Informed consent form for the clinical research project

Project name: The Efficacy of Watch and Wait Strategy or Surgery After Neoadjuvant Immunotherapy for Locally Advanced Colorectal Cancer with dMMR/MSI-H Guided by MRD Dynamic Monitoring(WINDOW): A Single-center, Open-label, Prospective, Phase II Clinical Trial.

Scheme version number and date：V1.0，July 02,2023

Research institution: Yunnan Provincial Cancer Hospital

Principal investigator: Zhang xuan, Li Yunfeng

Subject Name：　　Subject Name is abbreviated：

Subjects address：

Subjects telephone：

Dear_____ (Mr / Ms)

We invite you to participate in a clinical study. This informed consent form provides you some information to help you decide whether to participate in this clinical study. Please use the time to read the following content carefully. If there are any unclear questions or terminology, you can discuss with the doctor concerned.

Your participation in this study is completely voluntary. This study has been reviewed by the Ethics Committee of Yunnan Cancer Hospital.

1. research background：

Colorectal cancer is one of the most common malignant tumors of the digestive tract, with high morbidity and mortality rates. Among them, 60% of cases are in the locally advanced stage. For these patients, direct surgical resection often leads to high positive margin rates and local recurrence rates. Therefore, the NCCN guidelines recommend "neoadjuvant chemoradiotherapy (NCRT) + radical total mesorectal excision + postoperative adjuvant chemotherapy" as the standard treatment protocol for locally advanced rectal cancer. For locally advanced colon cancer, neoadjuvant chemotherapy with FOLFOX or CAPEOX is also recommended for patients with bulky lymph nodes or clinical T4 colon cancer. Studies have shown that 15% to 20% of patients can achieve pathological complete response (ypCR) after NCRT, and the

5-year overall survival rate for ypCR patients is approximately 90%, indicating excellent prognosis. Consequently, some scholars have proposed that non-surgical treatment may be feasible for patients predicted to achieve pCR based on imaging and tumor markers after neoadjuvant therapy. Patients with clinical complete response (cCR) who undergo a watch-and-wait (W&W) approach show no significant difference in survival rates compared to those with postoperative pathological pCR. With the advent of precision medicine and rapid advances in genetic testing, we have identified a specific subtype of mismatch repair deficiency (dMMR) or high microsatellite instability (MSI-H) in molecular classification of malignant tumors, accounting for approximately 15% of all colorectal cancers. These patients are insensitive to conventional neoadjuvant chemoradiotherapy, but multiple studies have shown that the pCR rate can reach approximately 60% after neoadjuvant immunotherapy. The W&W strategy seems to be an attractive option for patients with dMMR/MSI-H locally advanced colorectal cancer after neoadjuvant immunotherapy. Currently, the W&W approach utilizes imaging combined with tumor marker follow-up. However, with further research, it has been found that minimal residual disease (MRD) is a crucial factor for recurrence and metastasis after radical treatment of tumors. Traditional imaging or laboratory methods are unable to detect MRD, but circulating tumor DNA (ctDNA) in the blood is the most commonly used method for detecting MRD. Furthermore, ctDNA can predict recurrence 5 to 8 months earlier than imaging. Currently, the technological approaches for ctDNA-MRD detection in solid tumors are mainly divided into two major categories: Tumor-informed assays 和 Tumor-naïve assays，For the Tumor-informed approach, tumor tissue sequencing is first performed to obtain mutational information about the tumor. From this information, specific mutations are selected for personalized customization. Subsequently, blood samples are collected to detect ctDNA, and high-depth sequencing is used to track and identify the selected mutations from the tumor tissue. In contrast, the Tumor-naïve technique solely relies on plasma ctDNA detection. It employs a pre-determined set of primers/probes designed for cancer-specific panels to analyze ctDNA. The detection range is the same for everyone, and if a patient does

not have these mutations, they cannot be detected, resulting in a higher rate of missed detections and lower accuracy.

Currently, there is a lack of research worldwide on the use of MRD dynamic monitoring to guide the W&W strategy after neoadjuvant immunotherapy for dMMR/MSI-H locally advanced colorectal cancer. Therefore, this study aims to develop an individualized plan based on the Tumor-informed approach, utilizing ctDNA dynamic monitoring to assess MRD in patients with stage II/III dMMR/MSI-H locally advanced colorectal cancer after neoadjuvant immunotherapy. This approach aims to provide support for patients undergoing the W&W strategy or surgery.

2. purpose of research:：

The study is a single-arm, single-center, Phase II, prospective clinical trial designed to investigate the effectiveness and safety of adopting a watch-and-wait strategy after achieving clinical complete response following neoadjuvant immunotherapy for dMMR/MSI-H locally advanced colorectal cancer, guided by dynamic monitoring of MRD.

3. Research Process：

（1）Research Content

- Project Name: The Efficacy of Watch and Wait Strategy or Surgery After Neoadjuvant Immunotherapy for Locally Advanced Colorectal Cancer with dMMR/MSI-H Guided by MRD Dynamic Monitoring(WINDOW): A Single-center, Open-label, Prospective, Phase II Clinical Trial
- Researchers: Zhang Xuan, Li Yunfeng
- Declaration: Participants are enrolled in an experimental research project.
- Research Methodology: This is a single-arm, single-center, prospective Phase II clinical study, aiming to enroll 22 patients with locally advanced colorectal cancer with dMMR/MSI-H. The patients will receive neoadjuvant therapy

with PD-1 inhibitors, and the clinical complete response rate (cCR) will be assessed for patients with MRD-negative status.

- Trial Procedure: If you agree to participate in this study, each participant will be assigned a unique identifier. Relevant information will be collected and medical records will be established without affecting clinical testing, pathological diagnosis, and treatment.
- Duration of Participation for Subjects: August 2023 to August 2026.
- Follow-up Frequency and Procedure: Outpatient or telephone follow-up will be conducted for enrolled patients. The observation indicators include:

（1）Evaluation Indicators for the Effectiveness of Neoadjuvant Therapy: Objective Response Rate (ORR) and Modified Tumor Regression Grade (mrTRG);（2）Immune-Related Adverse Events；（3）Surgical Complications, Postoperative Pathological Remission Rate, Tumor Regression Grade (TRG), Major Pathological Remission (MPR), and Pathological Complete Remission (pCR).

- Safety Follow-up：A safety follow-up will be conducted 28 days after the last medication. The planned follow-up content includes: body weight; physical examination; ECOG PS score; 12-lead electrocardiogram; blood routine test; blood biochemistry; urine routine test; coagulation function; thyroid function; contrast-enhanced CT scans of the chest, abdomen, and pelvis. Note: For patients who discontinue treatment due to disease progression, further follow-up contrast-enhanced scans are not required. Adverse events will be continuously monitored until resolution or until the investigator determines that further observation is no longer necessary. Concomitant medications will be continuously collected until the treatment of adverse events is completed or until the investigator determines that further collection is no longer necessary.

- Survival Follow-up: After the patient's disease progresses, the patient's survival status will be collected every 3 months, allowing for telephone follow-up.
- Inclusion criteria：

Disease Characteristics

(1) Histologically confirmed colorectal adenocarcinoma.

(2) Immunohistochemically confirmed as dMMR or/and pCR or/and NGS as MSI-H.

(3) Locally advanced colorectal cancer (stage II-III, cT3-4 and/or N+) assessed accor detected ding to the UICC/AJCC TNM staging system (8th edition, 2017).

*Clinical staging method: Colon staging by CT, rectal staging by pelvic MRI combined with endorectal ultrasonography.

(4) No signs of intestinal obstruction; or obstruction relieved after proximal colostomy.

(5) No distant metastasis confirmed by comprehensive examination (distant organ or/and distant lymph node metastasis).

Patient Characteristics

(1) Age ≥ 18 years and ≤ 75 years at the time of signing the informed consent form.

(2) Eastern Cooperative Oncology Group (ECOG) performance status score ≤ 1, with no deterioration within 2 weeks before enrollment, and a life expectancy of not less than 12 weeks.

(3) Hematology: WBC > 4000/mm³; PLT > 100,000/mm³; Hb > 10g/dL.

(4) Liver function: SGOT and SGPT less than 1.5 times the normal value; bilirubin less than 1.5 mg/dL.

(5) Renal function: creatinine < 1.8 mg/dL.

Prior Treatments

(1) No prior colorectal cancer surgery.

(2) No prior chemotherapy, immunotherapy, or radiotherapy.

(3) No prior biological therapy.

(4) Prior endocrine therapy: no restrictions.

- Exclusion Criteria

(1) Tumor biopsy specimens showing pMMR or microsatellite stability (MSS) by immunohistochemistry or microsatellite instability testing.

(2) Patients with histological types of colorectal cancer other than adenocarcinoma (such as neuroendocrine carcinoma, sarcoma, lymphoma, squamous cell carcinoma, etc.).

(3) History of HIV infection or active chronic hepatitis B or C (high viral DNA copy).

(4) Autoimmune diseases.

(5) Other active clinically significant infections (> NCI-CTC 3.0 version).

(6) Patients with clinical stage I disease.

(7) Preoperative evidence of distant metastasis, including isolated, distant, or non-contiguous intra-abdominal metastases.

(8) Open surgical procedures ⩽ 14 days before enrollment, excluding colon surgery.

(9) Unable to provide surgical tissue for WES testing for customized personalized MRD detection panel or patients with failed customization of the personalized MRD detection panel.

(10) Unable to provide blood samples for MRD testing at treatment and follow-up monitoring points.

(11) Cachexia, organ dysfunction, or decompensation.

(12) History of pelvic or abdominal radiation therapy.

(13) Patients requiring treatment for seizures (e.g., steroid or anti-epileptic therapy).

(14) Chronic inflammatory bowel disease, intestinal obstruction.

(15) Other severe diseases that, in the opinion of the investigator, may affect follow-up and short-term survival.

(16) Patients with a history of blood transfusion within 2 weeks before treatment.

(17) Unable to undergo clinical follow-up using contrast-enhanced magnetic resonance imaging (MRI) or contrast-enhanced computed tomography (CT).

(18) Prior use of anti-tumor traditional Chinese medicine. Patients who have used anti-tumor traditional Chinese medicine for no more than 7 days and have stopped for 2 weeks or more before enrollment can be included.

(19) Evidence of severe or uncontrollable systemic diseases (e.g., severe mental, neurological diseases, epilepsy or dementia, unstable or decompensated respiratory, cardiovascular, liver, or kidney diseases, left ventricular ejection fraction (LVEF) < 50%, uncontrolled hypertension [i.e., hypertension grade 3 or higher after medication]). Patients with swallowing dysfunction, active gastrointestinal diseases, or other diseases that significantly affect the absorption, distribution, metabolism, and excretion of oral drugs. Patients who have undergone major gastrectomy.

(20) Fever with body temperature above 38°C or clinically significant active infection in the past week; active pulmonary tuberculosis; active fungal, bacterial, and/or viral infection requiring systemic therapy;

(21) Active bleeding or newly diagnosed thrombotic disease; currently taking anticoagulant medication at therapeutic doses or with a tendency to bleed;

(22) Clinically significant major abnormalities in rhythm, conduction, or morphology on resting electrocardiogram (ECG), such as complete left bundle branch block, second-degree or higher atrioventricular block, clinically significant ventricular arrhythmia or atrial fibrillation, unstable angina pectoris, congestive heart failure, chronic heart failure with New York Heart Association (NYHA) class $\geqslant$ 2; tendency to bleed;

(23) Myocardial infarction, coronary/peripheral artery bypass, or cerebrovascular accident within 3 months; tendency to bleed;

(24) QT interval (QTc) $\geqslant$ 450 ms for males and $\geqslant$ 470 ms for females on a 12-lead ECG; tendency to bleed;

(25) Presence of risk factors that cause QT interval prolongation or increase the

risk of arrhythmia, such as heart failure, ≥ CTCAE (version 4.03), second-degree hypokalemia (defined as serum potassium < lower limit of normal - 3.0 mmol/L, with symptoms requiring treatment), congenital long QT syndrome, family history of long QT syndrome; tendency to bleed;

(26) Use of any medication known to prolong the QT interval within 2 weeks before enrollment; tendency to bleed;

(27) Insufficient bone marrow reserve or organ function, reaching any of the following laboratory limits (no corrective treatment within 1 week before blood sampling for laboratory tests):

a. Absolute neutrophil count < 1.5 × 109 / L;

b. Platelet count < 90 × 109 / L;

c. Hemoglobin < 90 g/L (< 9 g/dL);

d. Alanine aminotransferase > 3 times the upper limit of normal (ULN);

e. Aspartate aminotransferase > 3 × ULN;

f. Total bilirubin > 1.5 × ULN;

g. Creatinine > 1.5 × ULN or creatinine clearance

h. Serum albumin (ALB) < 28 g/L;

(28) Female subjects who are pregnant, lactating, or plan to become pregnant during the study period;

(29) Subjects with drug abuse and medical, psychological, or social conditions that may interfere with their participation in the study or have an impact on the evaluation of study results;

(30) Subjects with known or suspected hypersensitivity to the study drugs or any drugs related to this trial;

(31) Subjects with any unstable condition or situation that may jeopardize their safety and compliance;

(32) Other situations where the investigator believes the subject should not participate in this study.

4. Matters Requiring Your Cooperation:

In order to ensure the smooth and successful conduct of this study, please cooperate with the following matters:

- Please follow the researcher's instructions for medication and examinations.
- Do not change your current treatment or start any new treatment without first confirming with the study doctor.
- It is important to inform the study doctor of any health issues, even if you think they are not significant.
- Please disclose to the study doctor any medications (including herbal medicines) you are taking or have taken before and during the study, in addition to the study medication.
- If you need to terminate the study treatment prematurely for any reason, we hope you can complete the final evaluation conducted by the study doctor.
- Routine examinations will be required to ensure your safety.
- Please truthfully record your medication diary as instructed and submit it to your study doctor during your next visit.

5. Risks and Discomforts of Participating in the Study:

The PD-1 inhibitor treatment regimen may cause the following adverse reactions:

（1）Very Common (Occurrence Rate of Over 10%)

- Anemia, leukopenia, neutropenia, thrombocytopenia, lymphocytopenia
- Hypothyroidism
- Decreased appetite, hyponatremia, hypoproteinemia, hypokalemia
- Sensory disorders
- Nausea, vomiting, constipation, diarrhea
- Elevated AST, ALT, blood bilirubin, and GGT
- Reactive capillary proliferation, rash, pruritus

- Elevated serum creatinine
- Fatigue, fever, edema

（2）Common (Occurrence Rate of >1% to <10%)

- Infectious pneumonia, upper respiratory tract infection, urinary tract infection
- Monocytopenia, febrile neutropenia
- Infusion-related reactions (infusion reaction, hypersensitivity reaction)
- Abnormal thyroid function tests, hyperthyroidism, thyroiditis, abnormal pituitary hormone tests
- Hyperlipidemia, hypophosphatemia, diabetes
- Insomnia, somnolence
- Dizziness, headache
- Dry eye syndrome, eye pain, increased tearing
- Tachycardia, hypertension, arrhythmia
- Pneumonia (pneumonitis, interstitial lung disease, immune-mediated pneumonitis, autoimmune lung disease), cough, sputum production, chest discomfort, epistaxis, pleural effusion
- Hiccups, abdominal distension, oral mucositis, abdominal pain, gingival periodontal disease, gastroesophageal reflux disease, colitis
- Elevated blood alkaline phosphatase
- Vitiligo, hair loss
- Musculoskeletal pain
- Proteinuria, nephritis
- Weight loss, phlebitis, weight gain
- Hearing loss, tinnitus
- Other laboratory test abnormalities, other electrolyte disorders, elevated blood sugar, hyperuricemia, positive fecal occult blood, other abnormalities in urine

routine examination, positive urine hemoglobin, abnormal coagulation function test, elevated blood lactate dehydrogenase

（3）Uncommon (incidence > 0.1% - < 1%)

- Local inflammation, septic shock
- Febrile neutropenia
- Hypophysitis
- Hypophosphatemia
- Syncope
- Peripheral neuropathy, myasthenia syndrome, cognitive impairment
- Conjunctivitis, blurred vision
- Abnormal myocardial enzyme profile, hypotension, heart failure, bradycardia, prolonged QT interval on electrocardiogram, hepatic hemangioma, venous thrombosis, myocarditis, pericardial effusion
- Shortness of breath, respiratory failure, dyspnea, hemoptysis, hypoxia
- Gastrointestinal bleeding, dry mouth, chronic gastritis, dysphagia, oral bleeding, functional gastrointestinal disorders, oral and pharyngeal discomfort, peptic ulcer, esophagitis
- Dermatitis, erythema, dry skin, hyperhidrosis, psoriasis
- Arthritis
- Oliguria, dysuria
- Chills, shock, chills, multiple organ dysfunction syndrome
- Sexual dysfunction
- Otitis media, deafness, earache
- Other electrocardiogram abnormalities, elevated amylase, decreased free urinary cortisol

Unknown Risks: There may be some unpredictable risks and adverse reactions currently unknown. You may not experience any adverse reactions, or you may

experience some with varying degrees of severity, ranging from mild to moderate to severe. If any of the aforementioned adverse events occur, please contact your research doctor or nurse immediately. Your doctor will provide you with appropriate treatment.

6. Benefits of Participating in the Study:

If you agree to participate in this study, there is a possibility that you may receive direct medical benefits, but there is also a chance that you may not. Through statistical analysis of your clinical pathological, molecular biological, and other data, we aim to assess the efficacy and safety of using MRD dynamic monitoring to guide the wait-and-watch strategy or surgery following neoadjuvant immunotherapy in achieving clinical complete response for locally advanced dMMR/MSI-H colorectal cancer.

We hope that the information gained from your participation in this study will provide guidance in the future for patients with similar conditions as yours.

7. Alternative Treatments:

If I choose not to participate in this study (or if I am not eligible to participate), are there any other medical options available to me?

The protocol of this study involves monitoring the changes in MRD after 4 cycles of neoadjuvant immunotherapy. If the MRD remains negative after two consecutive tests, a non-surgical watch-and-wait strategy will be adopted. However, if the MRD remains positive after 8 cycles of neoadjuvant immunotherapy, surgical treatment will be recommended.

Apart from participating in this study, you have the following alternative options:

- Traditional chemotherapy, radiotherapy, targeted therapy, etc.
- If disease progression (PD) is observed during the efficacy evaluation during participation in this study, an intra-hospital multidisciplinary team discussion (MDT) will be conducted to develop an individualized treatment plan, such as switching to direct surgical resection, chemotherapy, radiotherapy, targeted therapy, etc.

- For patients who undergo non-surgical watch-and-wait strategy after achieving MRD negativity in this study, if the MRD status changes from negative to positive during close follow-up, re-initiation of immunotherapy or direct surgical resection can be considered based on the patient's preference.

Please discuss these and other possible options with your doctor.

8. Participation Fees Related to the Study:

For this study, the administration of the Tirelizumab injection and MRD dynamic monitoring will be provided free of charge. However, all other medication, examination, and treatment costs will be borne by you.

Clarification: The tumor tissue and blood samples of enrolled participants will be sent to Tianjin Huada Medical Laboratory, which is equipped with a medical institution license and detection qualifications, for free monitoring.

9. Compensation：

This study does not involve any subsidies, thus you will be responsible for covering your own transportation and accommodation expenses during treatment and follow-up visits.

10. Indemnity：

Regarding compensation for participation in this study, insurance has been purchased for subjects. During the insurance period, if you suffer from adverse events or serious adverse events due to the study treatment during drug clinical trial activities, resulting in personal injury or death (including disability and death) to the trial subjects using the trial drug or trial-related products, you can obtain compensation through claims. The insurance company will be responsible for compensation within the agreed compensation limit according to the provisions of the insurance contract.

11. Right to Refuse or Withdraw from the Study：

You have the option to choose not to participate in this study, or you have the right to withdraw from the trial at any stage without giving any reason. Your medical treatment and rights will not be affected by this decision. However, please note that

any data processing done on your data before withdrawal is legal. If the data has already been integrated into the research project and cannot be removed due to cost considerations, it may still be used in this study while protecting your privacy.

If you wish for your data or biological samples to no longer be used after you withdraw from the study, you must inform the research doctor. In such cases, your remaining biological samples will be destroyed as soon as possible. Once you decide to participate in this study, please sign this informed consent form to indicate your agreement. Before entering the study, a physician will screen you to confirm your eligibility.

12. Privacy and Confidentiality Issues:

During the study, your personally identifiable information such as your name and gender will be replaced with code names or numbers and will be kept strictly confidential. Only relevant doctors will have access to your personal information, and your privacy rights will be well protected. The research results may be published in journals, but no personally identifiable information about you will be disclosed. The study involves whole-exome sequencing, which can screen for tumor genetics in your family. Therefore, the information regarding familial tumor genetics will also be kept strictly confidential.

If you agree to participate in this study, all your medical records may be accessed by the personnel of the research and development unit that initiated this study, relevant authorities, or an independent ethics committee to check the propriety of the research conduct. By signing this informed consent form, you are also agreeing to such access by these individuals.

13. How to Obtain Assistance During the Study:

You are entitled to access information and updates regarding this study at any time. If you have any questions related to this research, please contact Dr. Zhang Xuan at 17387911546. He will answer all your questions about the trial and can be reached in case of emergencies.

If you need to understand your rights and benefits as a participant in this study,

you can contact the Ethics Committee of Yunnan Cancer Hospital at 0871-68179625.

Informed consent signature page

If you fully understand the content of this research project and agree to participate in this study, you will sign this informed consent form in duplicate, each retained by the investigator and the subject or the client.

Signed by the subject himself or his legal representative:

Consent statement:

1. I confirm that I have read and understood the informed consent for this study, that possible problems and solutions during the study have been explained to me, and that I have the opportunity to raise my own questions.
2. I have made it clear that participation in the study is voluntary and refusal to participate in the study will not harm my due interests.
3. I have learned that the physicians involved in the study, the person in charge of this work and the medical ethics Committee have the right to review the study records and case data. I agree that the above personnel can directly obtain my study records and understand that the above information will be kept confidential.
4. I agree to take part in this study

   Subjects signed:_____ date:_____

   Subject contact details:_____

   (Note: If the subject is incompetent / limited capacity, guardian signature and signature date)

   Signature of guardian:_____ date:_____

   Contact information of the guardian:_____

   Guardian and subject relationship:_____

(Note: If the subject cannot read the consent form, an independent witness is required to prove that the investigator has informed the subject of all contents of the consent form and the independent witness needs signature and signature date)

Signature of the Independent Witness:_____date:_____

Contact Information of the Independent Witness:_____

Signature of the investigator:_____date:_____

Investigator Contact Information:_____