Informed Consent Form

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Research Unit: The Seventh Medical Center of the General Hospital of the Chinese People's Liberation Army

Principal researcher at this center: Professor Du Junfeng

Dear Subject:

We are inviting you to participate in an investigator-initiated clinical trial: "Preoperative neoadjuvant chemoradiotherapy combined with PD-1 monoclonal antibody (tislelizumab) and Probio-M9 for the treatment of pMMR/MSS in locally advanced mid-low rectal cancer: a single-center, prospective, randomized controlled trial." This informed consent form provides you with information to help you decide whether to participate in this study. Please take some time to carefully read the following content. If you have any questions or questions about terminology, please discuss them with your doctor.

Your participation in this study is entirely voluntary. This study has been reviewed and approved by the Medical Ethics Committee of the Seventh Medical Center of the PLA General Hospital.

I. Research Background

Rectal cancer is one of the most common digestive tract tumors in China, with approximately 253,000 new cases annually, accounting for 18.6% of global cases. With changes in modern lifestyles and improved living standards, the incidence of rectal cancer is also rising. Locally advanced rectal cancer (LARC) refers to rectal cancer where the tumor has invaded the muscular layer of the intestinal wall and may even have lymph node metastasis, but has not yet metastasized to distant sites. These patients are usually diagnosed at an intermediate or advanced stage, and the opportunity for surgical treatment

is limited. This is especially true for patients with mid-to-low rectal cancer (tumor less than 10 cm from the anus), whose lower tumor location reduces the chances of preserving the anus after surgery, resulting in a poorer postoperative quality of life.

Currently, the standard treatment for LARC is neoadjuvant chemoradiotherapy (nCRT) combined with total mesorectal excision (TME). Despite this, the pathological complete response (pCR) rate for patients receiving neoadjuvant chemoradiotherapy remains low, approximately 10 % -15%. Recent studies have shown that immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway (such as PD-1 monoclonal antibodies) have good efficacy in some patients (e.g., those with high microsatellite instability or mismatch repair deficiency in CRC). However, most LARC patients are not among these patients, and their response to immunotherapy remains limited. A study initiated by Professor Zhang Zhongtao of Beijing Friendship Hospital showed that neoadjuvant chemoradiotherapy combined with tislelizumab can improve the pCR rate and objective response rate in patients with pMMR/MSS locally advanced rectal cancer, with good safety.

In recent years, the role of the gut microbiota in regulating the immune environment has attracted attention. It is currently believed that the balance of the gut microbiota may be correlated with the response to immunotherapy. Chinese researchers isolated a probiotic strain, *Lactobacillus rhamnosus* Probio-M9, from human colostrum. In laboratory studies and animal models, Probio-M9 has been observed to have a certain regulatory effect on the gut microbiota. Based on these preliminary findings, researchers hope to explore whether this probiotic, as a nutritional supplement, can assist existing standard treatment regimens by improving the gut environment. It is important to note that Probio-M9 is currently marketed as a food product in China (Production License No.: SC10633070200513, Implementation Standard: Q/JYH0006S) . It is not a specific drug for treating cancer, and its synergistic effect with immunotherapy is still in the clinical exploration stage; it cannot directly replace existing anticancer drugs.

II. Research Objectives

effect of adding oral probiotic Probio-M9 to standard treatment in patients with locally advanced rectal cancer . Simultaneously, we will observe whether changes in gut microbiota have a potential positive impact on patients' short-term treatment response and long-term quality of life, providing scientific evidence for future combination therapy strategies.

III. Research Content

1) Research Overview

This study is a prospective clinical trial designed to evaluate whether the combined use of the probiotic Probio-M9, in addition to standard neoadjuvant chemoradiotherapy and immunotherapy, can provide additional benefits for patients like you with locally advanced rectal cancer.

50 participants nationwide . If you agree to participate, you will be randomly assigned to one of two groups:

Experimental group (Group A): Received neoadjuvant chemoradiotherapy + tislelizumab + Probio-M9

Control group (Group B): Received neoadjuvant chemoradiotherapy + tislelizumab + placebo

"Randomization" is like a coin toss; a computer program determines your group, and neither you nor your doctor can choose. This ensures fair grouping and avoids human bias influencing research results. Based on a 1 :1 allocation ratio, you have approximately a 50% chance of being placed in the experimental group and a 50% chance of being placed in the control group.

This study will not interfere with your standard surgical procedure or routine postoperative follow-up schedule. We will follow you up for 3 years post-surgery to collect information on your recovery and disease control.

2) Research Procedure

The entire research process will begin after you sign this informed consent form and mainly includes the screening period, treatment period, surgical period, and follow-up period.

Step 1: Informed Consent and Screening (approximately 1 week)

After you have fully understood this study and signed the consent form, the research physician will need to confirm whether you meet the participation criteria (i.e., "inclusion/exclusion criteria").

Therefore, we will select from the examinations performed during your routine medical treatment , including: blood collection for complete blood count, liver and kidney function tests, coagulation function tests, and screening for infectious diseases; urine collection for routine urinalysis; and electrocardiogram (ECG) examination.

Please note: These examinations are not additional requirements of this study protocol, but are routine examinations necessary for the diagnosis and treatment of your disease .

Step 2: Randomization and Treatment

If you pass the screening, you will officially enter the study and be randomly assigned by the computer system to either the experimental or control group mentioned above.

The specific intervention measures for the two groups are as follows:

Common treatment: Both groups received the same standard neoadjuvant chemoradiotherapy (long-course radiotherapy combined with capecitabine chemotherapy , followed by 2 cycles of CapeOx chemotherapy after radiotherapy) and PD-1 monoclonal antibody (tislelizumab) treatment.

the difference:

Experimental group (Group A): Starting from day one, take an extra dose of Probio-M9 probiotics orally once a day.

Control group (Group B): Starting from day one, an additional placebo with the same appearance as Probio-M9 was administered orally once daily.

Step 3: Surgical and Postoperative Procedures

After neoadjuvant therapy, you will undergo standard surgical procedures (total mesorectal excision, TME) according to treatment guidelines. This surgery is standard treatment for this condition and was not performed as part of this study.

After surgery, researchers will collect your surgical pathology specimens for analysis

to assess the tumor's response to previous treatment (i.e., pathological remission assessment), which is a key part of this study.

Step 4: Follow-up

Routine follow-up examinations: According to the guidelines for the diagnosis and treatment of colorectal cancer, you will need to have regular follow-up examinations after surgery (e.g., every 3-6 months for the first 3 years) at our hospital or a local hospital, including chest, abdominal and pelvic CT scans, tumor marker tests (CEA, CA19-9), and colonoscopies. These are routine medical procedures necessary for your recovery.

Study Follow-up: To collect the data required for this study, our research team will monitor your health and disease control every three months for the first three years after surgery via telephone, outpatient visit, or by submitting follow-up examination records. Our research team will record the results of your routine follow-up examinations but will not require you to undergo additional examinations or procedures.

III. Other Treatment Options

Participation in this study is entirely voluntary. In addition to participating in this study, you always have the right to opt out of this study protocol and choose other recognized treatment methods. Your doctor will respect any decision you make, and this will in no way affect your access to any standard medical care you are entitled to at our hospital.

Here are some other major treatment options you may consider, along with their main benefits and risks:

1. Standard neoadjuvant chemotherapy followed by surgery

This is the current standard treatment plan for your condition, and its benefits and risks have been fully verified.

Key benefits: This treatment plan is a standard therapy recommended by authoritative guidelines both domestically and internationally. It is technically mature and has a clearly defined procedure. It can effectively shrink tumors, reduce the risk of postoperative recurrence, and create more favorable conditions for surgical resection.

Key risks and limitations: Compared to the protocol used in this study, the

pathological complete response rate of this standard protocol is relatively limited. This means that the probability of complete tumor disappearance may be lower, and the opportunity to achieve organ preservation (anal preservation) may be correspondingly reduced.

2. Individuals not participating in this study but receiving neoadjuvant chemoradiotherapy combined with tislelizumab.

This regimen was used in the control group of this study and is also a cutting-edge exploratory treatment regimen.

Main benefits: Adding immunotherapy to standard radiotherapy and chemotherapy has been shown in studies to potentially improve the complete remission rate of tumors, and is expected to bring patients better treatment results and a higher chance of sphincter preservation than radiotherapy and chemotherapy alone .

Key risks and limitations: This regimen still requires the combined use of immune checkpoint inhibitors, and therefore carries the risk of corresponding immune-related adverse reactions (such as thyroid dysfunction, pneumonia, colitis, etc.). Furthermore, its long-term efficacy and safety are still under further investigation and observation.

3. Perform radical surgery directly.

For some patients with locally advanced rectal cancer, it may be considered to skip preoperative neoadjuvant therapy and undergo surgery directly .

Main benefits: Avoids the toxic side effects that may be caused by radiotherapy, chemotherapy and immunotherapy, and has a shorter treatment cycle.

Key risks and limitations: For patients with large tumors, direct surgery may result in incomplete resection, increased risk of recurrence, and extreme difficulty in preserving the anus . This approach is generally not the first choice for patients with advanced-stage tumors.

4. Patients do not receive anti-tumor treatment; only symptomatic, supportive, and palliative care is provided.

If you have a full understanding of the risks of disease progression, you may choose

not to receive any anti-tumor treatments, but only supportive care aimed at relieving symptoms and improving quality of life (such as pain relief, nutritional support, psychological support, etc.).

Main benefits: Avoidance of all adverse reactions associated with anti-tumor treatments, and a relatively simple treatment process.

Key risks and limitations: This option does not control tumor progression, and the disease may continue to develop.

5. Choose to participate in other clinical studies (if any).

In addition to this study, you may also choose to participate in other ethics-approved clinical studies if you meet the eligibility criteria. Your suitability for other studies will be assessed by the investigating physician based on your specific medical condition.

Important Note:

We recommend that you fully discuss all options with your family and attending physician. If you choose to participate in this study, you have the right to withdraw at any time without any reason. If you choose to withdraw, your doctor will discuss with you and switch to the other most suitable treatment option mentioned above.

IV. Potential Impacts of This Research

1. Time and Arrangement

The planned visits and examinations during the study may take up more of your time and may require special travel arrangements. If you have any questions about any step of the study, please feel free to consult the studying physician.

2. Medication restrictions

To ensure the scientific validity of the research results and your safety, you must adhere to the following medication guidelines during the research period:

Prohibited medications: You must not use any other immunotherapy drugs (such as PD-1/L1, CTLA-4, or other immune checkpoint inhibitors). Furthermore, any live virus vaccines are prohibited during the study period.

Medications requiring caution: You must consult your research physician before

starting any new medication (including prescription drugs, over-the-counter drugs, herbal remedies, or health supplements). Extra caution is needed with the following medications that may affect the immune system or interact with investigational drugs:

Systemic immunosuppressants (such as high-dose corticosteroids, unless used to treat immunotherapy-related adverse reactions).

Other biological agents or chemical drugs known to have immunomodulatory effects.

Your research physician will provide you with detailed medication guidance, clearly explaining which medications you can take and which you need to avoid.

3. Restrictions on participation in other research

From the date you sign this informed consent form until the end of this study, you may not participate in any other clinical studies involving drugs or medical devices.

V. Additional risks and adverse reactions associated with participating in this study

In addition to the known risks of standard treatment for locally advanced rectal cancer (including neoadjuvant chemoradiotherapy, immunotherapy, and surgery), participation in this study may bring you the following additional risks and uncertainties. All of these risks will be closely monitored in addition to routine surveillance.

(a) New risks in research: Potential risks of probiotic Probio-M9

This study aims to evaluate the safety and efficacy of adding the probiotic Probio-M9 to standard treatment regimens. Although Probio-M9, as a food- grade probiotic, is generally well-tolerated, as part of this study, you should be aware of its potential risks and areas of uncertainty:

1. Unknown interactions and surgical effects:

Currently, there are no clinical studies specifically evaluating the potential impact of Probio-M9 on surgical safety and postoperative recovery when used in combination with neoadjuvant chemoradiotherapy and immunotherapy.

Therefore, this study will establish an independent safety monitoring committee to pay particular attention to any signs that may affect perioperative safety , such as delayed wound healing, abnormal immune activation, or coagulation dysfunction . If any signs that may affect surgical safety are detected, the study medication will be discontinued

immediately and medical intervention will be provided.

2. Theoretical individual health risks:

Gut microbiota imbalance: In rare cases, the introduction of exogenous probiotics may cause a temporary imbalance in the gut microbiota, resulting in symptoms such as diarrhea, bloating, or constipation, which usually recover after discontinuation of the medication.

Allergic reactions: If you are allergic to probiotics or their excipients, you may experience symptoms such as rash, itching, and in severe cases, fever. If this occurs, stop taking the medication immediately and seek medical attention.

Risk of gut microbiota dependence: Some theories suggest that long-term reliance on exogenous synthetic probiotics may impair the gut's ability to naturally multiply beneficial bacteria. Therefore, this study will discontinue use after the treatment period to avoid long-term dependence.

(ii) Known risks of immunotherapy in standard treatment regimens

tislelizumab) used in both groups in this study is a cutting-edge immunotherapy regimen, which inherently carries a unique set of risks, namely immune-related adverse events (irAEs). This is because the drug-activated immune system may attack your normal organ tissues. These reactions can involve multiple organs and occur at any time during or after treatment.

Common organs that may be affected include:

Intestinal issues: May cause diarrhea, colitis, and in severe cases, rectal bleeding and severe abdominal pain.

Skin: Rash and itching may occur.

Endocrine organs: May cause thyroid dysfunction, pituitary inflammation, etc.

Liver: May lead to hepatitis, manifested as fatigue, jaundice, etc.

Lungs: Rare but serious pneumonia, characterized by cough, chest tightness, and difficulty breathing.

We have well-established management guidelines for these immune-related adverse reactions. Once they occur, immunotherapy will be paused or permanently discontinued

depending on the severity, and intervention will be initiated with medications such as glucocorticoids. Detailed grading and management principles are provided in the appendix.

Unknown risks:

There may be some risks and adverse reactions that are currently unpredictable.

sintilimab) and Probio-M9 used in this study are both immune-related or gut microbiota-modulating biologics, their preoperative use may have some impact on surgical safety and postoperative recovery. The following potential situations require special attention:

1. Increased intraoperative risks : Immune-related drugs may cause inflammatory responses or enhanced immune system activity, leading to increased risk of intraoperative tissue edema, abnormal vascular responses, or bleeding. 2. **Delayed postoperative recovery** : Some patients may experience delayed wound healing, increased risk of infection, or slow recovery of bowel function. 3. **Coagulation abnormalities** : Coagulation function indicators will be monitored during the study . If abnormalities are found, treatment may be suspended or surgery may be postponed as appropriate. 4. **Preoperative assessment and adjustment of surgical timing** : An observation and assessment window (usually no less than 4 weeks) will be established between the last preoperative medication and surgery to ensure that the effects of the medication gradually diminish and the patient's physical condition is suitable for surgery. If the assessment reveals a risk that immediate surgery is not advisable, surgery will be postponed or the treatment strategy will be changed. 5. **Risk of unsuitability for surgery** : In rare cases, such as severe immune-related complications or organ dysfunction, the surgeon's decision on whether to proceed with the planned surgical procedure may be affected.

placebo risk

Some patients may be taking a placebo (a blank medication). Taking a placebo means you are not taking any additional medication. If you have any questions about placebos, consult your research physician.

Please understand and accept that such risks are among the potential unknown risks

of this current research. The research team will closely monitor your progress and promptly address any adverse reactions to ensure your treatment safety to the greatest extent possible. Please contact your research physician immediately if you experience any discomfort or pre-operative abnormalities.

You should inform your family or close friends that you are participating in a clinical study so they can take note of the events described above. If they have any questions about your participation in the study, you can tell them how to contact your study physician.

VI. Research Benefits

The main potential benefit of participating in this study is that by monitoring and modulating your gut microbiota, research physicians can gain a deeper understanding of your body's response to treatment. While we anticipate that the addition of probiotics may help improve treatment efficacy, as this study is still in the exploratory stage, we cannot guarantee that you will personally experience direct therapeutic effects (such as tumor shrinkage or prolonged survival). Your participation will provide the medical community with crucial data for exploring "gut microbiota regulation in synergistic tumor immunotherapy," thereby benefiting future patients.

VII. Processing of biological specimens and medical information

1. Collection and use of biological samples

Sample type and quantity: This study will additionally collect your stool sample (approximately 10g, three times in total, immediately after enrollment, week 2 of neoadjuvant therapy, and before surgery) and tumor tissue sample (surgical specimen, soybean-sized, approximately 0.5cm in diameter).

Purpose: These samples will be used to analyze indicators related to treatment response, such as detecting biomarkers related to tumor immunity through technologies like gene sequencing, studying the correlation of gut microbiota changes in blood signals, and exploring potential factors affecting efficacy. These analyses aim to help us better understand the mechanism of action of this treatment regimen.

Preservation and Duration: All collected biological samples will be desensitized (i.e.,

your name, ID number, and other direct personal information will be hidden, and only the research number will be used) and then stored at low temperatures in the biobank of the General Surgery Laboratory of the General Hospital of the Chinese People's Liberation Army (General Surgery Center of the PLA), which has strict security measures. The samples are expected to be preserved for 5 years, calculated from the date you complete or withdraw from this study. After the preservation period expires, or if you request to withdraw your consent at any time, we will completely destroy your samples in accordance with biosafety regulations.

Future Use and Provision: These delabeled samples and data may be used for other scientific research related to this project in the future. However, please be aware that all future research projects must be reviewed and approved by our institution's ethics committee again. If future research exceeds the scope of this informed consent, the research team will contact you again to obtain your specific written informed consent. Your sample will not be used directly for commercial product development. Your biological sample will not be provided or shared with any third party without your explicit and repeated consent.

Sample Disposal: If you choose to withdraw during the study, your submitted samples will be de-identified and used for the analyses already initiated, unless you explicitly request their destruction. If you explicitly request destruction, any remaining unused samples will undergo thorough biosafety processing.

2. Confidentiality of research data and personal information

All your medical information (such as imaging reports, pathology reports, laboratory test data, etc.) and analytical data derived from your biological samples collected in this study will be used only by authorized personnel of this research team, and its sole purpose is to conduct scientific analysis and efficacy evaluation.

Data Sharing and Secondary Use: Your research data, after being fully de- identified, may be used for subsequent scientific research analysis or for publishing aggregated research results in high-level academic journals (without disclosing any personally identifiable information). All secondary use of data will be strictly in accordance with

confidentiality agreements and under supervision.

Confidentiality Measures: We will strictly protect your privacy through the following measures: all paper documents will be stored in locked filing cabinets; all electronic data will be stored on a dedicated, encrypted, password-protected research server; throughout the research process, your identity will be represented by a unique research number, and any information that can identify you personally will be kept separate.

Data Transfer Statement: All your original personally identifiable information and biosample analysis data will be strictly stored in the General Surgery Laboratory of the Seventh Medical Center of the PLA General Hospital . All data and information will not be transferred or provided to any third-party institution or company outside this research institution, or transferred overseas. In academic publications, only aggregated data that cannot be personally identifiable will be used.

Your rights: You have the right to withdraw your consent to the use of your biological samples and medical information at any time. Withdrawing consent will not affect your future medical treatment. If you choose to withdraw, we will cease collecting and using your samples and information and will destroy the collected samples in accordance with regulations.

VIII . Rights and Obligations of Subjects

You have ample time to consider and the right to ask questions at any time, and the final decision on whether to participate in this study rests with you. Your decision not to participate in this study will not affect any other medical attention you should receive; if you decide to participate, please truthfully inform the research physician about your medical history and physical condition, whether you have participated in other studies or are currently participating in other studies, and please sign this written informed consent form. Even after signing, you can still withdraw from this study at any stage. If any new, important information is discovered during the study that may affect your willingness to continue participating, your research physician or other research team members will notify you immediately. You can also inquire about and consult about the study at any time.

If you do not comply with the study plan, or if the study physician believes that your continued participation in this study is not in your best interest, the study physician may ask you to withdraw from the study; if you experience an adverse reaction to the study drug, or if new safety information regarding the study drug becomes available during the study, the study physician or sponsor may terminate your participation in this study without your consent.

If you withdraw from the study for any reason, you may be asked about your use of the study drug. You may also be required to undergo unscheduled physical and laboratory tests if the study physician deems it necessary, and the study physician will discuss medical matters following your withdrawal from the study with you.

IX. Related expenses for participating in the research

The investigational product Probio-M9 and placebo involved in this study will be provided free of charge by the sponsor. Furthermore, any additional costs incurred due to participation in this study related to gut microbiota sample collection (fecal collection) and related specialized laboratory tests (such as microbiota sequencing analysis , single-cell sequencing , etc.) will be covered by the study funds and will not be borne by you. Other standard medical expenses required for your disease treatment (such as routine chemotherapy and radiotherapy, tislelizumab , surgery fees, and routine CT scans and laboratory tests) will still be borne by the patient or their medical insurance as usual.

10. Remuneration or Compensation

This study was conducted without compensation or remuneration.

XI . Measures to handle damage caused by the research institute:

The physician will make every effort to prevent and treat any harm that may arise from this study. If you experience any discomfort or unexpected situation during the study, whether or not it is related to the study, please inform your study physician immediately. They will assess the situation and provide professional medical treatment. If an adverse event is confirmed to be related to this study, and any resulting injury is covered by this study, the research institution will bear the costs of treatment and provide corresponding

financial compensation in accordance with Chinese laws and regulations. This research institution has purchased clinical trial liability insurance for this study to ensure that the above commitments are fulfilled.

Even though you have signed this informed consent form, you still retain all your legal rights. If your rights are violated, you can contact the Medical Ethics Committee of the Seventh Medical Center of the PLA General Hospital at 010-66721926.

12. Confidentiality

If you decide to participate in this study, your participation and personal information during the study will be kept confidential. The physicians and other researchers in charge of the study will use your medical information for the research. Your file will only be accessible to the researchers. Your research information and laboratory test specimens will be identified by a number during the study. Your identity will not be identified; only the research physicians and research team members will have access to the number.

To ensure the research is conducted in accordance with regulations, government authorities or members of the ethics review committee may, when necessary, access your personal data at the research institution, without disclosing any of your information. Any public reports regarding the results of this research will not disclose your personal identity.

We will comply with relevant laws and regulations to ensure that the privacy of your personal medical information is fully protected.

Thirteen, Participation is voluntary

Participation in this study is entirely voluntary. You may refuse to participate or withdraw from the study at any time during the study process without giving any reason. This decision will not affect your future treatment.

If you decide to withdraw from this study, please notify your study physician in advance. For your safety, you may be required to undergo relevant examinations, which is beneficial to your health.

XIV. How to get help

If you have any questions or difficulties related to this research, please contact the

researcher, Dr. Du Junfeng, at 15810908850.

If you have any questions regarding the rights of participants in this study, you can contact the Medical Ethics Committee of the Seventh Medical Center of the PLA General Hospital at 010-66721926.

Informed consent signature page

Subject statement:

Having fully understood the contents of the informed consent form for this study and the potential risks and benefits of participating in this study, I voluntarily participate in this trial and make the following declaration:

1. I have read the contents of the above informed consent form and understand the nature, purpose, and possible adverse reactions of this study. My questions have been answered satisfactorily.

researchers , truthfully and objectively providing them with information about my health status and related circumstances before, during, and at each follow-up period of this study.

3. I understand that I can withdraw from the study at any time without any adverse impact on my subsequent treatment. I understand that the researchers have the right to terminate the study at any time based on my situation.

4. I am aware that I will receive a copy of a signed informed consent form.

5. I have learned that the doctors involved in this study, the heads of relevant authorities, and the Medical Ethics Committee of the General Hospital of the People's Liberation Army all have the right to review the research records and case data. I agree that the aforementioned personnel may directly obtain my research records and understand that the above information will be kept confidential.

6. After careful consideration, I volunteer to participate in this clinical study.

Subject's signature: Date:

Subject contact number:

Signature of legal guardian (if applicable): Date:

(Spouse/Parents/Adult Children)

Contact number for legal guardian:

Reasons for requiring the legal guardian's signature:

Researcher's statement:

I confirm that the details of this study have been explained to the patients, especially the potential risks and benefits of participating in this study, and that the participants have been given a copy of the informed consent form signed and dated by both parties.

Researcher's Signature: Date:

Researcher contact number: