsened).

Causality judgment between adverse event and study intervention.

Subject's symptom and sign changes, including vital signs such as temperature, blood pressure, heart rate.

Recording Method: All adverse events must be documented in detail in the case report form (CRF) and electronically recorded in the study database.

9.5 Adverse Event Management

Mild Adverse Events: Symptomatic treatment and supportive care, such as dietary adjustment, use of antiemetics, etc.

Moderate Adverse Events: Adjust treatment regimen, such as suspending or reducing drug dosage, closely monitoring subject's symptom changes.

Severe Adverse Events: Immediately suspend study intervention, take emergency treatment measures, such as using immunosuppressants to treat immune-related adverse events.

Continuous Monitoring: Continuously monitor all adverse events until symptoms resolve or stabilize.

9.6 Serious Adverse Event (SAE) Reporting Method

Definition: Serious adverse events requiring hospitalization, prolonging hospitalization, disability, affecting work capacity, life-threatening, or causing death.

Reporting Time: Within 24 hours of learning of SAE, researcher reports to principal investigator, ethics committee, and clinical monitor by phone or fax, and submits SAE report form within 24 hours.

Reporting Content:

Subject basic information (name, gender, age, inpatient number, etc.).

Detailed description of SAE (occurrence time, symptoms, severity, management measures, outcome).

Causality judgment between SAE and study intervention.

Follow-up plan and subsequent management measures.

9.7 Serious Adverse Event (SAE) Management Measures

Emergency Management: Immediately take appropriate treatment measures to ensure subject safety.

Suspend Study Intervention: After assessing causality between SAE and study intervention, decide whether to suspend study intervention.

Continuous Monitoring: Continuously monitor SAE until symptoms resolve or stabilize.

Subsequent Treatment: Provide appropriate subsequent treatment recommendations according to SAE severity and subject's overall condition.

9.8 Follow-up Method and Time

Follow-up Method: Maintain contact with subjects through phone calls, text messages, outpatient follow-up, etc., regularly conduct safety follow-up.

Follow-up Time:

For mild and moderate adverse events, follow-up every 1-2 weeks until symptoms resolve or stabilize.

For severe adverse events, follow-up every 1 week until symptoms resolve or stabilize.

For SAE, according to specific circumstances, may need more frequent follow-up until symptoms resolve or stabilize.

Documentation and Reporting: Document follow-up status in detail, including subject's symptom changes, management measures, and outcome, and electronically record in study database.

10. Study Quality Control and Quality Assurance

To ensure the scientific validity, reliability, and ethical integrity of this study, the research

team will implement strict quality control and quality assurance measures covering laboratory indicator testing, standard operating procedure (SOP) execution, researcher training, subject compliance, and study monitoring. The following is the specific quality control and quality assurance plan:

10.1 Quality Management Plan

10.1.1 Laboratory Indicator Testing

Standardized Operations: All laboratory tests (such as complete blood count, blood biochemistry, tumor markers, imaging examinations, etc.) must be conducted according to standard operating procedures (SOP) to ensure accuracy and reproducibility of test results.

Quality Control: Regularly calibrate and maintain laboratory equipment to ensure normal operation. Laboratory must pass internal quality control and external quality assessment to ensure test results meet standards.

Data Recording: Laboratory test results must be documented in detail in the case report form (CRF) and electronically recorded in the study database to ensure data integrity and traceability.

10.1.2 SOP Execution

SOP Development: Research team will develop detailed standard operating procedures (SOP) covering all aspects of the study, including subject screening, treatment process, data recording, adverse event management, etc.

Training and Execution: All research team members must receive SOP training to ensure strict execution of study operations according to SOP. During the study, regularly check and evaluate SOP execution to ensure standardization and normalization of study operations.

10.1.3 Researcher Training

Training Content: Research team members must receive training including study protocol, SOP, GCP (Good Clinical Practice) principles, data management, adverse event management, etc.

Training Methods: Training conducted through online courses, offline seminars, simulated

operations, etc., to ensure researchers are familiar with study processes and operational norms.

Training Records: Detailed recording of training content, training time, training personnel list, etc., to ensure traceability of training process.

10.1.4 Subject Compliance

Compliance Monitoring: Monitor subject treatment compliance through regular follow-up, phone reminders, questionnaires, etc., timely detect and solve problems encountered by subjects during treatment.

Compliance Assessment: Regularly assess subject compliance. For subjects with poor compliance, research team will take measures to improve compliance, such as providing additional guidance and support.

Documentation and Reporting: Document subject compliance status in detail, including treatment completion status, drug use status, follow-up status, etc., and electronically record in study database.

10.1.5 Study Monitoring

Monitoring Plan: Research team will develop detailed monitoring plan, regularly monitor study process to ensure scientific validity and ethical integrity.

Monitoring Content: Including subject screening, treatment process, data recording, adverse event management, SOP execution, etc., to ensure study operations comply with study protocol and GCP principles.

Monitoring Frequency: Monitoring frequency adjusted according to study progress and needs, generally comprehensive monitoring every 3 months, increase monitoring frequency if necessary.

Monitoring Report: After monitoring, monitor must write monitoring report, document monitoring findings and recommendations in detail, and timely feedback to research team for rectification.

10.2 Quality Control Measures

10.2.1 Data Quality Control

Data Entry and Review: All data must be entered by two persons to ensure data entry accuracy. After data entry, data manager reviews and corrects errors in a timely manner.

Data Integrity: Ensure integrity of all data, including subject basic information, treatment process, laboratory test results, adverse event records, etc. For missing data, use preset missing value processing methods.

Data Consistency: Regularly check data consistency to ensure consistency between data from different sources (such as CRF, laboratory reports, imaging examination reports, etc.).

Data Backup and Security: Regularly backup study database to ensure data security. Database must be password protected, accessible only to authorized researchers.

10.2.2 Specimen Quality Control

Specimen Collection and Processing: Strictly follow SOP for specimen collection and processing to ensure specimen quality and stability. Specimens must be sent for testing in a timely manner after collection, avoiding long-term storage.

Specimen Storage and Management: Specimens must be stored according to requirements, such as yFMT bacterial solution must be stored at -80 ° C, avoiding repeated freeze-thaw cycles. Specimen storage and management must comply with relevant regulations and ethical requirements.

Specimen Tracking and Recording: Detailed recording of specimen collection, processing, storage, and use to ensure specimen traceability. Specimen tracking log must record specimen flow and use status in detail.

10.3 Quality Assessment and Improvement

10.3.1 Quality Assessment

Internal Assessment: Research team regularly conducts internal quality assessment of the study, including data quality, SOP execution, subject compliance, adverse event management, etc. Internal assessment conducted every 3 months, with detailed recording of assessment results.

External Assessment: Invite external experts or institutions to assess study quality as needed, with assessment results serving as important basis for study improvement.

10.3.2 Improvement Measures

Problem Rectification: According to quality assessment results, research team timely rectifies identified problems to ensure continuous improvement of study quality.

Continuous Improvement: Research team will establish continuous improvement mechanism, regularly optimize study processes and operational norms to improve study efficiency and quality.

10.4 Document Management and Review

10.4.1 Document Management

Document Classification: Study documents must be classified and managed by category, including study protocol, CRF, questionnaires, laboratory reports, imaging examination reports, specimen tracking logs, etc.

Document Storage: All documents must be stored according to requirements, paper documents stored in secure file cabinets, electronic documents stored in secure databases.

Document retention period must comply with relevant regulations and ethical requirements.

Document Access Permissions: Document access must be permission-controlled, accessible only to authorized researchers. Document access must be recorded to ensure document security and traceability.

10.4.2 Document Review

Regular Review: Research team regularly reviews study documents to ensure document integrity and accuracy. Document review conducted every 3 months, with detailed recording of review results.

Problem Handling: Timely handle problems identified during review to ensure document quality and integrity.

10.5 Quality Control Officer

Quality Control Officer: Research team designates specific quality control officer

responsible for comprehensive study quality management. Quality control officer must have rich research experience and quality management knowledge.

Responsibilities: Quality control officer responsible for developing quality control plan, supervising implementation of quality control measures, conducting regular quality assessment and improvement, ensuring scientific validity and ethical integrity of the study.

11. Project Risk Pre-assessment and Risk Management Plan

11.1.1 Subject Safety Risks

Adverse Event Risk: Adverse events that may occur in subjects during yFMT, immunotherapy, and chemotherapy, including gastrointestinal reactions, immune-related adverse events, hematologic toxicity, etc.

Serious Adverse Event Risk: Possible serious adverse events (SAE), such as immune hepatitis, immune pneumonia, severe infection, etc.

Disease Progression Risk: Subjects may experience disease progression during treatment, leading to poor treatment efficacy.

11.1.2 Study Operation Risks

Operation Error Risk: Possible operational errors during yFMT operation, drug preparation, and administration.

Data Management Risk: Risks such as incomplete data recording, data loss, or data errors.

Subject Compliance Risk: Subjects may be unable to comply with study protocol due to various reasons, affecting reliability of study results.

11.1.3 Study Management Risks

Ethical Risk: Possible ethical issues during study, such as inadequate subject informed consent, imperfect privacy protection, etc.

Resource Allocation Risk: Possible insufficient or unreasonable resource allocation during study, affecting study progress.

Regulatory Risk: Possible regulatory issues during study, such as failure to complete ethical approval in time, failure to submit study progress reports on time, etc.

11.2 Risk Management Plan

11.2.1 Subject Safety Risk Management

Adverse Event Management:

Mild adverse events: Symptomatic treatment and supportive care, such as dietary adjustment, use of antiemetics, etc.

Moderate adverse events: Adjust treatment regimen, such as suspending or reducing drug dosage, closely monitoring subject's symptom changes.

Severe adverse events: Immediately suspend study intervention, take emergency treatment measures, such as using immunosuppressants to treat immune-related adverse events.

Serious Adverse Event (SAE) Management:

Emergency management: Immediately take appropriate treatment measures to ensure subject safety.

Suspend study intervention: After assessing causality between SAE and study intervention, decide whether to suspend study intervention.

Continuous monitoring: Continuously monitor SAE until symptoms resolve or stabilize.

Subsequent treatment: Provide appropriate subsequent treatment recommendations according to SAE severity and subject's overall condition.

Disease Progression Management:

Assess progression: Assess disease progression through imaging examinations and laboratory tests.

Adjust treatment regimen: Adjust treatment regimen according to disease progression, such as increasing treatment intensity or changing treatment regimen.

Provide subsequent treatment recommendations: Provide appropriate subsequent treatment recommendations according to subject's overall condition.

11.2.2 Study Operation Risk Management

Operation Error Management:

Training and retraining: Conduct regular training for research team members to ensure

familiarity with operation processes and SOP.

Dual operation: Key operations must be conducted by two persons to ensure operation accuracy and safety.

Operation recording: Document operation process in detail to ensure operation traceability.

Data Management Risk Management:

Data entry and review: All data must be entered by two persons to ensure data entry accuracy. After data entry, data manager reviews and corrects errors in a timely manner.

Data backup and security: Regularly backup study database to ensure data security. Database must be password protected, accessible only to authorized researchers.

Data integrity: Ensure integrity of all data, including subject basic information, treatment process, laboratory test results, adverse event records, etc. For missing data, use preset missing value processing methods.

Subject Compliance Risk Management:

Compliance monitoring: Monitor subject treatment compliance through regular follow-up, phone reminders, questionnaires, etc., timely detect and solve problems encountered by subjects during treatment.

Compliance assessment: Regularly assess subject compliance. For subjects with poor compliance, research team will take measures to improve compliance, such as providing additional guidance and support.

Documentation and reporting: Document subject compliance status in detail, including treatment completion status, drug use status, follow-up status, etc., and electronically record in study database.

11.2.3 Study Management Risk Management

Ethical Risk Management:

Informed consent: Ensure subjects or their agents fully understand study purpose, procedures, and possible risks, and sign informed consent.

Privacy protection: Strictly protect subject personal information and study data to ensure

privacy is not 泄露.

Ethical approval: Ensure study protocol, informed consent, etc. are approved by ethics committee, and comply with ethical requirements during study.

Resource Allocation Risk Management:

Resource assessment: Before study initiation, comprehensively assess required resources to ensure adequate and reasonable resource allocation.

Resource allocation: During study, timely allocate resources according to needs to ensure smooth study progress.

Emergency plan: Develop emergency plan to address possible resource shortage problems.

Regulatory Risk Management:

Regulatory compliance: Ensure study protocol, informed consent, etc. comply with relevant regulations and ethical requirements, and comply with regulatory requirements during study.

Regular reporting: Submit study progress reports on time to ensure transparency and traceability of study process.

Communication and coordination: Maintain close communication with ethics committee and regulatory departments, timely solve possible regulatory issues.

11.3 Risk Monitoring and Assessment

Regular Monitoring: Research team will regularly monitor risks during study process, assess risk severity and occurrence frequency.

Dynamic Adjustment: According to risk monitoring results, dynamically adjust risk management plan to ensure effectiveness and timeliness of risk control.

Documentation and Reporting: Document risk monitoring and management status in detail to ensure traceability and transparency of study process.

Through the above risk pre-assessment and risk management plan, the research team will ensure scientific validity, reliability, and ethical integrity of this study, providing strong guarantee for subject safety and credibility of study results.

12.Data Safety Monitoring

Clinical research will formulate corresponding data safety monitoring plans according to risk level. All adverse events will be documented in detail, properly managed, and tracked until properly resolved or condition stabilized. Serious adverse events and unexpected events will be reported to ethics committee, competent authorities, sponsor, and drug regulatory departments in a timely manner according to regulations. Principal investigator will regularly conduct cumulative review of all adverse events, convene investigator meetings to assess study risks and benefits when necessary. Emergency unblinding can be performed when necessary in double-blind trials to ensure subject safety and rights. Studies greater than minimal risk will arrange independent data monitors to monitor study data. High-risk studies will establish independent data safety monitoring committees to monitor cumulative safety data and efficacy data to make recommendations on whether to continue the study.

13. Statistical Analysis

This study's statistical analysis will adopt scientific and rigorous methods to ensure accuracy and reliability of data analysis. The following is the detailed statistical analysis plan:

13.1 General Methods

13.1.1 Descriptive Statistics

Categorical Data: Expressed as frequency and percentage, such as gender, ECOG score, etc.

Continuous Data: Expressed as mean, standard deviation (SD), median, range, etc., such as age, tumor marker levels, etc.

Data Display: Display descriptive statistical results through tables and graphs (such as bar charts, box plots).

13.1.2 Inferential Statistics

P-value and Confidence Interval: Calculate P-value and 95% confidence interval for assessing statistical significance.

Test Type: Select one-sided or two-sided test according to study design and data distribution.

Covariate Setting: Pre-set covariates that may affect study results, such as age, gender, baseline tumor burden, etc., and adjust in analysis.

Assumption Checking: Conduct normality test (such as Shapiro-Wilk test) and homogeneity of variance test (such as Levene test). For data not conforming to normal distribution, use appropriate transformation (such as logarithmic transformation) or non-parametric test methods (such as Mann-Whitney U test).

13.2 Primary and Secondary Endpoint Analysis

13.2.1 Primary Endpoint Analysis

Primary endpoints include serious adverse event (SAE) incidence, treatment-related adverse event (TRAE) incidence, and intervention modification rate due to adverse events, all categorical variables, using following analysis methods:

Descriptive Statistics: Calculate incidence rates of each indicator (number of events / total number in analysis set) and 95% confidence intervals (calculated using binomial distribution method), clarifying risk ranges.

Stratified Analysis:

Stratified by adverse event severity: TRAEs counted separately as "all grades" and " \geq Grade 3", focusing on high-risk toxicity;

Stratified by intervention-related category: SAE and TRAE split by "yFMT-related, immune-related, chemotherapy-related, mixed-related", clarifying safety risk characteristics of different interventions;

Stratified by baseline characteristics (if sample size permits): Stratified by age (≤ 65 years / >65 years), ECOG score (0 / 1), tumor burden (high/low, median of baseline target lesion maximum diameter sum as cutoff), exploring potential influencing factors.

Association Analysis: If sample size supports (number of events ≥ 5), use Fisher's exact test to analyze association between baseline characteristics (age, gender, ECOG score) and SAE/ \geq Grade 3 TRAE occurrence, judging whether high-risk populations exist.

13.2.2 Secondary Endpoint Analysis

Secondary endpoints include progression-free survival (PFS), objective response rate (ORR), overall survival (OS), respectively time-to-event data and binary variables, analysis methods

as follows:

Progression-Free Survival (PFS):

Statistical Description: Use Kaplan-Meier method to plot survival curve, calculate median PFS and 95% confidence interval, clarifying time benefit of disease control;

Survival Curve Comparison: If subsequent expanded sample size allows inter-group comparison, use Log-rank test to compare survival differences (this study is single-arm design, focusing on describing survival distribution characteristics);

Influencing Factor Analysis: Use Cox proportional hazards regression model for univariate analysis, exploring association between baseline age, gender, ECOG score, tumor burden and other variables and PFS; if ≥ 2 variables with $P < 0.1$ in univariate analysis, further include in multivariate model, adjust potential confounding factors, calculate hazard ratio (HR) and 95% confidence interval.

Objective Response Rate (ORR):

Descriptive Statistics: Calculate ORR (number of CR+PR cases / number of cases completing at least one post-baseline efficacy assessment) and 95% confidence interval (binomial distribution method); simultaneously describe frequency and percentage of CR, PR, SD, PD, presenting overall efficacy distribution;

Stratified Analysis: Stratified by baseline characteristics (age, ECOG score, tumor burden), describe ORR differences among different subgroups, providing subgroup efficacy clues for subsequent research;

Duration of Response (DoR): For subjects achieving CR/PR, use Kaplan-Meier method to calculate median DoR and 95% confidence interval, assessing efficacy stability.

Overall Survival (OS):

Statistical Description: Use Kaplan-Meier method to plot survival curve, calculate median OS and 95% confidence interval, simultaneously count 12-month survival rate and 95% confidence interval, intuitively presenting long-term survival benefit;

Survival Status Confirmation: Subjects still alive at last follow-up, last follow-up date as

censoring time; lost to follow-up subjects, last valid follow-up date as censoring time, detailed recording of censoring reasons;

Influencing Factor Analysis: Same as Cox proportional hazards regression model for PFS, exploring impact of baseline variables on OS.

13.3 Missing Value and Outlier Handling

Missing Value Handling:

Time-to-event data (PFS, OS): Use censoring treatment, no imputation, only record censoring reasons, ensuring authenticity of survival data;

Categorical data (SAE/TRAЕ occurrence, ORR): Missing data treated as "no event" for sensitivity analysis, simultaneously note missing proportion and handling method in results, assessing impact of missing on conclusions.

Outlier Handling:

Continuous derived data (such as tumor marker change amplitude): Identify outliers (beyond $Q1-1.5IQR$ or $Q3+1.5IQR$) through box plot (IQR method);

Sensitivity Analysis: Repeat analysis using "include outliers" and "exclude outliers" strategies respectively, if result trends are consistent, conclusion is robust; if differences are large, need to explain potential impact of outliers in discussion.

13.4 Statistical Significance Level

All statistical tests use two-sided test, significance level $\alpha = 0.05$. This study is exploratory single-arm study, mainly focusing on "descriptive results" of endpoint indicators (such as incidence rate, median survival time, confidence interval), no formal hypothesis testing inference, all P-values only used for descriptive association analysis, not as confirmation basis for efficacy or safety.

13.5 Results Presentation

Primary Endpoints: Presented as "incidence rate (95% CI)", with stratified statistical table, clarifying safety risk distribution;

Secondary Endpoints: PFS and OS presented as Kaplan-Meier survival curve + median

survival time (95% CI); ORR presented as "incidence rate (95% CI)" + efficacy distribution table;

Supplementary Instructions: Clearly label analysis set (ITT/PP) for each endpoint, missing data handling method, stratified analysis basis, ensuring results are traceable and reproducible.

13.6 Baseline Descriptive Analysis

Baseline Characteristics Comparison

Demographic Characteristics: Including age, gender, ECOG score, etc.

Laboratory Indicators: Including complete blood count, blood biochemistry, tumor markers, etc.

Statistical Methods: Use descriptive statistical methods, for categorical data, use chi-square test or Fisher's exact test.

Results Display: Display baseline characteristics of all patients through table summary.

14. Supporting Documents and Precautions

14.1 Informed Consent Process

Before study initiation, researchers should obtain ethics committee approval. Informed consent form should be provided to study subjects. The following is the detailed procedure for obtaining informed consent from study subjects:

Obtaining Informed Consent:

Informed consent should be completed before study subjects agree to participate in the study and continue throughout the entire study process. After informed consent form is approved by ethics committee, study subjects should read the informed consent form.

Researchers will explain study process in detail, including study purpose, methods, possible risks and benefits, and answer questions raised by study subjects.

Study subjects may discuss with family members or guardians before agreeing to participate.

Researchers must inform study subjects that participation is voluntary and they may withdraw from the study at any time without any form of discrimination or adverse effects.

Original or copy of informed consent form may be provided to study subjects for reference.

Study subjects' rights and welfare will be protected, emphasizing that their quality of medical care will not be affected by refusing to participate in the study.

Signing Environment:

Informed consent form signing should be conducted in quiet, private environment, ensuring study subjects have sufficient time and space to understand study content.

Researchers must ensure study subjects sign informed consent form with full understanding of study content and voluntarily.

Guardian Signature:

If study subjects are minors or persons without civil capacity, legal guardians must sign informed consent form on their behalf.

If study subjects cannot sign informed consent form due to special circumstances (such as coma), according to relevant regulations and ethical requirements, close relatives or legal agents must sign on their behalf, with detailed recording of reasons for proxy signature.

14.2 Privacy Protection

Detailed description of methods and procedures for protecting study subject data (including related forms, records, specimens, and subject privacy):

Data Protection Methods:

All study subjects' personal information and study data will be strictly confidential, accessible only to authorized researchers.

Study subjects' personal information will be anonymized, using codes instead of real names and identity information.

All paper documents will be stored in secure file cabinets, electronic documents will be stored in encrypted databases with password protection.

Data Access Permissions:

No study information may be disclosed to unauthorized third parties without approved consent.

If data needs to be provided to third parties due to study needs (such as statistical analysis units,

ethics committees, etc.), ensure third parties sign confidentiality agreements and strictly use data according to agreements.

14.3 Specimen and Data Collection and Use

Specimen and Data Collection:

All specimens (such as blood, feces, etc.) and data (such as CRF, questionnaires, laboratory results, etc.) collection must strictly follow study protocol and SOP.

Specimen collection, processing, and storage must comply with relevant regulations and ethical requirements to ensure specimen quality and stability.

Third-party Testing or Custody:

If involving third-party testing or custody, cooperation agreement must be signed with third party, clarifying rights and obligations of both parties.

Third party must have corresponding qualifications and capabilities to ensure security and confidentiality of specimens and data.

Specimen and Data Storage:

Retained remaining specimens, imaging data, and other data may be used for future research with study subject consent.

Storage location, method, and time of specimens and data must be clearly stated in informed consent form and strictly followed during study.

14.4 Data Processing and Record Retention

(1) Data Collection and Management

Data Collection:

Data collection conducted by clinical researchers under supervisor supervision, supervisor responsible for accuracy, completeness, and timeliness of reported data.

All data should be clear to ensure accurate interpretation and traceability.

Data Management:

Clinical data will be stored in database with password protection, logical verification procedures established when database is created.

Data entry must be conducted by two persons to ensure data entry accuracy. After data entry, data manager reviews and corrects errors in a timely manner.

(2) Study Data Retention

Data Retention Period:

Minimum retention time for all study data and original documents is 5 years after study

completion, specific retention time must comply with relevant regulations and ethical requirements.

Before data destruction, ethics committee approval must be obtained and destruction conducted according to relevant regulations.

14.5 Publication and Data Sharing Agreement

Publication Agreement:

Study results publication must be discussed and decided jointly by research team to ensure scientific validity and accuracy of published content.

Publication must clearly label study funding sources and participating units to ensure transparency and traceability of study.

Data Sharing Agreement:

Study data sharing must follow relevant regulations and ethical requirements to ensure data security and confidentiality.

Data sharing must obtain study subject consent, with purpose and scope of data sharing clearly stated in informed consent form.

14.6 Conflict of Interest Statement

Research team members must sign conflict of interest statement to ensure no conflicts of interest that may affect study results exist during study process.

If research team members discover potential conflicts of interest during study, they must promptly report to study supervisor and take corresponding measures for handling.

15. Ethics of Clinical Research

Clinical research will follow World Medical Association Declaration of Helsinki and other relevant regulations. Before study initiation, clinical research will be implemented only after ethics committee approves the trial protocol. Before each subject is enrolled in this study, researchers have responsibility to fully and comprehensively introduce study purpose, procedures, and possible risks to subjects or their agents, and sign written informed consent.

Subjects should be informed that they have the right to withdraw from this study at any time, with informed consent retained as clinical research document for future reference. Subject personal privacy and data confidentiality will be protected during study process.

16.Study Schedule

Phase 1: January 2026 - February 2026

Task: Study Preparation

After ethics review approval, organize research team to complete training, clarify responsibilities of researchers, coordinators, and safety monitors; sign cooperation agreement with yFMT preparation supplier, confirm preparation delivery, cold chain transportation, acceptance, storage, and clinical administration processes; complete laboratory testing project docking, and establishment of serious adverse event (SAE) monitoring and reporting mechanism, ensuring all conditions meet ethical and regulatory requirements before study initiation.

Phase 2: March 2026 - December 2026

Task: Subject Enrollment and Intervention

Strictly according to inclusion and exclusion criteria, fully inform eligible patients of study content, complete baseline assessment and enrollment after they voluntarily sign informed consent. Immediately initiate intervention after enrollment: first yFMT (defined as Day 0), thereafter every 2 weeks for a total of 6 times (complete all yFMT by Week 10-12); simultaneously receive PD-1 inhibitor and FOLFIRI chemotherapy according to standard protocol.

Phase 3: January 2027 - October 2027

Task: Follow-up Implementation

Each subject enters follow-up period after completing 6 yFMT treatments (approximately 12 weeks). Study-defined follow-up time points are Month 1, Month 3, Month 6, and Month 9 after last yFMT dose. Follow-up content includes tumor assessment (according to RECIST 1.1 criteria), laboratory tests, vital signs monitoring, adverse event recording, and quality of

life assessment, etc. This phase will simultaneously complete treatment implementation and follow-up of all subjects, ensuring complete collection of primary safety data (SAE incidence) and preliminary efficacy indicators (such as ORR, PFS).

Phase 4: November 2027 - January 2028

Task: Data Analysis and Reporting

Complete final data analysis, write research report, including study results, conclusions, and recommendations.

Paper Writing and Publication: Write academic papers according to study results, submit to relevant academic journals for publication.

Study Summary and Feedback: Report study results to ethics committee, study funders, and research team, summarize study experience and lessons.

Data Archiving and Preservation: Archive and preserve all study data, original documents, research reports, etc., ensuring data security and traceability.

Preparation for Subsequent Research: Prepare plans for subsequent research or clinical application according to study results.

Research Team Summary: Summarize research team work, evaluate team performance and improvement directions.

Objective: Complete archiving and preservation of study data, preparing for subsequent research or clinical application.

17. Study Personnel

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INFORMED CONSENT FORM

Informed Consent Statement Page

Protocol Title: Young Fecal Microbiota Transplantation (yFMT) Combined with Immunotherapy and Chemotherapy in Microsatellite Stable Metastatic Colorectal Cancer (MSS mCRC): An Exploratory Study

Protocol Version Number and Date: Version: V3.0

Version Date: February 26, 2026

Informed Consent Version Number and Date: Version: V3.0

Version Date: February 26, 2026

Principal Investigator:

Study Department: Gastrointestinal Oncology Surgery

Dear Participant:

You are invited to participate in the exploratory study of Young Fecal Microbiota Transplantation (yFMT) Combined with Immunotherapy and Chemotherapy in Microsatellite Stable Metastatic Colorectal Cancer (MSS mCRC). Please read this informed consent form carefully and make a thoughtful decision about whether to participate in this study. When your study doctor or research staff discusses the informed consent form with you, you may ask them to explain any parts you do not understand. We encourage you to fully discuss with your family and friends before making the decision to participate in this study. If you are currently participating in other studies, please inform your study doctor or research staff. The purpose, background, study procedures, and other important information of this study are as follows:

I. Study Background

In recent years, immunotherapy has made significant progress in cancer treatment; however, patients with microsatellite stable (MSS) metastatic colorectal cancer (mCRC) show poor response to immunotherapy. Young Fecal Microbiota Transplantation (yFMT) is a novel intervention that reshapes intestinal microecology and regulates the immune microenvironment, potentially improving first-line treatment resistance. Preliminary studies both domestically and internationally suggest that yFMT combined with immunotherapy may provide new treatment strategies for MSS mCRC patients.

II. Study Purpose

This study aims to evaluate the safety, tolerability, and preliminary efficacy of young fecal microbiota transplantation (yFMT) combined with immunotherapy and chemotherapy in MSS mCRC patients, and to explore the feasibility of this novel combination treatment regimen.

III. Study Procedures

How many people will participate in this study?

Approximately **(10)** people will participate in this study at this hospital.

Who can participate in this study?

Age ≥ 18 years and ≤ 75 years, male or non-pregnant female;

ECOG performance status score 0-1;

Pathologically confirmed microsatellite stable metastatic colorectal cancer;

Progression after failure of first-line chemotherapy and targeted therapy;

Women of childbearing potential must have negative urine or serum pregnancy test within 7 days before enrollment and agree to use highly effective contraception during the study and for at least 120 days after the last dose;

Non-sterilized males must agree to use highly effective contraception during the study and for at least 120 days after the last dose;

Patient or family member able to understand the purpose of the trial, voluntarily participate, and sign informed consent;

Subjects must have adequate organ function to tolerate immunotherapy combined with chemotherapy regimen.

Study Procedures

If you agree to participate in this study, please sign this informed consent form.

Blood Collection: A total of **5 blood collections** are planned during the entire study period, with a total volume of approximately **50 mL** (10 mL each time, for complete

blood count, liver and kidney function, electrolytes, coagulation function, tumor markers, and immunological indicators).

After confirming your eligibility for this study, you will enter the following process:

3.1. Pre-FMT:

All subjects meeting inclusion criteria will be assigned to the yFMT combined chemotherapy and immunotherapy group. Each enrolled subject will be assigned a unique study number, coded according to consecutive enrollment sequence, used for identification and data management.

3.2. FMT Phase:

3.2.1. Intestinal Microbiota Testing and Matching

Approximately 2 weeks before yFMT treatment, baseline fecal samples need to be collected for bacterial solution matching.

Test results are only responsible for the submitted samples. Test results serve only as reference for professionals, not as clinical diagnostic opinions, and cannot be used as diagnostic basis for determining whether patients have certain diseases.

You need to complete fecal sample collection, preservation, and mailing according to relevant requirements. If sample quality does not meet testing requirements due to force majeure or operational errors during sampling, repeat sampling is needed. This process will not incur additional costs.

According to your disease severity, clinical symptoms, and intestinal microbiota test results, we will perform precise matching for you.

3.2.2. yFMT Bowel Preparation

3 days before yFMT: Routine intestinal antibiotic pretreatment; antifungal drugs for fungal infections; sensitive antibiotics for complex intestinal infections; antiviral treatment for patients with concurrent viral infections.

24 hours before yFMT: Routine administration of bowel cleansing agents (such as polyethylene glycol, etc.) for intestinal cleansing.

Specific preparation work needs to be determined by doctors according to condition and actual situation.

Follow dietary adjustment instructions before and after fecal microbiota transplantation.

3.2.3. yFMT Administration Method

Single dose of **50 mL fecal bacterial solution** via nasoenteric tube infusion, followed by oral FMT capsules, **12 capsules single dose**. Every **2 weeks** for a total of **6 times**. Tube placement may cause some inconvenience to daily life, but these inconveniences are relatively minor.

3.2.4. Visit Time Points and Examination Contents

Baseline Visit (Day 0):

History Collection and Physical Examination: Detailed recording of subject's medical history, including past medical history, family history, allergy history, etc., and comprehensive physical examination.

Laboratory Tests: Including complete blood count, liver and kidney function, electrolytes, coagulation function, tumor markers, etc.

Imaging Examinations: Baseline imaging examinations such as CT or MRI to assess tumor size and location.

Intestinal Microbiome Analysis: Fecal sample collection for analysis of baseline intestinal microbiota composition.

Endoscopy: Collection of intestinal baseline data.

Treatment Period Visits (Every 2-4 weeks):

Physical Examination: Monitoring subject's signs such as temperature, blood pressure, heart rate, etc.

Symptom Assessment: Detailed recording of any symptoms reported by subjects, assessing treatment tolerability.

Treatment Response Assessment: Assessing tumor size changes through imaging examinations (CT or MRI every 6 months) to determine treatment efficacy.

Intestinal Microbiome Monitoring: Regular fecal sample collection to monitor changes in intestinal microbiota.

3.3. Post-yFMT Follow-up (1, 3, 6, and 9 months after treatment completion):

Physical Examination and Laboratory Tests: Comprehensive physical examination and laboratory tests to assess overall health status.

Imaging Examinations: Every 6 months, abdominal/pelvic enhanced CT/MRI to assess tumor efficacy according to RECIST 1.1 criteria.

Treatment Summary: Summarizing all data during treatment period, including efficacy, safety, adverse events, etc.

Intestinal Microbiome Monitoring: Regular fecal sample collection to monitor changes in intestinal microbiota.

How long will this study last?

This study consists of two parts: treatment phase and follow-up phase:

Treatment Phase: From signing informed consent and completing baseline examination to completing all combination treatments (yFMT + immunotherapy + chemotherapy), totaling approximately **12 weeks (3 months)**.

Follow-up Phase: After you complete the last study medication, the research team will continue regular follow-up with you at **1, 3, 6, and 9 months**. Follow-up content includes physical examination, laboratory tests, imaging examinations (if tumor progression is suspected), and survival status assessment.

Therefore, your total participation time in this study is approximately **12 months** (3 months treatment period + 9 months follow-up period). You may choose to withdraw from the study at any time without any unfair treatment and without losing any benefits you should receive. However, if you decide to withdraw from this study during the study, we encourage you to discuss with your doctor first. Considering your safety issues, relevant examinations may be performed once after withdrawal.

IV. Risks and Benefits

1.What are the risks of participating in this study?

The following risks may be brought to you by participating in this study. You should discuss these risks with your study doctor, or if you wish, with your regular attending physician.

Known risks include, but are not limited to:

Psychological discomfort with fecal microbiota transplantation;

Gastrointestinal System: Transient fever, chills, belching, nausea, vomiting, abdominal pain, bloating, diarrhea, intestinal spasm, constipation, blood in stool, with or without elevated inflammatory indicators such as CRP, ESR, IL-6. Most of these symptoms are mild and self-limiting, requiring no special treatment. This study combines immunotherapy and chemotherapy; subjects have risk of bacteremia or sepsis due to decreased immunity or intestinal mucosal barrier damage causing intestinal bacterial translocation. Once you show warning signs such as temperature $\geq 38.3^{\circ}\text{C}$ or significantly elevated inflammatory indicators, the research team will immediately activate the emergency plan. Primary measures include suspending FMT and immunotherapy, simultaneously conducting fecal pathogen and blood culture tests, while testing complete blood count, CRP, PCT, and intestinal mucosal barrier markers to clarify infection status. On this basis, infectious disease specialists will provide consultation and initiate anti-infective treatment to ensure effective infection control before pathogen identification results return, ensuring your life safety. Immediate pathogen-directed culture and metagenomic retrospective testing will be conducted on retained samples of the same batch of microecological preparations to exclude preparation contamination risk and track homologous pathogens.

Immune System: Prostration, tongue vesicles, etc., with reports of peripheral neuropathy, Sjögren's syndrome, idiopathic thrombocytopenic purpura, and rheumatoid arthritis;

Metabolic/Endocrine System: Possible increased or decreased insulin sensitivity;

Musculoskeletal System: Hip paresthesia;

Other Rare Adverse Reactions: Nasal congestion, rhinorrhea, sore throat, increased purulent bloody stool, condition deterioration, etc., with reports of bacteremia and positive *Clostridioides difficile* toxin detection. Given the complexity of diseases, unknown risks beyond those described above may also occur after microbiota transplantation.

If any of the above complications occur, you agree that doctors will provide corresponding beneficial adjuvant treatment. When these complications are severe, they may prolong hospitalization and thus increase medical costs, or even cause permanent damage.

2. What are the benefits of participating in the study?

If you agree to participate in this study, you may obtain direct medical benefits, including:

Improvement of first-line treatment resistance: Through young fecal microbiota transplantation (yFMT) reshaping intestinal microecology, potentially enhancing efficacy of immunotherapy (such as PD-1 inhibitors), thereby delaying tumor progression or reducing lesion size.

Individualized treatment opportunity: The study will develop combination regimens based on your intestinal microbiota characteristics and tumor molecular features, potentially obtaining more precise intervention than conventional treatment.

This study may delay disease progression, prolong survival, or improve quality of life, but cannot guarantee cure of tumor or effectiveness for all participants. We hope that data obtained from your participation in this study (such as association between intestinal microbiota changes and efficacy) can provide better treatment strategies for future MSS mCRC patients, benefiting more patients with similar conditions.

V. Alternative Treatment Options

In addition to participating in this study, you have the following options:

Conventional Treatment Options

Chemotherapy Combined with Immunotherapy: In first-line treatment of gastric cancer (GC) and gastroesophageal junction cancer (GEJC), immune checkpoint inhibitors (ICIs) combined with chemotherapy show certain efficacy. For example, pembrolizumab combined with chemotherapy significantly prolonged overall survival (OS) and progression-free survival (PFS) in the KEYNOTE-859 study. Additionally, nivolumab combined with chemotherapy in the CheckMate 649 study also showed significant OS and PFS benefits for patients with PD-L1 CPS \geq 5.

New Treatment Options

Dual-target Drugs: SHR-1701 is a PD-L1/TGF- β bispecific antibody drug. At the 2024 ESMO and ASCO conferences, it demonstrated significant survival benefits compared to chemotherapy alone, and significantly reduced chemotherapy myelosuppression toxicity. This dual-target mechanism simultaneously inhibits

PD-L1 and TGF- β pathways, solving the immunosuppression dilemma in the tumor microenvironment.

Immunotherapy Combined with Targeted Therapy: For HER2-positive gastric cancer patients, chemotherapy combined with immune checkpoint inhibitor regimens are being explored. Additionally, for patients with specific molecular markers (such as dMMR, EB virus infection, CLDN18.2-positive), personalized comprehensive treatment regimens are also under study.

Immunotherapy Combined with Other Therapies: In colorectal cancer (CRC), although most microsatellite stable (MSS)/mismatch repair proficient (pMMR) CRC patients show poor response to immunotherapy, some new combination treatment strategies show potential. For example, one study reported that triple therapy combining PD-1 inhibitor with radiotherapy and granulocyte-macrophage colony-stimulating factor (GM-CSF) achieved complete remission in an MSS/pMMR metastatic CRC patient, with progression-free survival exceeding 2 years. Please discuss these and other possible options with your doctor.

VI. Use of Study Results and Confidentiality of Personal Information

With your and other participants' understanding and assistance, study results obtained from this project (including clinical efficacy, safety, laboratory, and intestinal microbiota data) may be published in domestic and international medical journals or academic conferences in anonymous aggregated form to promote medical progress.

Your personal information and medical records will be treated as confidential and handled according to the following principles:

Data Identification: All information that can identify you (such as name, hospital number, ID number, etc.) will be replaced with unique codes. Original identification information will be separately encrypted and saved, accessible only to authorized personnel.

Access Permissions: In addition to the research team, only national drug regulatory authorities, hospital ethics committees, or legally authorized institutions may access your original materials according to prescribed procedures when needed for compliance review, supervision, or legal proceedings.

Information Sharing: Study data may be shared in anonymous form with domestic and international cooperating units (such as universities, research institutions) for further analysis of the relationship between intestinal microbiota and tumor immunotherapy, but no information that can identify you will be disclosed.

Results Publication: Your name, photos, or other identifiable information will not appear in any publicly published materials.

Unless required by national laws and regulations, your personal information will not be disclosed to unrelated third parties. After study completion, all materials involving your identity will be preserved for at least 15 years according to hospital regulations, and then destroyed according to standards.

VII. Study Costs and Related Compensation

1. Costs for Study Drugs and Related Examinations

Study Medication: Costs related to young fecal microbiota transplantation (yFMT) and immunotherapy drugs (PD-1 inhibitors) will be provided free of charge by the sponsor (The First Affiliated Hospital of Xiamen University).

Study-related Examinations: Including additional laboratory examinations specified in the study protocol (such as intestinal microbiota sequencing, immunological indicators), endoscopy, imaging examinations (CT/MRI), and visit examination costs increased due to study needs, borne by the sponsor.

Routine Medication and Examinations: If you have other diseases requiring non-study drugs (such as antihypertensive drugs, hypoglycemic drugs) or routine medical care (such as routine complete blood count, electrolyte re-examination), related costs will be settled according to medical insurance policy, paid by you or medical insurance.

Potential Additional Costs: Costs incurred due to personal reasons (such as requesting additional examinations, non-study-related complications) need to be borne by yourself and will not be compensated.

2. Compensation for Study Participation

To compensate for possible transportation, lost work, and other expenses incurred by your participation in this study, you will receive **¥200.00 (Two Hundred Yuan)** cash

compensation for each visit, totaling no more than **¥2,000.00 (Two Thousand Yuan)** (calculated according to actual completed visit times). Compensation will be distributed on-site by research assistants after each visit and confirmed by signature.

3. Compensation for Injuries

If determined by the study doctor to be directly related to the study (such as infection caused by yFMT, serious adverse reactions caused by immunotherapy, etc.), you will receive:

Free Treatment: Necessary medical treatment provided by The First Affiliated Hospital of Xiamen University until condition stabilizes.

Legal Compensation: According to Good Clinical Practice (GCP) and relevant Chinese laws and regulations, The First Affiliated Hospital of Xiamen University will bear corresponding responsibilities, including medical costs, lost wages, disability or death compensation, etc. Specific amounts will be negotiated based on judicial appraisal results or resolved through legal channels.

VIII. Participants' Rights and Responsibilities

1. Your Rights

Throughout the entire study participation process, you are voluntary. If you decide not to participate in this study, it will not affect other treatments you should receive. If you decide to participate, you will be asked to sign this written informed consent form. You have the right to withdraw from the study at any stage without discrimination or unfair treatment, and your corresponding medical treatment and rights will not be affected.

2. Your Responsibilities

As a participant, you need to provide true information about your medical history and current physical condition; inform the study doctor of any discomfort you discover during this study period; avoid taking drugs or foods that may interfere with treatment effects or increase safety risks (such as antibiotics, proton pump inhibitors, other probiotics/prebiotic preparations, and alcohol, etc.). If you indeed need to use them due to your health condition, please communicate with the study doctor in advance,

and the research team will provide individualized advice after evaluating risk-benefit; inform the study doctor whether you have recently participated in other studies or are currently participating in other studies.

IIV. Relevant Contact Information

If you have any questions related to this study, please contact at phone number **15980809201**.

If you have any questions related to your rights/interests, or if you want to report difficulties, dissatisfaction, and concerns encountered during participation in this study, or want to provide opinions and suggestions related to this study, please contact the Ethics Committee at phone number **0592-2137569**, email: xdfyec@sina.com.

INFORMED CONSENT FORM

Consent Signature Page

I have been informed about the purpose, background, procedures, risks, and benefits of this study. I have had sufficient time and opportunity to ask questions, and the answers are satisfactory to me.

I have also been informed about whom to contact when I have questions, want to report difficulties, concerns, suggestions for the study, or want to obtain further information or provide help for the study.

I have read this informed consent form and agree to participate in this study.

I know I can choose not to participate in this study, or withdraw from this study at any time during the study period without any reason.

I have been informed that if my condition worsens, or if I experience serious adverse reactions, or if my study doctor feels that continued participation in the study is not in my best interest, he/she will decide to withdraw me from the study. Without my consent, the sponsor or regulatory authorities may also terminate the study during the study period. If this occurs, the doctor will notify me promptly, and the study doctor will also discuss other options with me.

I will receive a copy of this informed consent form containing signatures of both myself and the researcher.

Participant Signature: _____ **Date:** _____

Contact Phone:

(Note: If the participant lacks capacity or has limited capacity, the guardian needs to sign in the guardian signature section below)

Guardian Signature: _____ **Date:** _____

Contact Phone:

I have accurately informed the participant about this document. He/she has accurately read this informed consent form, and I certify that the participant had the opportunity to ask questions and voluntarily agreed.

Researcher Signature: _____ **Date:** _____

Contact Phone: