# 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.

study protocol

Protocol version number: V1.0
Version date: July 02, 2023

Study director: Associate senior doctor Zhang xuan,
Chief physician Li Yunfeng
Research unit: Yunnan Provincial Cancer Hospital

# Scheme signature page

I, as the physician / statistical analyst involved in the study, have read the protocol for this study.

I have fully discussed the purpose of the study and the content of the protocol.

I agree to conduct the study under this protocol, to comply with the ethics requirements, and to conduct this clinical study under the guidance of Good Clinical Practice (GCP).

I agree that the contents of this protocol are confidential and will not be disclosed to third parties, and the contents of the protocol are only treatment for this study.

I understand that if the decision to prematurely terminate or suspend it is made at any time and for any reason, I will be informed in writing. Similarly, if I decide to withdraw from the study, I will immediately notify the study leader and the principal investigator.

Name：_____

Signature：_____

Date：_____

# Protocol summary

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| research topic | 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 |
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| by stages | Il designated time |
| Bid unit | Yunnan Provincial Cancer Hospital |
| Principal investigator | Associate senior doctor Zhang xuan, Chief physician Li Yunfeng |
| Participating in research units | Single center |
| Subject investigated | dMMR/MSI-H Locally advanced colorectal cancer |
| research design | This study is a single arm, single center, phase II, prospective clinical study aimed at exploring the effectiveness and safety of watch and wait strategy guided by dynamic MRD monitoring to achieve clinical complete response after neoadjuvant immunotherapy for locally advanced colorectal cancer with dMMR/MSI-H. |
| Study duration | The study is scheduled to begin in July 2023 |
| Study objective / endpoint | <u>Main end point</u><br>MRD-negative clinical complete response (cCR).<br><u>Secondary end point</u><br>(1) Optimal number of neoadjuvant immunotherapy cycles.<br>(2) Consistency ratio between MRD-negative cCR rate and cCR rate assessed by traditional methods.<br>(3) 3-year local recurrence/relapse rate.<br>(4) 3-year disease-free survival (DFS).<br>(5) 3-year overall survival (OS). |

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| | (6) Objective response rate (ORR).<br>(7) Tumor downstaging rate (mrTRG).<br>(8) Incidence of immune-related adverse events.<br>Exploratory endpoints<br>(1) Clearance rate of ctDNA in blood MRD-positive patients 2 weeks after completion of neoadjuvant immunotherapy.<br>(2) High-risk period for MRD conversion or relapse in blood MRD-negative patients 2 weeks after completion of neoadjuvant immunotherapy.<br>(3) Dynamic changes and change rates of MRD and their correlation with treatment efficacy.<br>(4) Detection sensitivity, specificity, positive predictive value, and negative predictive value of individualized MRD detection protocols (tumor-informed).<br>(5) Other multi-omics exploratory analysis results. |
| study population | Inclusion criteria:<br>**1) Disease Characteristics**<br>• Histologically confirmed colorectal adenocarcinoma.<br>• Immunohistochemically confirmed as dMMR or/and pCR or/and NGS as MSI-H.<br>• 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).<br>*Clinical staging method: Colon staging by CT, rectal staging by pelvic MRI combined with endorectal ultrasonography.<br>• No signs of intestinal obstruction; or obstruction relieved after proximal colostomy.<br>• No distant metastasis confirmed by comprehensive examination (distant organ or/and distant lymph node metastasis).<br>**2) Patient Characteristics**<br>• Age ⩾ 18 years and ⩽ 75 years at the time of signing the informed consent form. |

- 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.
- Hematology: WBC > 4000/mm³; PLT > 100,000/mm³; Hb > 10g/dL.
- Liver function: SGOT and SGPT less than 1.5 times the normal value; bilirubin less than 1.5 mg/dL.
- Renal function: creatinine < 1.8 mg/dL.
- No other malignant diseases (except non-melanoma skin cancer or cervical carcinoma in situ) within 5 years or concurrently.
- No psychiatric disorders that would prevent informed consent.
- No other severe diseases that would shorten survival.
- Female patients of childbearing age should adopt appropriate contraceptive measures from screening to 3 months after cessation of study treatment and should not breastfeed. Pregnancy test should be negative before treatment starts, or one of the following criteria should be met to prove no risk of pregnancy:
- Postmenopausal defined as age > 50 years and amenorrhea for at least 12 months after stopping all exogenous hormone replacement therapy.
- Women < 50 years who have stopped all exogenous hormone therapy for 12 months or more and have luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels within the postmenopausal reference range in the laboratory can also be considered postmenopausal.
- Those who have undergone irreversible sterilizing surgery, including hysterectomy, bilateral ovariectomy, or bilateral salpingectomy, except for bilateral tubal ligation.
- Male subjects should use barrier contraception (i.e., condom) from screening to 3 months after cessation of study treatment.

- Patients and their families can understand the research protocol and are willing to participate in this study, signing a written informed consent form.
- Patients have good compliance and are willing to receive follow-up, treatment, laboratory tests, and other research steps as planned.

**3) Prior Treatments**

- No prior colorectal cancer surgery.
- No prior chemotherapy, immunotherapy, or radiotherapy.
- No prior biological therapy.
- Prior endocrine therapy: no restrictions.

**4) Exclusion Criteria:**

- Tumor biopsy specimens showing pMMR or microsatellite stability (MSS) by immunohistochemistry or microsatellite instability testing.
- Patients with histological types of colorectal cancer other than adenocarcinoma (such as neuroendocrine carcinoma, sarcoma, lymphoma, squamous cell carcinoma, etc.).
- History of HIV infection or active chronic hepatitis B or C (high viral DNA copy).
- Autoimmune diseases.
- Other active clinically significant infections (> NCI-CTC 3.0 version).
- Patients with clinical stage I disease.
- Preoperative evidence of distant metastasis, including isolated, distant, or non-contiguous intra-abdominal metastases.
- Open surgical procedures $\leqslant$ 14 days before enrollment, excluding colon surgery.
- 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.

- Unable to provide blood samples for MRD testing at treatment and follow-up monitoring points.
- Cachexia, organ dysfunction, or decompensation.
- History of pelvic or abdominal radiation therapy.
- Patients requiring treatment for seizures (e.g., steroid or anti-epileptic therapy).
- Chronic inflammatory bowel disease, intestinal obstruction.
- Other severe diseases that, in the opinion of the investigator, may affect follow-up and short-term survival.
- Patients with a history of blood transfusion within 2 weeks before treatment.
- Unable to undergo clinical follow-up using contrast-enhanced magnetic resonance imaging (MRI) or contrast-enhanced computed tomography (CT).
- 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.
- 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.
- 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;

- • Active bleeding or newly diagnosed thrombotic disease; currently taking anticoagulant medication at therapeutic doses or with a tendency to bleed;
- 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 ⩾ 2; tendency to bleed;
- Myocardial infarction, coronary/peripheral artery bypass, or cerebrovascular accident within 3 months; tendency to bleed;
- QT interval (QTc) ⩾ 450 ms for males and ⩾ 470 ms for females on a 12-lead ECG; tendency to bleed;
- 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;
- Use of any medication known to prolong the QT interval within 2 weeks before enrollment; tendency to bleed;
- 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);

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| | e. Aspartate aminotransferase > 3 × ULN;<br>f. Total bilirubin > 1.5 × ULN;<br>g. Creatinine > 1.5 × ULN or creatinine clearance<br>h. Serum albumin (ALB) < 28 g/L;<br>• Female subjects who are pregnant, lactating, or plan to become pregnant during the study period;<br>• 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;<br>• Subjects with known or suspected hypersensitivity to the study drugs or any drugs related to this trial;<br>• Subjects with any unstable condition or situation that may jeopardize their safety and compliance;<br>• Other situations where the investigator believes the subject should not participate in this study. |
| The planned sample size | Utilizing the non-inferiority test, we set the non-inferiority margin with reference to the DYNAMIC II study as -8.5%. Based on a power of 0.8, a one-sided alpha of 0.05, a post-conventional treatment PD-1 efficacy rate of 30%, and an expected post-treatment MRD-negative rate of no less than 50%, initial calculations using the Non-Inferiority Tests for the Difference Between 1 Proportion and 1-Sample function in the PASS (Power Analysis and Sample Size) software indicate that a sample size of 20 cases is required for enrollment.Furthermore, considering factors such as the dropout rate during dynamic monitoring, the follow-up capabilities of the hospital's execution team, patient compliance, and the length of follow-up, the overall dropout rate for the 3-year follow-up is expected to be no more than 10%. Therefore, the estimated minimum number of subjects required for enrollment is 22 cases. |
| Treatment and monitoring programs | **I. Neoadjuvant Immunotherapy Phase:**<br>**1.Immunotherapy Drug:** Tislelizumab: Administered at 200mg intravenously, every three weeks (Q3W), for a minimum of four |

cycles. The specific number of neoadjuvant immunotherapy cycles will be determined based on the results of MRD dynamic monitoring.

**2.MRD Dynamic Monitoring Time Points:** Dynamic monitoring will be performed at the time of initial diagnosis and after the fourth cycle of neoadjuvant immunotherapy. Blood sampling for monitoring will occur 1-2 weeks after immunotherapy. If consecutive MRD monitoring results are negative for two consecutive times, a wait-and-observe strategy will be adopted. If MRD remains positive after eight cycles of neoadjuvant immunotherapy, surgical intervention will be considered.

(Specific Example: If MRD turns negative after the fourth cycle of neoadjuvant immunotherapy, further monitoring of MRD will be performed after the fifth cycle. If MRD remains negative, a wait-and-observe strategy will be adopted. If MRD is still positive after the fourth cycle, immunotherapy will continue for another cycle, and MRD monitoring will be performed after the fifth cycle. This process will continue accordingly. If MRD remains positive after eight cycles, surgical resection will be undertaken.)

**II. "Watch and Wait" Strategy:**

After adopting the MRD dynamic monitoring for the "watch and wait" strategy, the time points for recurrence or metastasis monitoring are: 1 month, 4 months, 7 months, 13 months, 19 months, 25 months, and 37 months after the initiation of the "watch and wait" period. The follow-up and review strategies, along with current routine clinical examination items, include: digital rectal examination and tumor markers every 1 to 3 months for 3 years, contrast-enhanced CT of the chest, abdomen, and pelvis every 3 months, pelvic MRI, transrectal ultrasound, colonoscopy with biopsy, and PET-CT if necessary.

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| | **III. Surgery:**<br><br>After adopting MRD dynamic monitoring following surgery, the time points for recurrence or metastasis monitoring are: 3 to 4 weeks, 4 months, 7 months, 13 months, 19 months, 25 months, and 37 months after radical surgery. The follow-up and review strategies, along with current routine clinical examination items, include: tumor markers, contrast-enhanced CT of the chest, abdomen, and pelvis every 3 months for 3 years, colonoscopy every 12 months, and MR and PET-CT if necessary. |
| Safety Evaluation Criteria | All subjects will be observed for any adverse events during the clinical study, including abnormal clinical symptoms and vital signs, as well as abnormalities in laboratory tests. The clinical manifestations, severity, onset time, duration, treatment methods, and prognosis of these adverse events will be recorded. Furthermore, the correlation between these adverse events and the investigational drug will be determined. The safety of the drug will be evaluated using the NCI-CTC AE 5.0 version criteria. |
| Evaluation Criteria for Effectiveness | During the entire study period, tumor evaluation will be conducted primarily through dynamic monitoring of minimal residual disease (MRD) to assess the proportion of patients achieving clinical complete remission (cCR). The analysis will be performed using the Response Evaluation Criteria in Solid Tumors (RECIST) standard (version 1.1). Radiological imaging scans (CT, MRI) will be performed to measure and calculate the cCR, objective response rate (ORR), and major pathological tumor regression grade (mrTRG) for all patients. |
| Statistical Methods | Statistical analysis will be performed using SPSS23.0 or SAS software. The PPS and FAS data will be analyzed for efficacy indicators, while the SS data will be analyzed for safety indicators. Most of the data in this study are descriptive. For continuous data, they will be expressed as mean and standard deviation; categorical |

indicators will be expressed as the number of cases and percentage for each category.

(1) Baseline Demographic and Disease Characteristics:

Descriptive statistical methods will be used to summarize the characteristics of the observed patients, including age, gender, clinical stage, ECOG score, etc.

(2) Primary Endpoint Analysis Method:

During neoadjuvant immunotherapy, patients with two consecutive negative MRD assessments will be evaluated as having clinical complete remission (cCR).

Secondary Endpoint Analysis Methods:

A. Imaging results will be evaluated using the RECIST 1.1 standard to calculate the objective response rate (ORR) and major pathological tumor regression grade (mrTRG). After MRD becomes negative, two radiologists will jointly assess whether the patient has achieved clinically complete remission (cCR) in the traditional sense.

B. Quality of life (QoL) will be assessed using the Chinese version of the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) (V3.0).

C. Safety indicators: Based on the SS set, adverse events and serious adverse event incidence rates in this trial will be described using the NCI-CTC AE v5.0 standard, along with their clinical manifestations, severity, onset time, duration, treatment methods, and prognosis.

D. Tumor downstaging rate, sphincter-preserving rate, surgical complication rate, adverse event incidence rate, and serious adverse event incidence rate will all be expressed as percentages.

F. Survival analysis will be performed using the Kaplan-Meier method to estimate the 3-year overall survival (OS) and disease-free survival（DFS）；

# Flow chart of the clinical trial

| Study Step / Visit 1 | Screening period | Treatment period 3 | | | | | | | W&W/Surgery | | | | Safety follow-up 4 | Survival follow-up 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Neoadjuvant immunotherapy | | | | | | | | | | | | |
| Visit time | -28~0 | C2 D1 | C3 D1 | C4 D1 | C5 D1 | C6 D1 | C7 D1 | C8 D1 | One month after W&W/Surgery | Once every 1-3 months after W&W/Surgery | Once every 3 months after W&W/Surgery | Once every 3 months, once every 6 months after half a year, once every year after 2 years | 28 days after the last dose | once every 3 mouths |
| Window time | 0 | ±3 Days | ±3 Days | ±3 Days | ±3 Days | ±3 Days | ±3 Days | ±3 Days | ±7 Days | ±7 Days | ±7 Days | ±7 Days | ±7 Days | ±7 Days |
| Signed the informed consent form 2 | ■ | | | | | | | | | | | | | |
| Demographic data 6 | ■ | | | | | | | | | | | | | |
| History of past illness 7 | ■ | | | | | | | | | | | | | |
| Tumor diagnosis | ■ | | | | | | | | | | | | | |
| Tissues WES | ■ | | | | | | | | | | | | | |
| Height, weight | ■ | | ■ | | ■ | | ■ | | | | | | ■ | |
| Vital sign | ■ | | ■ | | ■ | | ■ | | | | | | ■ | |
| Physical examination 8 | ■ | | ■ | | ■ | | ■ | | | ■ | | | ■ | |
| ECOG PS Score | ■ | | ■ | | ■ | | ■ | | | | | | ■ | |
| Enteroscopy+ biopsy | ■ | | ■ | | ■ | | ■ | | | | ■ | | | |
| A 12-lead ECG | ■ | | ■ | | ■ | | ■ | | | | | | ■ | |
| MRD dynamic monitoring 9 | ■ | | ■ | | ■ | | ■ | | ■ | | | ■ | | |
| CEA | ■ | | ■ | | ■ | | ■ | | | ■ | | | ■ | |
| Routine blood test | ■ | | ■ | | ■ | | ■ | | | | | | ■ | |
| Blood biochemical | ■ | | ■ | | ■ | | ■ | | | | | | ■ | |
| Coagulation function | ■ | | | | | | | | | | | | ■ | |
| Fecal hidden blood | ■ | | | | | | | | | | | | ■ | |
| Routine urine test | ■ | | | | | | | | | | | | ■ | |
| Thyroid function | ■ | | ■ | | ■ | | ■ | | | | | | ■ | |
| HIV、HBV、HCV | ■ | | | | | | | | | | | | ■ | |
| Myocardial enzymes, | ■ | | ■ | | ■ | | ■ | | | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **myocardial markers** | | | | | | | | | | | | | | |
| **Peripheral blood assay 10** | ■ | | ■ | | ■ | | ■ | | | | | | | |
| **Genetic testing 11** | ■ | | | | | | | | | | | | | |
| **Thoracal/ abdominal/ pelvic enhanced CT** | ■ | | ■ | | ■ | | ■ | | | | ■ | | ■ | |
| **Pelvic cavity MRI 12** | ■ | | | | | | | | | | ■ | | | |
| **Endorectal ultrasonography 13** | ■ | | | | | | | | | | ■ | | | |
| **PET-CT** | **Conducted according to clinical needs** | | | | | | | | | | | | | |
| **Neoadjuvant immunotherapy** | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | | | | |
| **Adverse events 14** | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| **Simultaneous medication 15** | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| **Quality of life assessment 16** | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| **Survival condition** | | | | | | | | | | | | | | ■ |

# Flow-chart description of the clinical trial

1. One period in this study was defined as 21 days. The inspection items should be completed within the time window listed in the test process. In case of statutory holidays, the reason for the over window can be recorded in advance and recorded in the CRF. In addition to the follow-up in the flow chart, the investigator may increase the examination items or increase the visit frequency based on the clinical condition oft h e subject.
2. The informed consent form for the screening period should be signed within 28 days before treatment. Except for the existing tumor imaging examinations and tumor tissue biopsies within the specified time limit before the first medication, written informed consent must be obtained prior to performing any clinical research procedures. This study allows subjects who have failed previous screenings to undergo re-screening, and a new informed consent form must be signed and a new subject number must be registered for re-screening.
3. Treatment period: The specific medication is tirelizumab: 200mg, intravenous drip, Q3W, for at least 4 cycles. The specific number of neoadjuvant immunotherapy cycles will be determined based on the MRD dynamic monitoring results.
4. Safety follow-up will be performed 28±7 days after the last medication or before starting a new anti-tumor treatment, whichever occurs first. All AEs occurring before the safety follow-up visit should be recorded until they resolve to grade 0-1 or baseline levels, or until the investigator determines that further follow-up is not necessary for reasonable reasons (such as unrecoverable or improved condition), whichever occurs first. SAEs occurring within 90 days after the last medication or before the subject starts a new anti-cancer treatment (whichever occurs first) should be followed up and recorded. If the patient is not due to disease progression, imaging examinations should be performed during this follow-up. If there are imaging data within 4 weeks before this follow-up, the investigator may assess whether a re-examination is needed.

Survival follow-up begins after the end of the safety follow-up period, with telephone follow-up every 3 months to record the subject's survival status until the last subject's follow-up is completed or the trial ends.

5. Demographic data includes: age, gender, etc.
6. Past medical history includes: tumor location, disease stage, histological stage, history of perforation or obstruction.
7. Physical examination: During the treatment period, rectal examination by digital palpation is mandatory for rectal cancer patients. Other examination items may be specified based on clinical symptoms or clinical needs.
8. MRD monitoring: Dynamic monitoring will be performed at the time of initial diagnosis and after the 4th cycle of neoadjuvant immunotherapy. Blood sampling for MRD monitoring should be performed 1-2 weeks after immunotherapy. If two consecutive MRD monitoring results are negative, a wait-and-observe strategy will be adopted. If MRD remains positive after 8 cycles of neoadjuvant immunotherapy, surgical treatment will be considered.

9. Tumor peripheral blood: This includes CD3+, CD4+, and CD8+ T cell expression levels.
10. Genetic testing: This includes routine testing for colorectal cancer, such as MSI status (or immunohistochemical assessment of MMR status), POLE/POLD1 mutation status, PTEN mutation status, TMB status, RAS and BRAF mutation status, etc.
11. Pelvic MRI: This will only be performed for rectal cancer patients.
12. Rectal endosonography: This will only be performed for rectal cancer patients.
13. Adverse event collection begins at the start of treatment. Once a subject terminates the trial treatment, only new or unresolved adverse events related to the trial treatment should be recorded.
14. Concomitant medication records should include any medication taken within 28 days of the screening period and during the trial period. Once a subject interrupts the trial treatment, only concomitant medications and treatments used for new or unresolved adverse events related to the trial treatment should be recorded. Concomitant medications for other diseases do not need to be recorded.
15. Quality of life assessment will be performed using the Wexner scoring scale, EORTC QLQ-C30 scale, and EORTC-QLQ-CR29 scale.

# 1. research background

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive tract. According to the global cancer statistics in 2018, CRC ranks third in incidence (10.2%) and second in mortality (9.2%) [1]. Locally advanced colorectal cancer (LACRC) is defined as CRC in stage II (cT3-4, N0) or stage III (cT1-4, N+). If these patients undergo direct surgical resection, it will lead to a high local recurrence rate and a low overall survival rate. Therefore, for locally advanced rectal cancer (LARC), the globally recognized standard treatment is the "neoadjuvant chemoradiotherapy (NCRT) + total mesorectal excision (TME) + postoperative adjuvant chemotherapy" recommended by the National Comprehensive Cancer Network (NCCN) guidelines. For locally advanced colon cancer (LACC), the NCCN guidelines also recommend considering neoadjuvant chemotherapy with FOLFOX or CAPEOX for patients with bulky lymph nodes or clinical T4 colon cancer [2].

In clinical practice, among LARC patients who undergo radical surgery after NCRT, 15% to 20% of specimens show no residual tumor cells, achieving pathological complete response (ypCR). The 5-year overall survival rate for ypCR patients is approximately 90%, indicating excellent prognosis [3]. Based on this, some scholars have proposed the idea of non-surgical treatment for patients predicted to achieve pCR after neoadjuvant therapy. Subsequent studies, including our own, have confirmed that patients with LARC who achieve clinical complete response (cCR) after NCRT and undergo a watch-and-wait (W&W) approach have no statistical difference in overall survival compared to those who undergo surgery and achieve pCR [4]. Additionally, patients on the W&W protocol avoid unnecessary surgical trauma and risks, significantly improving their quality of life. For patients who achieve near-clinical complete response (near-cCR), over half have the opportunity to adopt W&W for organ preservation. The recurrence-free survival (RFS) and metastasis-free survival (MFS) in the near-cCR group are not significantly different from those in the cCR group [5]. In summary, NCRT has been proven to significantly improve tumor downstaging rates, radical resection rates, and even allow some patients to avoid surgery altogether. However, the incidence of perioperative complications is relatively high in LARC patients undergoing radical surgery after NCRT, including anastomotic leakage, impaired bowel function, sexual function, and reproductive function caused by radiotherapy and chemotherapy. Patients undergoing low anterior resection also

face the possibility of temporary stoma failure and long-term anterior resection syndrome (ARS). Similarly, LACC patients undergoing neoadjuvant chemotherapy (NCT) may experience adverse reactions such as nausea, vomiting, and diarrhea during treatment, and some may even discontinue treatment due to intolerance to these side effects.

Certainly, are there alternative neoadjuvant treatment modalities, beyond neoadjuvant (chemo)radiotherapy, that are compatible with the Watch & Wait strategy while circumventing the associated acute and chronic toxic side effects, as well as the decline in quality of life? Amidst the advent of precision oncology and the burgeoning field of genetic testing, researchers have uncovered a distinct molecular subset of malignancies characterized by deficient mismatch repair (dMMR) or high microsatellite instability (MSI-H). This subgroup comprises approximately 15% of all colorectal cancer patients, exhibiting robust immunogenicity, profound lymphocytic infiltration within the tumor microenvironment, and favorable prognosis. However, these patients often display insensitivity to traditional neoadjuvant (chemo)radiotherapy [6, 7]. Mismatch repair (MMR) is a pivotal DNA repair mechanism, encompassing four essential proteins: MLH1, MSH2, MSH6, and PMS2. This intricate system precisely identifies and rectifies base mismatches, as well as small-scale base deletions or insertions that arise during DNA replication or recombination, thereby safeguarding genomic stability. dMMR represents mutations in MMR-related genes, leading to dysfunctional repair capabilities and diminished or absent mismatch repair activity. MSI-H, on the other hand, refers to alterations in the length of microsatellite sequences, often triggered by insertions or deletions during DNA replication, frequently stemming from MMR defects associated with dMMR [8].

Tumor cells in MSI-H/dMMR colorectal cancer patients exhibit significantly upregulated expression of programmed death-ligand 1 (PD-L1) on their surface. This PD-L1 can bind to the programmed death receptor 1 (PD-1) on effector T cells, inhibiting their immune killing effect against tumor cells. However, immune checkpoint inhibitors can block the binding of PD-L1 to PD-1 receptors, thereby enhancing the body's immune system's ability to kill tumor cells [9]. In 2021, the NCCN guidelines formally recommended pembrolizumab as a 1A-level evidence-based treatment for first-line therapy in patients with dMMR/MSI-H metastatic colorectal cancer (mCRC) [10]. However, dMMR/MSI-H mCRC accounts for less than 5% of all CRC patients, and most

dMMR/MSI-H CRC cases are still locally advanced. Therefore, neoadjuvant immunotherapy (NIT) for dMMR/MSI-H locally advanced colorectal cancer (LACRC) has gradually become a current research hotspot.

Numerous studies on neoadjuvant immunotherapy conducted by our research team have been reported as follows: In 2018, the NICHE study [11], as the world's first neoadjuvant immunotherapy trial for non-metastatic colon cancer, has garnered significant attention since its inception. The study included two patient populations: those with dMMR colon cancer and those with proficient mismatch repair (pMMR) colon cancer. Among the 32 patients with dMMR colon cancer, 100% achieved pathological remission after neoadjuvant immunotherapy, with 69% achieving pathological complete response (pCR). However, due to the small sample size of only 32 dMMR patients in the NICHE study, larger-scale studies are needed to further validate the findings. Against this backdrop, researchers embarked on the NICHE-2 study [12], targeting a larger patient population.In 2022, the NICHE-2 study enrolled 112 patients with non-metastatic dMMR colon cancer. They received one dose of ipilimumab (1mg/kg) and two doses of nivolumab (3mg/kg) within 6 weeks before surgery. The primary endpoint was safety and 3-year disease-free survival (DFS), while secondary endpoints included major pathological response (MPR) and pCR. The results showed that in terms of safety, the incidence of adverse events (AEs) of any grade was 61%, with 4 patients (4%) experiencing grade 3-4 AEs. Only 2 patients had surgery delayed for more than 2 weeks due to immune-related AEs. All patients underwent R0 surgical resection, and pathological remission was observed in 99% of patients, including 95% MPR and 67% pCR rates. After a median follow-up of 13.1 months, no patient experienced disease recurrence. The NICHE-2 study further demonstrated that neoadjuvant immunotherapy for non-metastatic dMMR colon cancer achieves significant efficacy with good tolerability. Based on the NICHE/NICHE-2 studies, our research team believes that neoadjuvant immunotherapy regimens contribute to the preservation of organ function in patients with dMMR/MSI-H colon cancer. In 2021, the PICC study conducted by Professor Deng Yanhong's team at the Sixth Affiliated Hospital of Sun Yat-sen University [13] also confirmed the favorable efficacy of neoadjuvant immunotherapy. The study enrolled 34 patients with dMMR/MSI-H CRC and randomly divided them into two groups: the Toripalimab monotherapy group and the Toripalimab combined with

Celecoxib (COX-2 inhibitor) group. The results showed that the pCR rate in the 17 patients receiving PD-1 monotherapy was 65%, while the pCR rate in the 17 patients receiving Toripalimab combined with Celecoxib was as high as 88%.In 2023, a nationwide multicenter retrospective study conducted by Professor Ding Peirong from the Sun Yat-sen University Cancer Center, along with Professor Li Yunfeng from our hospital, analyzed the efficacy of PD-1 inhibitor-based neoadjuvant therapy in terms of tumor remission, surgical resection rate, long-term survival, and recurrence rate [14]. A total of 73 patients were enrolled in this study, most of whom had LACRC. 79.5% of the patients received PD-1 inhibitor monotherapy, and ultimately, 62 patients achieved objective remission in imaging assessment, with 17 achieving complete response (CR) and opting for the watch-and-wait (W&W) approach instead of surgery. 45 patients achieved partial remission, with a median response time of 9.6 weeks. There was no difference in remission rates between cT4a/4b patients and cT2-3 patients (84.0% vs. 85.4%; P=0.999). With a median follow-up time of 17.2 months, among patients who underwent surgery or achieved CR, the 2-year tumor-specific disease-free survival (DFS) rate and overall survival (OS) rate were both 100%. This is the largest study to date in the field of CRC NIT treatment worldwide and the first report on the long-term efficacy of NIT in this area.Moreover, in 2022, our research team also published a retrospective analysis exploring the efficacy and safety of neoadjuvant immunotherapy monotherapy for patients with dMMR/MSI-H LACRC [15]. A total of 32 patients were included, with a median NIT duration of 6 cycles. The objective response rate (ORR) was 100%. Ultimately, 3 patients achieved clinical complete response (cCR) and adopted the W&W strategy, while 29 patients underwent radical surgery. The pathological remission rate was 100%, with a major pathological response (MPR) of 86.2% and a pCR rate of 75.9%. In summary, it is evident that patients with dMMR/MSI-H CRC can achieve a high pCR rate with NIT, and preoperative immunotherapy alone can achieve efficient and safe results.

So, can these patients who achieve pCR avoid surgery through preoperative assessment as cCR? Based on this idea, more and more researchers are exploring the direction of organ function preservation for patients with dMMR/MSI-H CRC. In June 2022, MSKCC published a significant study in the New England Journal of Medicine [16]. This study enrolled 12 patients with stage II/III rectal cancer with dMMR/MSI-H, who were treated with 500mg Dostarlimab q3w for 6 months and followed up for at least

6 months. Ultimately, all 12 patients achieved cCR and adopted the W&W approach. No cases of progression or recurrence were reported during the follow-up period, and no adverse events of grade 3 or higher occurred.In April 2023, a study led by Professor Xu Ruihua, Professor Chen Gong, and their gastrointestinal oncology team from the Sun Yat-sen University Cancer Center [17] also found that dMMR or MSI-H LARC patients, without the need for "sandwich therapy" (neoadjuvant chemoradiotherapy → surgery → adjuvant chemotherapy), could achieve cCR after PD-1 antibody treatment alone, avoiding the damage caused by chemoradiotherapy and surgical treatment. This study enrolled 17 newly diagnosed patients with dMMR/MSI-H LARC from October 2019 to June 2022, who received 4 cycles of neoadjuvant treatment with Sintilimab and had the option of choosing the following subsequent treatment options based on their treatment response. Among the 16 patients evaluable for efficacy, 15 patients showed tumor shrinkage after treatment. Six patients underwent radical surgery, with 3 achieving pCR. Another 3 patients had residual tumor cells after surgery, but they achieved radical treatment after surgical resection. Nine patients achieved cCR and chose the W&W approach. Follow-up of this group until November 2022 showed no tumor recurrence. This study once again demonstrates that neoadjuvant PD-1 monoclonal antibody immunotherapy can give patients the opportunity to achieve cCR, thus avoiding surgery and preserving organ function, fundamentally changing the treatment approach for this disease. Similarly, in 2022, another domestic multi-center real-world retrospective study led by Professor Ding Peirong's team from the Cancer Center of Sun Yat-sen University and involving our hospital [18] included 19 patients with dMMR/MSI-H rectal cancer who achieved cCR after PD-1 inhibitor treatment. Among them, 16 patients received PD-1 inhibitor as first-line treatment, and 11 patients received PD-1 inhibitor monotherapy. The median time from the start of treatment to cCR was 3.8 months. After achieving cCR, the median follow-up duration was 17.1 months, during which no local or distant recurrence was observed. The 2-year local RFS rate, 2-year distant metastasis-free survival rate, 2-year DFS rate, and 2-year OS rate were all 100%. This is the largest sample size and longest follow-up study on the exemption of surgery and radiotherapy after immunotherapy for dMMR/MSI-H rectal cancer in the international arena to date. A recently accepted retrospective study by this research team also found that the W&W strategy may become a new treatment model after NIT for dMMR/MSI-H LARC

patients. We included a total of 20 patients with dMMR/MSI-H LARC who received NIT. Among them, 90% of patients achieved CR after a median of 7 cycles, including 11 patients with postoperative pCR and 7 patients who chose the W&W strategy after being assessed as cCR or near-cCR. The median follow-up duration for both groups was 25 months, with 2-year DFS and OS both at 100%. There was no statistical difference in the incidence of immune-related adverse events (irAEs) between the two groups. However, in the pCR group, 2 patients underwent permanent colostomy, and 2 patients experienced surgical-related adverse events (srAEs). The above studies suggest that the W&W strategy may be a safe and reliable option for patients with dMMR/MSI-H rectal cancer, and even colon cancer, after achieving cCR through neoadjuvant immunotherapy.

However, in previous clinical practice, we have observed that although most patients exhibited significant radiographic regression after neoadjuvant immunotherapy (NIT), the rate of complete clinical response (cCR) was relatively low. Paradoxically, a high rate of pathological complete response (pCR) was confirmed in postoperative pathological analysis. This inconsistency in radiographic and pathological CR rates has made it difficult for clinicians to accurately assess the true efficacy of preoperative immune neoadjuvant therapy, resulting in many patients with deficient mismatch repair (dMMR)/microsatellite instability-high (MSI-H) colorectal cancer (CRC) undergoing potentially avoidable surgeries and incurring associated risks and complications. Therefore, clinical medicine experts are further exploring whether there exist more sensitive and precise detection technologies that can overcome the limitations of radiographic assessment of NIT efficacy, thereby guiding and optimizing wait-and-watch (WW) strategies.

The emergence of minimal disease residual (MRD) has brought us a ray of hope. Studies have found that MRD is a significant factor for recurrence and metastasis after radical tumor treatment, yet it represents tumor remnants at the cellular level that are undetectable by traditional imaging or laboratory methods. The next-generation sequencing (NGS) technology based on circulating tumor DNA (ctDNA) in blood serves as a favorable means for detecting MRD. Initial research on MRD detection in colorectal cancer tends to guide the selection of adjuvant therapy for patients in stages II and III. Adjuvant chemotherapy can improve overall survival (OS) in patients with stage III colon cancer. However, 40% of patients may achieve cure even without adjuvant therapy, while

30% may experience recurrence despite adjuvant treatment [19]. Therefore, especially in stage II and III colon cancer, we have the opportunity to intensify treatment for patients who are likely to benefit, thereby increasing the cure rate, and reduce treatment for those who are unlikely to benefit, thus lowering treatment-related toxicity.

The DYNAMIC study [20] is a prospective study specifically targeting patients with stage II colon cancer. This study aimed to assess whether ctDNA-guided therapy could reduce the need for adjuvant treatment without increasing the risk of recurrence compared to standard treatment methods. Additionally, it further explored the prognosis of patients with positive ctDNA who received adjuvant chemotherapy and those with negative ctDNA who did not receive adjuvant therapy. From August 10, 2015, to August 2, 2019, a total of 455 patients with stage II colon cancer were enrolled. Blood samples were collected from all patients at weeks 4 and 7, and they were randomly assigned to a 2:1 ratio into a ctDNA-Guided group (ctDNA-positive - adjuvant chemotherapy; ctDNA-negative - observation) and a standard treatment group. Ultimately, 289 patients entered the ctDNA-Guided group, and 147 entered the standard treatment group. With a median follow-up time of 37 months, the results showed that the ctDNA-guided management group had a 46% lower acceptance rate of adjuvant chemotherapy compared to the standard treatment group, significantly sparing some patients from unnecessary chemotherapy. The 2-year recurrence-free survival (RFS) rates were 93.5% vs 92.4% for the ctDNA-guided management group and the standard management group, respectively, with an HR of 0.96, indicating no significant difference and achieving the predefined non-inferiority endpoint. Among the ctDNA-guided management group, the 3-year RFS rate was better for ctDNA-negative patients than for ctDNA-positive patients, suggesting that ctDNA-positive patients could benefit from adjuvant therapy. The approach of using ctDNA-guided therapy for stage II colon cancer reduced the use of adjuvant chemotherapy without affecting recurrence-free survival. This study's findings may potentially change the guidelines' recommendation level for ctDNA testing, and ctDNA testing may even become one of the clinical standard detection methods. A prospective study led by Professor Chen Gong's team from the Cancer Center of Sun Yat-sen University [21] utilized patient-specific tumor-informed ctDNA analysis to detect MRD in patients with stage I to IV colorectal cancer. The study enrolled 117 patients with surgically resected stage I to IV colorectal cancer. An individualized tumor-informed

technology called brPROPHET was used to detect MRD at baseline (n=117), 7 days after surgery (n=117), and 30 days after surgery (n=93). With a median follow-up time of 213 days, the overall positive rate of baseline ctDNA was 97%. Among patients with stage I, II, III, and IV colorectal cancer, the positive rates of ctDNA were 88%, 98%, 98%, and 100%, respectively. The median ctDNA level was higher in patients with advanced colorectal cancer and was significantly correlated with tumor volume. In two patients who relapsed, ctDNA detection identified the risk of recurrence one and two months earlier than imaging methods. In the subgroup analysis, two other MRD detection technologies - a fixed panel (covering 168 genes, 273kb) tumor-informed method (fixed panel with informed calling [FI method]) and a tumor-agnostic method (fixed panel with agnostic calling [FA method]) - were compared head-to-head with brPROPHET. Among the 74 patients included in the parallel comparison of the three MRD detection methods, the preoperative ctDNA positive rates were 97.3%, 75.7%, and 68.9% when detected by brPROPHET, FI method, and FA method, respectively. Only 15 patients could be detected with ctDNA by the brPROPHET method at baseline. The ctDNA levels in these patients were below the detectable level of the FI and FA fixed panel methods. A total of 135 postoperative blood samples were tested using the three methods, and the positive rates of brPROPHET, FI, and FA fixed panel methods were 14.8%, 8.1%, and 6.7%, respectively. This study demonstrated the clinical performance of patient-specific brPROPHET in detecting ctDNA in patients with colorectal cancer, showing higher sensitivity in detecting preoperative and postoperative ctDNA compared to fixed panel detection methods.

ctDNA detection, as a non-invasive method, boasts high accessibility, safety, and convenience, allowing for continuous dynamic monitoring. Moreover, its short half-life enables real-time reflection of tumor status. Currently, there are two major technical approaches for monitoring MRD using ctDNA in patients with early or locally advanced colorectal cancer: the tumor-informed approach based on tissue sequencing and the tumor-naive approach solely relying on plasma analysis [22]. The tumor-informed approach involves sequencing tumor tissue to obtain mutation information, selecting specific mutations for personalized customization, and then detecting ctDNA through blood sampling and high-depth sequencing to track the selected mutations in tumor tissue. In contrast, the tumor-naive technique solely relies on plasma ctDNA detection using a

fixed-pattern approach, where the detection range is the same for everyone. If a patient does not have these mutations, they cannot be detected, resulting in a higher false-negative rate and lower accuracy. The Signatera technology, based on the tumor-informed approach, can reliably detect tumor-specific mutations with a variant allele frequency (VAF) of 0.01%. By filtering out clonal hematopoiesis and germline-derived variations of unknown significance from the analysis, it significantly reduces the false-positive rate. In contrast, tumor-naive detection has lower sensitivity with a reliable detection limit of 0.1% to 1% VAF. The GALAXY study [23] reported a preoperative ctDNA positive rate of over 90% in stage II-III colorectal cancer, suggesting that tumor-informed individualized customization may have better application prospects in MRD monitoring.

The role of dynamic monitoring of ctDNA in advanced colorectal cancer and the post-surgical adjuvant phase has been increasingly recognized, but its role in the neoadjuvant therapy stage before surgery for colorectal cancer remains largely unexplored. Currently, there is only one study [24] on dynamic monitoring of ctDNA after radical treatment for anal squamous cell carcinoma (ASCC) that included 31 patients receiving definitive chemoradiation (dCRT). Among them, 65% had stage III tumors, with a median radiation dose of 54 Gy/27 fractions. 84% of patients received fluorouracil (5-FU or capecitabine) combined with mitomycin chemotherapy, while 16% received capecitabine monotherapy. The median follow-up time was 32 weeks. The final results showed that 27 patients successfully underwent baseline ctDNA testing, of which 23 patients had detectable baseline ctDNA, with a positive detection rate of 85%. Except for one patient, all patients with negative ctDNA achieved clinical complete response (cCR) in subsequent clinical assessments. Patients who did not achieve cCR were assessed as having residual tumor despite negative ctDNA, and the researchers are conducting follow-up monitoring to further evaluate this patient's condition. Survival analysis showed that the 1-year disease-free survival (DFS) rate for all patients was 96%, and the 1-year overall survival (OS) rate was 100%. Notably, the time to ctDNA remission was significantly shorter than the time to cCR.

Similarly, there is a lack of research on the use of MRD dynamic monitoring to guide the wait-and-watch (W&W) strategy after achieving complete response (CR) with neoadjuvant immunotherapy for dMMR/MSI-H locally advanced colorectal cancer (LACRC). Therefore, this study aims to customize individualized protocols based on the

tumor-informed approach, dynamically monitoring MRD status after neoadjuvant immunotherapy for dMMR/MSI-H stage II/III CRC patients using ctDNA technology. This will provide support for patients considering the W&W strategy or selecting surgical treatment.

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# 2. research contents

## 2.1 fundamental purpose

MRD-negative Clinical Complete Response Rate (cCR)

## 2.2Secondary purpose

1) Optimal Number of Neoadjuvant Immunotherapy Cycles
2) Inconsistency Rate between MRD-negative cCR Rate and cCR Rate Using Traditional Assessment Methods
3) 3-Year Local Recurrence/Regrowth Rate
4) 3-Year Disease-Free Survival (DFS)
5) 3-Year Overall Survival (OS)
6) Objective Response Rate (ORR)

7) Tumor Downstaging Rate (mrTRG)

8) Incidence Rate of Immune-Related Adverse Events.

## 2.3 Exploratory research purpose

1). Clearance rate of ctDNA in blood MRD-positive patients after 1-2 weeks of completion of neoadjuvant immunotherapy.;

2) High-risk period for MRD conversion or recurrence in blood MRD-negative patients after 1-2 weeks of completion of neoadjuvant immunotherapy；

3) Dynamic changes and rates of change in MRD and their correlation with treatment efficacy；

4) Detection sensitivity, specificity, positive predictive value, and negative predictive value of the tumor-informed, individually customized MRD detection scheme;

5) Results of other exploratory multi-omics analyses.

# 3. study protocol

## 2.3Treatment plan：

### 2.3.1 Neoadjuvant Immunotherapy Protocol

1. Immunotherapy Drug: Tirelizumab: 200mg, intravenous drip, Q3W (every 3 weeks), for at least 4 cycles. The specific number of neoadjuvant immunotherapy cycles will be determined based on the results of dynamic MRD (Minimal Residual Disease) monitoring.

2. Time Points for Dynamic MRD Monitoring: The first monitoring will be conducted at the initial diagnosis, and subsequent monitoring will be performed after the 4th cycle of neoadjuvant immunotherapy. Blood sampling for monitoring will be done 1-2 weeks after immunotherapy. If consecutive monitoring of MRD reveals negative results twice, a wait-and-watch strategy will be adopted. If MRD remains positive after 8 cycles of neoadjuvant immunotherapy, surgical intervention will be considered.

(Specific Example: If MRD turns negative after the 4th cycle of neoadjuvant immunotherapy, continued monitoring of MRD after the 5th cycle will be performed. If MRD remains negative, the wait-and-watch strategy will be maintained. If MRD is still positive after the 4th cycle, an additional cycle of immunotherapy will be administered, and MRD will be monitored after the 5th cycle. This process will continue accordingly. If MRD remains positive after 8 cycles, surgical resection will be performed.)

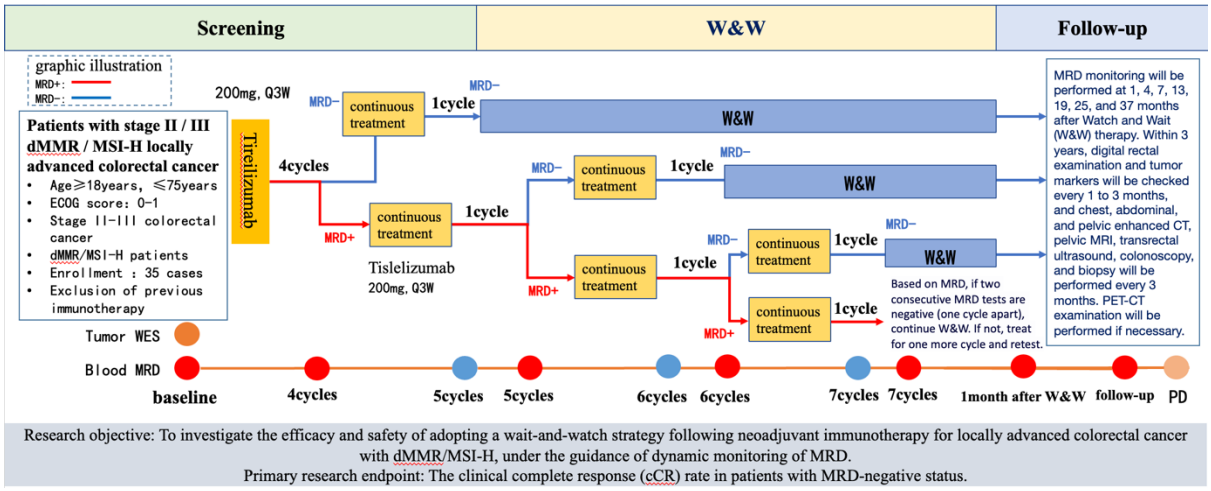
### 2.3.2 Wait-and-Watch" Strategy

After four cycles of neoadjuvant immunotherapy, if consecutive monitoring of MRD reveals negative results twice, a wait-and-watch strategy will be adopted. Following the wait-and-watch period, the time of recurrence or metastasis will be observed through dynamic MRD monitoring, with MRD testing conducted at 1 month, 4 months, 7 months, 13 months, 19 months, 25 months, and 37 months after the observation period. The follow-up and review strategy, along with current routine clinical examination items, include: digital rectal examination and tumor markers every 1 to 3 months for 3 years, chest, abdomen, and pelvic contrast-enhanced CT, pelvic MRI, transrectal ultrasound, colonoscopy with biopsy every 3 months, and PET-CT if necessary.

### 2.3.3 Surgical Resection

If MRD remains positive after eight cycles of neoadjuvant immunotherapy, surgical resection will be performed. After surgery, the time of recurrence or metastasis will be observed through dynamic MRD monitoring, with MRD testing conducted at 3-4 weeks, 4 months, 7 months, 13 months, 19 months, 25 months, and 37 months after the radical surgery. The follow-up and review strategy, along with current routine clinical examination items, include: tumor markers, chest, abdomen, and pelvic contrast-enhanced CT every 3 months for 3 years, colonoscopy every 12 months, and MR and PET-CT if necessary.

The research process is as follows：




MRD: Minimal Residual Disease; +: Positive; -: Negative; dMMR: Deficient Mismatch Repair; MSI-H: Microsatellite Instability-High; pMMR/MSS: Proficient Mismatch Repair/Microsatellite Stable; W&W: Wait-and-Watch Strategy; WES: Whole Exome

Sequencing; PD: Progression of Disease; MRD Status Assessment: Based on the gene mutation results of WES detection in tumor tissue from each patient, variant sites are screened to develop an individualized MRD detection panel for the patient.

## 2.4MRD Monitoring Technology and Sequencing Platform

### 2.4.1 MRD Detection Technique

Through Whole Exome Sequencing (WES) of tumor tissue and control blood samples, variations unrelated to tumor progression and of unknown significance are filtered out to obtain the gene mutation spectrum of tumor lesions for each subject. The Signatera proprietary algorithm is utilized to select 16 primary clonal somatic mutations, thereby customizing an individualized MRD monitoring panel. Based on this customized MRD monitoring panel, ultra-high-depth ctDNA-NGS sequencing is performed on the subject's blood samples to evaluate the status and content of ctDNA/MRD. By continuously monitoring changes in blood ctDNA, the disease progression of the subject can be indicated.

### 1.2.2 MRD Sequencing Platform

1. Whole Exome Sequencing Platform: MGISEQ-2000.
Sequencing Strategy: PE100;
Sequencing Depth: Tumor Sample > 500X, Control Blood > 200X.

2.ctDNA Sequencing Platform: MGISEQ-2000.

Sequencing Strategy: PE50;
Sequencing Depth: > 100,000X.
3. Tumor tissue specimens and blood samples from enrolled patients, i.e., the subjects undergoing testing, will be sent to Tianjin BGI Medical Testing Lab, which possesses a medical institution practice license and testing qualifications, for free monitoring.

## 2.5Treatment Evaluation

1.Effectiveness: Tumor evaluation will be conducted throughout the study period, primarily through dynamic MRD monitoring to assess the proportion of patients achieving clinical complete response (cCR). The Response Evaluation Criteria in Solid Tumors (RECIST) standard (version 1.1) will be adopted for analysis. Radiological imaging (CT, MRI) scans will be performed to measure and calculate cCR, objective response rate (ORR), and modified tumor regression grade (mrTRG) for all patients.

2.Safety: Adverse events will be observed and graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Tumor markers, blood routine tests, blood biochemistry, liver and kidney function, thyroid function, etc., will be checked every 2 cycles. During the treatment process, adverse reactions such as edema, gastrointestinal reactions, leukopenia, anemia, hand-foot syndrome, rash, muscle spasms, diarrhea, etc., will be closely monitored. The time, severity, management methods, and outcomes of these adverse reactions will be recorded.

## 2.6 Dose adjustment and adverse reaction treatment

Hematological toxicity dose adjustment

All dose changes should be documented and treated with the most adequate supportive therapy. If the symptoms resolve immediately after supportive care and continued study treatment is considered appropriate and the investigator believes the treatment is beneficial to the patient, consider continuing the same dose of study treatment plus appropriate continued supportive care. If the reduction is medically required, the reduction of chemotherapy is allowed. Adjust this cycle dose according to the lowest blood count after last dose (refer to the table below)：

| low | | The next cycle dose |
|---|---|---|
| Neutrophil number（×109/L） | Platelet count（×109/L） | |
| ≥0.5 | Platelet count≥50 | invariant |
| ＜0.5 | Or＜50 | The subsequent dose of chemotherapy will bereduced by 20% at the discretion of the investigator |

After two reductions, another 4 degrees of neutropenia or III degree grain deficiency with fever (> 38.5°C) or above 3 degrees occurred, without the third reduction, the investigator will discuss whetherto continue the trial according to the specific situation.

hepatotoxicity

- Bilirubin: Ifthe bilirubin levels are abnormal in the study, the next cycle should be delayed. If 4 weeks, the trial;；

- Liver enzymes: AST and / or ALT and / or alkaline phosphatase levels abnormal in the absence o fdisease progression, liver protection treatment, within 2 weeks, not returning to normal, dose can be adjusted according to the table below.

If the liver fuction recovers in the next cycle, the dose should be increased to the previous level.

| AST/ALT price | Alkaline phosphatase values | dose titration |
|---|---|---|
| ＜1.5×ULN | ＜5×ULN | No dose adjustment is required |
| ＞1.5×ULN 至＜2.5×ULN | ＜2.5×ULN | No dose adjustment is required |
| 2.5×ULN 至＜5×ULN | ＜2.5×ULN | Subsequent dose reduction of chemotherapy was 20-25% as determined by the investigator |
| ＞1.5×ULN 至＜5×ULN | ＞2.5×ULN＜5×ULN | |
| ＞5×ULN and /or＞5×ULN | | Delay by up to two weeks. if still not Recovery, at the discretion of the investigator. Ill Whether the person withdrew from the study. |

Immune-related adverse effects

Based on the mechanism of action, patients receiving its treatment may develop immune-related adverse reactions, including severe and fatal cases. Most immune-related adverse effects are reversible and can be managed by suspension or cessation of the treatment and administering corticosteroid therapy and / or supportive therapy. Depending on individual patient safety and tolerability, dose suspension or permanent discontinuation may be required. Increase or dose reduction is not recommended. See the table below for recommended Tirelizumab.

As recommended for Tirelizumab

| Immune-related adverse effects | order of severity ‡ | Treatment adjustment protocol |
|---|---|---|
| pneumonia | Level 2 | Suspension until adverse effects return to grade 0-1 |
| | Grade 3 or 4 or recurrent grade 2 | Permanent withdrawal of drugs |

| | | |
|---|---|---|
| Diarrhea and colitis | Level 2 or 3 | Suspension until adverse effects return to grade 0-1 |
| | Level 4 | Permanent withdrawal of drugs |
| hepatitis | Grade 2, aspartate aminotransferase (AST) or alanine aminotransferase (ALT)> 3 to 5 times the upper limit of normal (ULN); and / or total bilirubin (TBIL)> 1.5 to 3 times ULN | Suspension until adverse effects return to grade 0-1 and prednisone 10mg / day or equivalent dose |
| | Grade ,3 AST or ALT> 5x ULN, and / or TBIL> 3 x ULN | Permanent withdrawal of drugs |
| Nephritis and renal dysfunction | Grade 2, creatinine> 2 – 3 times ULN | Suspension until adverse effects return to grade 0-1 |
| | Grade 3, creatinine>3 times ULN or>4.0 mg/dL, with indication for hospitalization; life- threatening, indication for dialysis treatment | Permanent withdrawal of drugs |
| endocrine disease | ≥ Grade 2 hyperthyroidism | Suspension until symptom improvement or adverse response remission to grade 0-1 |
| | ≥ Grade 2 hypothyroidism | Continue the medication, by standard therapy, by hormone replacement therapy Control |
| | ≥ Grade 2 hyperglycaemia or type 1 diabetes mellitus | Suspension until adverse effects return to grade 0-1. Start insulin replacement therapy as clinically indicated, and use hypoglycemic drugs for hyperglycemia |
| | Grade 2 hypophysitis | Suspension until the subject is clinically stable |

| | | |
|---|---|---|
| | Grade 3 or 4 hypophysitis | Suspension until hormone replacement therapy To when the subject was clinically stable |
| | ≥ Grade 2 hypadrenia | Suspension until the subject is clinically stable |
| Skin adverse effects | Grade ,3 or suspected Stevens-Johnson syndrome (Stevens Johnson syndrome, SJS) or toxic epidermal necrolysis (Toxic Epidermal Necrolysis, TEN) | Suspension until adverse effects return to grade 0-1 |
| | Level 4, or to confirm the SJS or TEN | Permanent withdrawal of drugs |
| carditis | Level 1 | Suspension of dosing |
| | ≥ level 2 | Permanent withdrawal of drugs |
| thrombocytopenia | Level 3 | Suspension until adverse effects return to grade 0-1 |
| | Level 4 | Permanent withdrawal of drugs |
| Other immune-related adverse effects | Grade 1 encephalitis<br>Grade 2 or 3 pancreatitis<br>Grade 2 myasthenia gravis<br>Grade 3 or 4 with elevated blood amylase or elevated lipase<br>Other immune-related adverse reactions that first occurred in grade 2 or 3 include dermatitis, etc | The administration should be suspended until the adverse reaction returns to grade 0-1, and the encephalitis should lipase decide whether to continue the medication based on clinical judgment |

| | | |
|---|---|---|
| | ≥grade 2 encephalitis<br>Grade 4 pancreatitis<br>Grade 3 or 4 myasthenia gravis<br>Guillain Barre Svndrome（Guillain-Barrésyndrome，GBS）<br>Other adverse immune-related reactions first occurring in grade 4 include dermatitis | Permanent withdrawal of drugs |
| Recurrent or persistent adverse effects | Recurrent Grade 3 or Grade 4 (except for endocrine disorders)<br>Within 12 weeks after the last dose: Grade 2 or 3 adverse effects did not improve to Grade 0-1 (except endocrine disease) or the corticosteroid dose was not reduced to prednisone 10mg daily, or equivalent dose | Permanent withdrawal of drugs |
| Iniection site reaction | Level 2 | continue |
| | Level 3-4 | Permanent withdrawal of drugs |
| hypersensitivity | Level 2 | Stop the drug administration immediately. Depending on the observed intensity of response, antihistamines should be given in advance in the next cycle of treatment and the subcutaneous injection is slowed. |
| | Level 3-4 | Immediately continue immediately with subsequent permanent withdrawal |

Toxicity grading was determined using theNational Cancer Institute Common Terminology Assessment Criteria forAdverse Events version 4.03 (NCICTCAE v4.03).

›For advice on hormone replacement therapy, see the envafolimab insert [Notes].

## Treatment of adverse reactions

The treatment of other adverse reactions can refer to the drug instructions or the routine treatment principles of the test center.

## 2.7 Follow-up

1）Before treatment

All patients participating in this trial should sign an informed consent form and receive a copy of the informed consent form. If the patient agrees to participate in the trial, the participation form should be completed and signed immediately, and thereafter the patient can be enrolled. Within 28 days prior to initiation oftreatment, the investigator should evaluate the following clinical and laboratory indicators.

1 Past history, including: age, sex, tumor location, disease stage, histological stage, history of perforation or obstruction, duration of surgery, and the procedure

2 Physical strength status score (see Appendix

3 Clinical examination, height (pre-treatment measurement only), body weight

4 Electrocardiogram

5 Collection of clinical laboratory data:：

Routine blood indicators: hemoglobin, white blood cells, platelets and neutrophils;

Blood biochemical indexes: creatinine, alkaline phosphatase, total bilirubin, SGOT, SGPT;

Coagulation function: prothrombin time (PT), activated partial thromboplastin time (APTT) and international standardized ratio (INR);

Thyroid function: thyroid-stimulating hormone (ISH), free I3, free T4;

Urine routine: including urine specific gravity, pH value, white blood cells, red blood cells, protein, glucose, ketone body, and tube type. Two consecutive routine urine testing of urine protein + +, it is recommended to test 24 hours of urine protein quantification as soon as possible (such as within 72 hours;

fecal occult blood testing；

HIV check;

HBV infection: including hepatitis B five and HBV DNA;

HCV-infected persons: including HCV RNA.。

6 Detectionof tumor markers: CEA

7

8 pelvic cavity M R I Ctoluminal ultrasound

9 Genetic testing: MSI status (or MMR status assessed by immunohistochemistry), POLE / POLD 1, PTEN mutation status, TMB status, RAS and BRAF gene mutation testing

10 13

11 Peripheral tumor blood: CD3 +, CD4 +, and CD8 + I cell

12 14

13 Concomitant medication: collect medication within the 3 months before treatment 15

14 Quality of life assessment

2）During the treatment process

During the treatment process, researchers can add follow-up content or increase the frequency of follow-up according to needs. The follow-up items during the treatment period need to be completed after the 2nd, 4th, 6th, and 8th cycles of treatment.

The follow-up content of the trial plan is as follows:

1．Body weight: Measurements will be taken before neoadjuvant immunotherapy, after 2 cycles, 4 cycles, 6 cycles, and 8 cycles of neoadjuvant immunotherapy.

2．ECOG PS (Eastern Cooperative Oncology Group Performance Status) Score: Assessment will be conducted before neoadjuvant immunotherapy, after 2 cycles, 4 cycles, 6 cycles, and 8 cycles of neoadjuvant immunotherapy.

3．12-lead electrocardiogram (ECG): This test will be performed before neoadjuvant immunotherapy, after 2 cycles, 4 cycles, 6 cycles, and 8 cycles of neoadjuvant immunotherapy.

4．Complete blood count (CBC): Blood samples will be collected for CBC before neoadjuvant immunotherapy, after 2 cycles, 4 cycles, 6 cycles, and 8 cycles of neoadjuvant immunotherapy.

5．Blood biochemistry: Biochemical analysis will be done before neoadjuvant immunotherapy, after 2 cycles, 4 cycles, 6 cycles, and 8 cycles of neoadjuvant immunotherapy.

6．MRD (Minimal Residual Disease) detection: MRD will be assessed before neoadjuvant immunotherapy, after 2 cycles, 4 cycles, 6 cycles, and 8 cycles of neoadjuvant immunotherapy. If MRD becomes negative after neoadjuvant immunotherapy for the first time, a repeat MRD test will be performed after the next cycle of immunotherapy.

7．Tumor Marker Detection: To be performed before neoadjuvant immunotherapy, and after 2, 4, 6, and 8 cycles of neoadjuvant immunotherapy.

8．Thyroid Function Test: To be performed before neoadjuvant immunotherapy, and after 2, 4, 6, and 8 cycles of neoadjuvant immunotherapy.

9．Peripheral Blood Testing (Th1/Th2, Lymphocyte Immune Panel): To be performed before neoadjuvant immunotherapy, and after 2, 4, 6, and 8 cycles of neoadjuvant immunotherapy.

10．Colonoscopy with Biopsy: To be performed before neoadjuvant immunotherapy, and after 2, 4, 6, and 8 cycles of neoadjuvant immunotherapy.

11．Thoracic, Abdominal, and Pelvic CT Scan with Contrast: To be performed before neoadjuvant immunotherapy, and after 2, 4, 6, and 8 cycles of neoadjuvant immunotherapy.

12．Pelvic MRI (for rectum only): To be performed before neoadjuvant immunotherapy, and after 2, 4, 6, and 8 cycles of neoadjuvant immunotherapy.

13．Adverse Events: Collection of adverse events after the start of treatment.

14．Concomitant Medications: Continuous collection.

15．Quality of Life Assessment: To be collected every cycle.

3）After the completion of the treatment

After completion of treatment, patients should be reviewed at the items and time in the table below; until tumor recurrence or at least 3 years. Review beyond the time of the following table can be conducted according to the habits of different centers, with PET / CT if necessary.

| proiect | After the first moon | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 / | 3 / | 6 / | 9 / | 12 / | 15 / | 18 / | 21 / | 24 / |
| symptom | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| check-up | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| routine blood test | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Blood biochemical | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| MRD detection | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| CEA | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Peripheral blood detection | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Chest/abdominal/pelvic CT | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| colonoscope* | √ | √ | √ | √ | √ | √ | √ | √ | √ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Assessment of quality of life | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Observe the efficacy | √ | √ | √ | √ | √ | √ | √ | √ | √ |

* If the lesion is found by colonoscopy, the colonoscopy period can be shortened according to the specific situation.

4）Safety follow-up

Safety follow-up was performed 28 days after the last dose and the trial planned follow-up is as follows:

1 weight
2 check-up
3 ECOG PS Score
4 And a 12-lead ECG
5 routine blood test
6 Blood biochemical
7 routine urine test
8 coagulation function
9 thyroid function
10 Enhanced thoracic, abdominal, and pelvic CT scans: Patients who are discontinued due to disease progression do not need further follow-up enhancement
11 Adverse events: Continuous follow-up until adverse event recovery or further observation is not required by the investigator.
12 Concomitant medication: Continuous collection until treatment for the adverse event is completed or the investigator.
13 Quality of life assessment

5）Survival follow-up

After disease progression, patient survival status was collected every 3 months allowing telephone follow-up.

6）Patient compliance observation

The investigator's responsibility to ensure patient compliance with the trial will be examined by itinerant representatives.

## 2.8 data collection

All data shall be timely, truthfully and detailed in the medical record register (Case Report Form, CRF) or CRF software. The medical record form should be filled in by special personnel and signed by the project leader. The medical records (CRFs) shall be checked and collected regularly, and recorded according to the time node required by the clinical trial management center of the hospital.

After the clinical trial, the unit shall write the clinical summary report according to the standard requirements.。

## 2.9 Clinical safety assessment

1）Adverse events

Definition o fthe adverse events

Adverse event (or adverse experience, AE: any adverse medical event occurring in a subject or clinical subject, not necessarily causally related to treatment.

The AE can therefore be any bad or non-intended signs (e. g., including abnormal laboratory results), symptoms, or transient drug-related disease, which should be considered for medication involvement.

Adverse events occurring both before and after treatment were considered as adverse events according on management needs. Therefore, safety monitoring (reporting adverse events or serious adverse events) should be performed from subject enrollment to the end of the study. Therefore, adverse events occurring during signing the informed consent and initiation of study treatment were also considered as AEs.

Adverse drug reactions (ADRs): Al toxicities and non-intended reactions to the drug related to any dose should be considered as adverse drug reactions (ADRs).

Response to the drug means that there is at least a reasonable possibility of a causal relationship between the drug and the AE, meaning that this relationship cannot be excluded.

Serious Adverse Event (SAE: means all adverse medical events occurring at any drug dose:

- Lead to death
- life-threatening

Note: "serious" and "life threatening" are defined as an adverse event when the subject has the adverse event; rather than an adverse event that the more serious may lead to death.。

- Patients require hospitalization or extension of existing hospitalization
- Leading in persistent or significant incapacity / disability
- Congenital malformations or birth defects

Important medical events: important medical events, do medical and scientific appraisal to decide whether to report is appropriate, these important medical events may not immediately life threat or cause death or hospitalization, but may harm subjects or may need interference to prevent the occurrence of the other results, usually should also be considered serious. For example, some adverse events require severe treatment in the emergency room, or management of allergic bronchial asthma at home; malignant fluid or convulsions without hospitalization; drug dependence and abuse, or malignant tumors histologically different from the primary tumor.

Other events that should be addressed as SAEs: Drug exposure during pregnancy / lactation: In principle, pregnancy and lactation are under the exclusion criteria. If a pregnancy occurs during the study, the patient should immediately withdraw from the study and inform the investigator immediately and follow up the patient throughout the pregnancy and postpartum. Even if the mother and child are completely normal without any adverse events, the consequences should be recorded. Even if the pregnancy is not a SAE, use the SAE report form.

Events that shouldb e handled as SAEs: disease progression.

2）Record and evaluation methods ofadverse events

All A s should be recorded in the appropriate part ofthe Medical Record Report Form (CRF). SAE report forms (including initiation or follow-up reports) should also be completed.

The following aspects of each event should be recorded in the CRF:：

- AEs in medical terms, not as a subject;
- Date of occurrence (start date);
- Octime (start time);
- Recovery date (end date);
- Recovery time (end time).

The grading was assessed by the investigator as defined by NCI-CTC version 5.0.

- Level 1= mild;
- Level 2= moderate;
- Level 3= Heavy;

- Grade 4= life-threatening or disabling;;
- Level 5 = death.

The investigator should assess the causal relationship between the adverse events and the study drug; the decisive factor assessed in the record is the temporal correlation between the AEs and the study drug. The causal relationship between the adverse event and the study drug or study protocol is judged as follows:

- Uncorrelation = no temporal relationship with the study drug (too early, too late or not medication), or a reasonable causal relationship between the A and another drug, associated disease or environment;
- Impossible = a temporal relationship with study drug, but no reasonable causal relationship between AE and study drug;
- Probably = a reasonable causal relationship between A and study drug. Lack of withdrawal information (withdrawal information) or unclear;
- It is likely that there is a reasonable causal relationship between AE and study drug. Discontinuation (withdrawal from the drug study) had an effect on the response. No redose to prove;
- Clear / definite = a reasonable causal relationship between A and study drug. Discontinuation has an effect on response and can occur if readministered when clinically feasible.

Measures taken for this study treatment (none, discontinuation, dose reduction, delayed treatment, slowing of intravenous infusion) and other measures (no, concomitant medication, need for hospitalization, or prolonged hospitalization, surgery, delayed chemotherapy, discontinuation of chemotherapy, chemotherapy reduction) were defined as follows:

- Recovery with sequelae
- Healing without sequelae
- Not healed, but no treatment is needed
- Not healed and need treatment
- die

Toxicity grade / severity was changed

- Serious: Yes or No;
- If the patient has the same AE several times, each must be recorded and re-evaluated.

3) Reporting procedures for serious adverse events3

Any serious or medically significant clinical adverse event or laboratory abnormality

occurring during the study obligates the investigator to report every serious adverse event to the ADR by telephone or email within 24 hours.

After the telephone report, the written information should be sent by fax. The report shall provide the information of the reporter and recipient including name, address, telephone and fax number, and indicate that the report is "preliminary" report or "follow-up" report. If necessary, the report form shall be accompanied by the relevant medical record report form.

The investigator shall guarantee to the public ethics committee or competent authority any additional information regarding the death of the subject.

All forms must be dated and signed by the responsible person, or signed by an authorized colleague ofthe responsible person.

4）Monitoring of the adverse events of the subjects

Any adverse events occurring during the study were monitored and followed up until the end of the study. In addition, the SAEs must be reported through the SAE table.

5）Laboratory Indicator Assessment

All laboratories involved in clinical evaluations in the hospital should comply with the basic principles of Good Laboratory Practice (GLP) and the requirements of their respective hospitals.

6）Clinical Safety Assessment

NCI-CTCAE version 5.0 will be used to evaluate the clinical safety of the treatment in the study. The occurrence of adverse events in the subjects must be assessed during each clinical visit.

## 2.10 statistical analysis

tatistical analysis technique

Using computer (SPSS or R) software package, count data adoption rate, mean and 95% credible interval for measurement data: t-test for measurement data when comparing two groups, chi-square test for count data, Kaplan-Meier and Log Rank methods for survival analysis, and Cox proportional risk model for prognosis analysis. The probability P-value was used at any stage, and the statistical significant difference bound was used at 0.05.

Safety analysis: The toxicity evaluation criteria were evaluated according to the NCI-CTC 5.0 criteria. Specific statistical methods are the same as above.

Type of analysis

Prior to database lock, the following three datasets were identified.

The primary efficacy analysis will be performed in all enrolled populations (the intent-to-treat population or the ITT population) and the per-protocol population (PP). Secondary efficacy analysis and exploratory analysis will be performed in the intention-to-treat population. Non-inferiority analysis will be performed in the per-protocol population.

1.Intention-to-treat population

The intention-to-treat population consists of all patients randomized to any of the two treatment arms. Analysis will be performed according to the treatment groups randomassigned by the patients.

2.Population conforming to the protocol

Patients who have not received any dose of chemotherapy or chemoradiotherapy, or those who have suffered a serious violation of the inclusion or exclusion criteria. Analysis will be performed according to the treatment groups randomassigned by the patients.

3.Safety population

The safety population includes all patients who have received at least one dose of chemotherapy or radiotherapy. The Safety population was used to analyze all safety parameters. Patients were assigned to the treatment group based on the medication they actually received.

4.The data that should be excluded during the analysis

There were no excluded data available.

Medium-term analysis

An interim analysis of the efficacy endpoints is not performed in this study. An independent data security monitoring team will monitor the safety data throughout the study. All safety data descriptive analysis will be performed after study treatment for all patients enrolled in this study. This analysis will be strictly limited to the safety data and will not include any efficacy endpoints.

# 3 research contents

## 3.2 Sample size of the study

Using a non-inferiority test, we have set the non-inferiority margin based on the DYNAMIC II study as -8.5%. With a power of 0.8, one-sided alpha of 0.05, an effective rate of PD-L1 after conventional treatment of 30%, and an expected MRD-negative rate after treatment of no less than 50%, an initial calculation using the Non-Inferiority Tests for the Difference Between 1 Proportion and 1-Sample function in the PASS (Power Analysis and

Sample Size) software indicates that a sample size of 20 patients is required for enrollment. The dropout rate for dynamic monitoring is related to the follow-up capabilities of the hospital's execution team, patient compliance, and the length of follow-up. The overall dropout rate for 3-year follow-up is expected to be no more than 10%. Therefore, the estimated minimum number of patients required for enrollment is 22.

## 3.3 subject investigated

The research subjects of this project originate from 22 newly diagnosed patients with dMMR/MSI-H locally advanced colorectal cancer who were admitted to Yunnan Cancer Hospital (The Third Affiliated Hospital of Kunming Medical University) after the project's launch.

## 3.4 Enrollment criteria

**1）1) Disease characteristics**

- Histological confirmation of rectal adenocarcinoma:
- Immunohistochemistry confirmed pMMR or / and pCR or / and NGS as MSS:
- The tumor location is within 12cm from the anal margin:
- Local advanced rectal cancer (stage II-III, cT 3-4 and / or N +):

* Preoperative staging method: pelvic MRI / transrectal ultrasound.

- No signs of intestinal obstruction: or obstruction relieved after proximal colostomy surgery:
- Preoperative thoracic, abdominal, and pelvic CT excluded distant metastases.；

**2）Patient characteristics**

- Age: from 18 years old to 75 years old:
- Activity status score: ECOG 0-1:
- Life expectancy: greater than 2 years:
- Hematology: WBC> 3500106/L ; PLT>100000a 106/L ; Hb>10g/dL:
- Liver function: SGOT and SGPT were less than 1.5 times the normal value: bilirubin was less than 1.5 mg/dL:
- Renal function: creatinine was ml.8 mg/dL:

• Other: non-pregnant or lactating women: no other malignant disease (except non-melanoma or carcinoma of the cervix) within 5 years or concurrent: no mental illness that prevents informed consent: no other serious disease that leads to shortened survival.

• Patients or family members can understand the study protocol and are willing to participate in the study, and sign a written informed consent form:

• Patients had good compliance and volunteered for scheduled follow-up, treatment, laboratory tests, and other study procedures.。

**3）Previous treatment**

• No previous rectal cancer surgery:

• No prior chemotherapy or radiotherapy:

• No previous biological treatment:

• Previous endocrine therapy: no restriction.

## 4.4 Exclusion criteria

- • Tumor biopsy specimens with immunohistochemical indications of pMMR or microsatellite stability (MSS).
- Patients with histological types of colorectal cancer other than adenocarcinoma (such as neuroendocrine carcinoma, sarcoma, lymphoma, squamous cell carcinoma, etc.).
- History of HIV infection or active chronic hepatitis B or C (high viral DNA copy).
- Autoimmune diseases.
- Other active clinically severe infections (> NCI-CTC v3.0).
- Patients in clinical stage I.
- Preoperative evidence of distant metastasis, including isolated, distant, or non-contiguous intra-abdominal metastases.
- Patients who have undergone open surgery on sites other than the colon within 14 days prior to enrollment.
- Patients unable to provide surgical tissue for WES testing for customized personalized MRD detection panel or those with failed customization of the personalized MRD detection panel.
- Patients unable to provide blood samples for MRD testing during the treatment and follow-up periods.
- Cachexia or organ dysfunction.
- History of pelvic or abdominal radiation therapy.
- Patients requiring treatment for seizures (such as steroid or anti-epileptic therapy).

- Chronic inflammatory bowel disease or intestinal obstruction.
- Patients with other severe diseases that may affect follow-up and short-term survival, as determined by the investigator.
- Patients with a history of blood transfusion within 2 weeks before treatment starts.
- • Patients unable to undergo clinically required contrast-enhanced magnetic resonance imaging (MRI) or contrast-enhanced computed tomography (CT) for follow-up.
- Prior use of traditional Chinese medicine (TCM) with anti-tumor effects. However, if the duration of use was less than 7 days and the patient has discontinued the medication for 2 weeks or more before enrollment in this study, they may be eligible for inclusion.
- Evidence of severe or uncontrollable systemic diseases, such as severe psychiatric, neurological disorders, epilepsy, dementia, unstable or decompensated respiratory, cardiovascular, liver, or kidney diseases, left ventricular ejection fraction (LVEF) < 50%, uncontrolled hypertension (defined as greater than or equal to CTCAE grade 3 hypertension despite medication), dysphagia, active gastrointestinal diseases, or other conditions significantly affecting the absorption, distribution, metabolism, and excretion of oral medications. Patients who have undergone subtotal gastrectomy in the past are also excluded.
- Fever with a temperature of 38°C or higher in the past week, clinically significant active infections, or active pulmonary tuberculosis. Active fungal, bacterial, and/or viral infections requiring systemic treatment.
- Active bleeding, new-onset thrombotic disease requiring therapeutic anticoagulation, or patients with a bleeding tendency.
- Clinically significant major abnormalities in rhythm, conduction, or morphology on resting electrocardiogram (ECG), such as complete left bundle branch block, second-degree or higher heart block, clinically significant ventricular arrhythmias or atrial fibrillation, unstable angina, congestive heart failure, or chronic heart failure with New York Heart Association (NYHA) class ≥ 2. Patients with a bleeding tendency are also excluded.
- • History of myocardial infarction, coronary/peripheral artery bypass, or cerebrovascular accident within the last 3 months.
- Patients with a bleeding tendency.
- 12-lead electrocardiogram (ECG) QTc interval ≥ 450 ms for males and ≥ 470 ms for females.

- Presence of risk factors for QT interval prolongation or increased risk of arrhythmia, such as heart failure, grade 2 hypokalemia (defined as serum potassium < lower limit of normal - 3.0 mmol/L, with symptoms requiring treatment), congenital long QT syndrome, or a family history of long QT syndrome.
- Use of any medication known to prolong the QT interval within 2 weeks before enrollment.
- Insufficient bone marrow reserve or organ function, meeting any of the following laboratory limits (with no corrective treatment within 1 week before blood sampling):
    - Absolute neutrophil count < 1.5 × 109 / L
    - Platelet count < 90 × 109 / L
    - Hemoglobin < 90 g/L (< 9 g/dL)
    - Alanine aminotransferase > 3 times the upper limit of normal (ULN)
    - Aspartate aminotransferase > 3 × ULN
    - Total bilirubin > 1.5 × ULN
    - Creatinine > 1.5 × ULN or creatinine clearance < 45 mL/min (calculated using the Cockcroft-Gault formula)
    - Serum albumin (ALB) < 28 g/L
- Female subjects who are pregnant, breastfeeding, or plan to become pregnant during the study.
- Substance abuse and medical, psychological, or social conditions that may interfere with patient participation in the study or impact the assessment of study results.
- Known or suspected hypersensitivity to the study drugs or any drugs related to this trial.
- Any unstable condition or situation that may compromise patient safety and compliance.
- Any other situation deemed inappropriate for participation in the study by the investigator.

## 4.5Exit standard

- The subject withdrew his informed consent and requested his withdrawal:
- Poor compliance, not administered according to the study protocol:
- After enrollment from the study, the patient requests withdrawal from the investigator or fails to complete the study schedule for any reason:
- Other circumstances deemed necessary to conclude the study.

## 4.6 Termination of treatment

- Serious adverse events or serious adverse reactions (SAEs) during the study:
- Delay treatment for more than 4 weeks for any reason (meaning all drugs in the delayed regimen).

## 4.7 Evaluation of the study findings

1）Efficacy evaluation o f chemoradiotherapy

- CR: Pelvic MR: complete regression of primary tumor or only fibrotic scar, MR no mesangial lymph node enlargement: ultrasound colonoscopy: complete regression of primary tumor or only erythematous tiny ulcer or scar, no cancer remaining on biopsy: digital rectal tumor disappeared and no stiffness or nodules of intestinal wall. PR: tumor volume reduced by more than 30%, no new lesions:
- PD: tumor volume increased more than 20%, or new lesions.
- SD: The tumor volume did not change significantly, the reduction degree did not reach PR, and the increase degree did not reach PD:

2）Evaluation of the degree of radical operation

- R0 microscopic pathology examination cut margin has no residual tumor cells: The R1 microscopic pathological examination of the resection margin showed residual tumor cells:
- Residual tumor cells were visible at the macroscopic lower margins of R2.

3）Tumor response assessment

It is recommended to evaluate postoperative pathological specimens according to the tumor pathological withdrawal grade (tumor regression grading, TRG) criteria (see Annex 2): if not, a complete pathological response must be reported.

4） Evaluation of local recurrence or metastasis

When clinical symptoms occur (anal pain, hematochezia, lower limb edema, etc.), progressive elevation of CEA, or suspicious signs in chest or abdominal imaging, further examination is needed to find local recurrence or disease metastasis progression. Regional recurrence mainly refers to tumor recurrence in the local area or near the adjacent organs: distant metastasis refers to tumor recurrence outside the above areas. Disease-free survival is

the absence of tumor recurrence or the occurrence of new colorectal cancer by systematic evaluation. The diagnosis of clinical recurrence and metastasis must meet at least one of the following criteria:

1.Imaging suggested recurrence (ultrasound, CT, MRI, PET-CT)

2.Positive cytology biopsy (ascites, anastomotic recurrence, suspicious imaging findings)

The reported date of recurrence is the date when recurrence was detected using the diagnostic method described above. In case of recurrence, the investigator should specify the site of recurrence and the diagnostic method used. When definitive imaging evidence cannot be obtained, a positive result of cytology or biopsy should be obtained. Elevated CEA alone could not be used as evidence of local recurrence or metastasis of rectal cancer.

5）Calculation of the survival period

Local-regional recurrence-free survival was defined as the time interval from the onset of randomization to the appearance of a local recurrence event. Tumor evaluation (CT / MRI of the abdomen and pelvic cavity or ultrasound and chest CT) and CEA testing must be performed every 6 or 12 months after randomization, or when the patient shows signs of progression (i. e., clinically indicated).

Disease-free survival (DFS) is defined as the time interval from the start of randomization and the occurrence of the event in the next segment. Tumor evaluation (CT / MRI of the abdomen and pelvic cavity or ultrasound and chest CT) and CEA testing must be performed every 6 or 12 months after randomization, or when the patient shows signs of progression (i. e., clinically indicated). The suspected lesion detected by ultrasound must be confirmed by CT / MRI. All re-operations or further anticancer therapy should also be documented.

Based on the purpose of this study, events that identified patients were no longer in the disease-free condition were defined as follows:

- The patient showed signs of the original tumor recurrence:
- The patient showed signs of a new colorectal cancer,
- Death from any cause.

Note: recurrence of any original tumor or occurrence of new colorectal cancer should be determined by cytological or histological methods Confirm. No supporting evidence of other specific test findings (such as radiography, histology / cytology) cannot be based on isolated events such as increased CEA or unexplained clinical deterioration of the disease. The date of

relapse was defined as the date of final confirmation of the targeted findings. Patients will thereafter be followed up for survival as planned.

If a recurrence of a confirmed colorectal cancer or the occurrence of a new colorectal cancer occurs during the study treatment period, patients will be withdrawn from the study treatment for survival follow-up. If the recurrence of colorectal cancer or the occurrence of a new colorectal cancer occurs during the study treatment period, the patient may be treated further according to the guidance of the investigator.

All tested patients will be followed for at least 2 years. For a biopsy, a biopsy report should be provided. Overall survival was the time from randomization to death. When the patient survived, the time to the last follow-up was taken as the overall survival period.

6）Evaluation of the toxic and side effects of adjuvant therapy

Toxicity evaluation according to the CTC criteria (version 5.0)

Follow-up of patient safety should include during treatment and 30 days after the end of the last cycle. The reason for the delay or interruption should be recorded in the CRF table

7）ECOG (Appendix)

## 3.5 Ethical and informed consent aspects

### 3.5.1 Ethical requirements

The investigator ensured that the study was conducted in accordance with the Declaration of Helsinki principles for maximum protection of the individual. This study was reviewed and approved by the ethics committee of Yunnan Provincial Cancer Hospital.

### 3.5.2 informed consent

The investigator or a person assigned by the investigator is responsible (as permitted by local regulations) to obtain written informed consent for the purpose, methods, prospective benefits, and potential hazards of the study. Subjects who fail or fail to sign a legal consent must sign an informed consent by their legal representative. If the subject and his legal representative will not read, a notary should be present during the informed consent discussion. After the subject and his representative verbally agree to participate in the trial, the witness should sign the informed consent form to prove that the contents of the agreement are accurately interpreted and understood. The Investigator or designee should also state that the subject may refuse to participate in the study or withdraw from the study at any time for any reason. The CRF of this study includes the section documenting the subject's informed consent, which should be completed accurately. If new safety information leads to significant changes in the hazard /

benefit evaluation, the informed consent should be re-reviewed or updated as needed. All subjects (including those who have started treatment) should be informed of the new information, given an updated informed consent form, and obtained their consent to continue the study.

### 3.5.3 confidentiality

During the study, your name, gender and other personal data will be replaced by code names or numbers, and kept strictly confidential. Only the relevant doctor knows your information, and your privacy will be well protected. The principal investigator of this project will publish the final research results in a journal after the end of the project for academic communication and promoting medical progress, but will not disclose any of your personal information.

# 4 Project implementation years and annual plan

| The total length of the project implementation | 3 years |
|---|---|
| General schedule: June 2023 – June 2026 | |
| Phase time schedule | Main contents and results of the stage objectives |
| June 2023 - December 2023<br>（6 Mouths） | Study Enrollment Phase: 10 patients were enrolled for the initial assessment of efficacy, safety, and other relevant factors. |
| December 2023- June 2024<br>（6 Mouths） | Completion of Enrollment with Follow-up Phase: Upon completion of enrolling 22 patients, follow-up assessments were conducted to evaluate the cCR rate of MRD, safety, feasibility, toxic side effects of neoadjuvant immunotherapy, tumor regression, and other factors. |
| June 2024- December 2024<br>（6 Mouths） | Six-Month Follow-up Report after Enrollment of All Cases: Following the enrollment of all cases, a six-month follow-up report was issued to assess the cCR rate of MRD, safety, local recurrence rate, distant metastasis rate, and the incidence of surgical complications. |
| December 2024- June 2025<br>（6 Mouths） | One-Year Follow-up Report after Enrollment of All Cases: After the enrollment of all cases, a one-year follow-up report was issued to analyze the local recurrence rate, distant metastasis rate, and other relevant factors. |
| June 2025- June 2026<br>（12 Mouths） | Two to Three-Year Follow-up Report after Enrollment of All Cases: After the enrollment of all cases, a two to three-year follow-up report was issued to analyze the 3-year local recurrence rate, |

| | |
|---|---|
| | 3-year distant metastasis rate, 3-year DFS (Disease-Free Survival), and 3-year OS (Overall Survival).<br>Conclusion and Reporting: The study evaluated the safety and effectiveness of utilizing MRD dynamic monitoring results to guide the wait-and-watch strategy after neoadjuvant immunotherapy for dMMR/MSI-H colorectal cancer. |

# 6. appendix

## 6.1Attachment 1: ECOG PS Scoring criteria

| classify | Behavioral state |
|---|---|
| 0 | Ability to perform all daily activities without restriction |
| 1 | Severe physical activity is limited, but he can walk and can perform light physical work. |
| 2 | Can walk, live can take care of themselves, but can not engage in any work, awake, more than half of the time can get out of bed and walk |
| 3 | Can only have limited self-care, more than half of the waking time to bed or chair. |
| 4 | Unable to move: unable to take care of themselves: bed or chair. |

## 6.2 Attachment 2: Efficacy Evaluation Criteria for Solid Tumor Version (Response Evaluation Criteriain Solid Tumors RECIST Version1.1)

### 1Measureability of tumors at baseline levels

### 1.1Definition

At baseline, tumor lesions / nodes will be measurable and not measurable as defined below：

#### 1.1.1Measureable lesions

Tumor lesion: at least one diameter (which can be recorded as maximum) with the following minimum length:

- R CT scan 10mm (CT scan layer thickness not greater than 5mm)
- 0 Clinical routine examination instrument 10mm (tumor lesion cannot be accurately measured with diameter instrument should be recorded as unmeasurable)
- 0 Chest X-ray at 20mm
- 0 Malignant lymph nodes: Pathologically enlarged and measurable, short CT scan diameter 15mm (CT scan thickness recommended not more than 5mm). At baseline and follow-up, only short paths were measured and followed up.

#### 1.1.2Non-measurable lesions

All other lesions, including small lesions ( 10mm or 10mm to 15mm) and unmeasurable lesions. Unmeasured lesions include meningeal disease, ascites, pleural or

pericardial effusion, inflammatory breast cancer, cancerous lymphangitis of the skin / lung, imaging of unconfirmed and follow-up abdominal mass, and cystic lesions.

### 1.1.3 Special considerations regarding lesion measurement

Bone lesions, cystic lesions, and previously locally treated lesions should be specifically noted:

Bone lesions:

- Bone scan, PET scan or plain film are not suitable for measuring bone lesions, but can be used to confirm the presence or disappearance of bone lesions:
- If an osteolytic lesion or a mixed osteogenic lesion has a defined soft tissue component that meets the above measurable definition, these lesions can be considered as measurable lesions if they can be evaluated by tomographic imaging techniques such as CT or MRI:
- Osteogenic lesions are non-measurable lesions.

Cystic lesions：

- Lesions that meet the criteria of the definition of pure cyst by radioimaging should not be considered malignant because they are a simple cyst by defmition, neither measurable lesions nor unmeasurable lesions:
- R If it is a cystic metastatic lesion and meets the above measurable definition, it may be a measurable lesion. However, if non-cystic lesions exist in the same patient, non-cystic lesions should be preferred as the target lesion.

Topically treated lesions:

- Lesions located at sites previously irradiated or treated with other regional areas are generally considered as non-measurable lesions, unless the lesion has clearly progressed. The study protocol should describe in detail the conditions under which these lesions are measurable lesions.。

## 1.2 Description of the measurement method

### 1.2.1 Lesion measurement

For clinical evaluation, all tumor measurements were recorded in the metric rice system. All baseline assessments of tumor lesion size should be completed as close as possible prior to treatment initiation and must be completed within 28 days (4 weeks) before treatment initiation.

### 1.2.2 Evaluation methot

The same technique and methods should be used for the baseline assessment and subsequent measurements of the lesions. All lesions must be evaluated using imaging, except for those that cannot be evaluated by imaging but only by clinical examination.

Clinical lesions: Clinical lesions can only be considered as measurable lesions (such as skin nodules) when they are superficial and measured at 10mm in diameter. For patients with skin lesions, color photographs containing a scale to measure the size of the lesion, are recommended for archiving. When the lesions are evaluated using both imaging and clinical examination, imaging evaluation should be used as far as possible because the imaging is more objective and reproducible at the end of the study.

Chest X X: When tumor progression is an important study endpoint, chest CT should be preferred because CT is more sensitive than X-ray, especially for new lesions. Chest X-ray detection is applicable only if the measured lesions are well defined and the lungs are well ventilated.

CT, MRI: CT is currently the best available and reproducible method for efficacy evaluation. The definition of measurable ability is based on the 5mm thickness of the CT scan layer. If the CT layer thickness is greater than 5mm, the minimum measurable lesion should be 2 times the layer thickness. MRI is also acceptable in some cases (e. g. whole body scan).

Ultrasound: Ultrasound should not be used as a measurement method to measure the lesion size. Because of its operation dependence, the ultrasound examination is not reproducible after the end of the measurement, which cannot guarantee the identity of the technology and measurement between different measurements. If new lesions are identified using ultrasound during testing, they should be confirmed using CT or MRI. If radiation exposure to CT is considered, MRI can be used instead.

Endoscopy, laparoscopy: These techniques are not recommended for objective tumor evaluation, but they can be used to confirm CR in biopsy specimens obtained or in trials of recurrence after the study endpoint of CR or surgical resection.

Tumor markers: Tumor markers cannot be used alone to evaluate objective tumor response. However, if the marker level exceeds the upper normal limit at baseline, it must return to normal when used to evaluate complete remission. Because tumor markers vary by disease, this factor needs to be taken into account when writing the measurement criteria into the protocol. Specific criteria for CA-125 remission (recurrent ovarian cancer) and PSA (recurrent prostate cancer) remission have been published. In addition, the International Gynecological Cancer Organization has formulated the CA-125 progression criteria, which

will be added to the tumor objective evaluation criteria for the first-line treatment of ovarian cancer.

Cytology / histology techniques: in the specific circumstances specified in the protocol, these techniques can be used to identify PR and CR (such as residual benign tumor tissue often present in lesions of germ cell tumors). When exudation may be a potential side reaction of a therapy (e. g., treatment with taxane compounds or angiogenesis inhibitors), and a measurable tumor meets the criteria for remission or disease stabilization, tumor-related exudation occurrence or aggravation during treatment can be confirmed by cytology to distinguish between remission (or disease stabilization) and disease progression.

**2 2 It was used for tumor remission assessment**

**2.1Evaluation of all tumors and measurable lesions**

To evaluate the objective response or possible future progression, a baseline assessment of the total tumor burden in all tumor lesions is necessary for the subsequent measurements. In a clinical regimen with objective remission as the primary treatment endpoint, only patients with measurable lesions at baseline will be enrolled. A measurable lesion was defined as the presence of at least one measurable lesion. For trials with disease progression (time to progression or degree of fixed date progression) as the primary treatment endpoint, the protocol inclusion criteria must be limited to patients with measurable lesions or no measurable lesions can be included.

**2.22.2 Baseline recordings of target lesions and non-target lesions**

When there are more than one measurable lesions during the baseline assessment, all lesions should be recorded and measured, a total of not more than 5 (no more than 2 each organ), as the target lesions represent all involved organs (that is, patients with only one or two cumulative organs up to choose two or four target lesions as the baseline measurement lesions).

The target lesion must be selected based on size (longest diameter), representative of all organs involved, and the measurements must be well reproducible. Sometimes when the largest lesion cannot be repeatedly measured, the largest lesion can be reselected.

Lymph nodes need special attention because they are normal tissue and can be detected by imaging even in the absence of tumor metastasis. Pathological lymph nodes defined as measurable nodules or even target lesions must meet the following criteria: CT measurement of a short diameter of 15mm. Baseline only detects the short diameter. Radiologists usually use the short diameter of the nodule to determine whether the nodule has a metastatic tumor. The cle size is generally expressed by two-dimensional data from imaging detection (CT

axial plane and MRI selects a plane from the axial, sagittal or coronal plane). The minimum value is the short diameter. For example, a 20mm 30mm abdominal nodule with a short diameter of 20mm can be regarded as a malignant, measurable nodule. In this example, 20mm is the measurement of the nodule. Nodules with 10mm diameter but 15mm should not be considered as target lesions. However, nodules 10mm do not belong to the category of pathological nodules and do not need to be recorded and further observed.

The sum of the total diameter calculated together (including the longest diameter of the non-nodular lesion and the short diameter of the nodular lesion) will be reported as the sum of the baseline diameters. If containing the lymph node diameter, as mentioned above, only the short diameter is included. The sum of the baseline diameters will serve as the reference value for the disease baseline level.

All remaining lesions including pathological lymph nodes may be considered non-target lesions, but should be recorded at baseline assessment. If recorded as "present", "missing" or in rare cases "clear progress". Widespread target lesions can be recorded with target organs (e. g., massive expanded pelvic lymph nodes or large liver metastases).

**2.3 Mitigation criteria**

**2.3.1 Evaluation of the target lesions**

Complete response (CR): All target lesions disappear, and the short diameter of all pathological lymph nodes (including target nodules and non-target nodules) must be reduced to 10mm.

Partial response (PR): The sum of target lesion diameter decreased by at least 30% from baseline.

Disease progression (PD): Using the minimum value of the sum of all measured target lesions throughout the experimental study, the diameter and relative increase is at least 20% (baseline value if the minimum): otherwise, the absolute increase of at least 5mm must be met (occurrence of one or more new lesions is also considered as disease progression).

Disease stability (SD): the degree of target lesion reduction does not reach the PR, the degree of increase does not reach the PD level, in between the two, the minimum value of the sum of the diameter can be used as a reference.

**2.3.2 2.3.2 Precautions for target lesion evaluation**

Lymph node: Even if the lymph nodes identified to be the target lesion decrease to less than 10mm, the actual short diameter value corresponding to the baseline should be recorded at each measurement (consistent with the anatomical plane at the baseline measurement).

This means that if the lymph node belongs to the target lesion, even if the criteria for complete remission are achieved, it cannot be said that the lesion has all disappeared, because the short diameter of the normal lymph node is defmed as 10mm. The target lymph short lesion needs to be recorded at a specific location in the CRF table or other recording methods: for CR, all lymph short diameters must be 10mm: for PR, SD, and PD, the actual measurements of the target lymph short diameter will be included in the sum of the target lesion diameter.

Small to unmeasurable target lesions: In clinical studies, all baseline recorded lesions (nodules or non-nodules) should be recorded again in the later evaluation, even if the lesions are very small (e. g. 2mm). But sometimes it may be too small to cause the CT scan to be blurred, and the radiologist is struggle to define the exact value, potentially it as "too small to measure."In this case, it is important to record a value on the CRF table. If the radiologist believes that the lesion may have disappeared, it should also be recorded as Omm. If the lesion is indeed present but is vague and a precise measurement cannot be given, the default can be 5mm.(Note: lymph nodes are unlikely to do this because they normally have a measurable size or are often enclosed by adipose tissue as in the retroperitoneal cavity: but if this measurement cannot be given, the default is 5mm). The default value of 5mm stems from the cut thickness of the CT scan (this value is not changed by the different cut thickness values of the CT scan). Since the same measurement is not repeated, providing this default will reduce the risk of misassessment. But it should be reiterated that if the radiologist can give the exact value of the lesion size, the actual value must be recorded even if the lesion is less than 5mm in diameter.

Isolated or combined lesion: When the non-nodular lesion divides into fragments, the longest diameter of the separated part together calculates the total diameter of the lesion. Similarly, for the binding lesions, they can be distinguished by the plane between the bound parts, and then the respective maximum diameter is calculated. However, if the combination is inseparable, the longest diameter should be taken as the longest of the fusion lesion.

**2.3.3Assessment of non-target lesions**

This section defines the remission criteria for non-target lesion tumors. Although some non-target lesions are actually measurable, no measurement requires only qualitative assessment at the time points specified in the protocol.

Complete response (CR): All non-target lesions disappeared and tumor markers returned to normal levels. All lymph nodes were of non-pathological dimensions (short diameter 10mm).

Non-complete response / non-disease progression: presence of one or more non-target lesions and / or persistence of tumor markers at levels greater than normal.

Disease progression: a definite progression of preexisting non-target lesions. Note: The presence of one or more new lesions is also considered as disease progression.

**2.3.42.3.4 Special considerations for the progression assessment of non-target lesions**

The supplementary explanation of the definition of non-target lesion progression is as follows: When a patient has a measurable non-target focus, even if the target lesion is assessed as stable or partial remission, to make a clear definition of progression based on the non-target focus, the overall deterioration of the non-target lesion has reached the point that the treatment must be terminated. However, the general increase in the size of one or more non-target lesions is often not enough to meet the progression criteria. Therefore, the overall tumor progression is almost rare in the target lesion when the target lesion is stable or partially relieved.

This occurs when none of the non-target lesions are measurable: in some phase trials, when measurable lesions are not specified in the inclusion criteria. The overall assessment was based on the above criteria, but because there was no measurable data for the lesion in this case. The deterioration of the target lesions is not easy to assess (according to the definition: must all non-target lesions are not measured), so when the target lesions change to increase the overall disease burden is the target of the disease progression, according to the definition of the target focus progress, need to establish an effective detection method to evaluate. As described, an increase in tumor burden is equivalent to an additional 73% increase in volume (equivalent to a 20% increase in measurable lesion diameter). Or peritoneal exudation from "trace" to "massive": lymphangiopathy from "local" to "widespread spread": or described in the protocol as "sufficient to change treatment".

Examples include pleural exudate from trace to mass, spread of lymphatic involvement from the primary site to the distance, or may be described as "necessary therapeutic changes" in the protocol. If definite progression is found, the patient should be treated as disease progression at that time point. It is best to have objective criteria that can be applied to the assessment of unmeasurable lesions, noting that the increased criteria must be reliable

**2.3.5New lesions**

The appearance of new malignant lesions indicates the progression of the disease: therefore some evaluation of new lesions is very important. There are no specific criteria for imaging detection of lesions, however the discovery of a new lesion should be clear. For example, progression cannot be attributed to differences in imaging techniques, changes in imaging morphology, or other lesions other than tumors (for example, some so-called new

bone lesions are merely the cure of the original lesion, or the recurrence of the original lesion). This is important when a patient's baseline lesion is partially or completely reactive, for example, a necrosis of a liver lesion may be identified as a new cystic lesion on the CT report, but not.

Lesions detected at follow-up but not detected at baseline will be considered as new and estive of disease progression. For example, if a patient with a visceral lesion on baseline examination has metastases on CT or MRI, his intracranial metastases will be considered as the basis for disease progression, even if he did not have a cranial examination at baseline examination.

If a new lesion is unclear, for as due to its small morphology, further treatment and follow-up evaluation are needed to confirm whether it is a new lesion. If repeated examinations confirms it is a new lesion, the time of disease progression should be counted from the time of its initial discovery.

FDG-PET assessment of lesions generally requires additional testing for complementary confirmation, and the combination of FDG-PET and supplementary CT test results is reasonable to evaluate progression (especially for new suspected diseases). New lesions may be identified by FDG-PET according to the following procedures:

Baseline FDG-PET was negative and the following up FDG-PET was positive, indicating progression of the disease.

No baseline FDG-PET test was performed, and the subsequent FDG-PET test result was positive:

If the follow-up FDG-PET positive test results matched the CT test results, the disease progression was proved.

If the new lesion found by the positive test result of the follow-up FDG-PET is not confirmed by the CT test result, the CT test should be confirmed again (if confirmed, the time of disease progression starts from the previous FDG-PET abnormality).

If the follow-up FDG-PET results with a preexisting lesion by CT and the lesion does not progressive on imaging tests, no disease progression.

### 2.42.4 Best of the overall efficacy evaluation

The best overall efficacy evaluation is the best efficacy record from the beginning of the trial to the end of the trial, with any necessary conditions taken into consideration for confirmation. Sometimes the efficacy response occurs after the end of treatment, so the regimen should specify whether the efficacy evaluation after the end of treatment should be considered in the best overall efficacy evaluation. The protocol must clarify how any new

treatment affects optimal efficacy response. The best response response depends on the outcome of the target and non-target lesions and the performance of the new lesions. In addition, it relies on the nature of the trial, protocol requirements, and outcome measures. Specifically, the efficacy response profile is the primary goal in non-randomized trials, and the confirmation of efficacy by PR or CR is mandatory to confirm which is the best overall efficacy response.

**2.4.1Time-point reaction**

It is assumed that an efficacy response will occur at the specific time point of each regimen. Table 6 will provide a summary of the overall efficacy response of a patient population with disease measurable at baseline level at each time point.

If the patient has no measured lesions (no target lesion), the evaluation is presented in Table 7.

**2.4.2 Missing evaluation and non-evaluable description**

If lesion imaging or measurement cannot be performed at a particular point in time, the patient will not be evaluated at that time point. If only some of the lesions can be evaluated in an evaluation, it is usually considered impossible to evaluate at that time point, unless there is evidence that the missing lesion does not affect the efficacy response evaluation at the specified time point. This situation is very likely to occur in the case of disease progression.

For example, a patient with three lesions with a total of 50mm at baseline, but then only two lesions, with a total of 80mm, will be evaluated as disease progression, regardless of the impact of the missing lesion

**2.4.3 Best total response: all time points**

Once all the patient data are available, the best total response can be determined.

Assessment of best total response when the study does not require confirmation of a complete or partial efficacy response: best efficacy response in the trial was best response at all time points (e. g., a patient was evaluated as SD in the first cycle, PR in the second cycle, PD in the last cycle, but the best total response as PR. When the best total response is evaluated as an SD, it must meet the minimum time from the baseline level specified in the protocol. If the standard of the shortest time is not met, even if the best overall response evaluation as SD is not approved, the best overall response of this patient will depend on the subsequent evaluation. For example, a patient was evaluated as SD in the first cycle and the second cycle as PD, but it did not meet the shortest time requirement of SD, and its best overall response was evaluated as PD. The same patient lost to follow-up after the first cycle evaluation of SD will be considered as nonevaluable.

Assessment of the best total response when the study requires confirmation of complete or partial response: a complete or partial response is confirmed only if each subject meets the partial or complete response criteria specified in the trial and is specifically mentioned in the protocol at subsequent time points (usually four weeks later). In this case, the best total response is as described in Table 7.

**2.4.4Special tips for efficacy assessment**

When nodular lesions are included in the total target lesion assessment and the nodule size decreases to a "normal" size ( 10mm), they will still have a lesion size scan report. To avoid excessive assessment based on the increased nodule size, the measurements will be recorded even if the nodule is normal. As already mentioned, this means that subjects with complete response will not be recorded as 0 on the CRF table.

During the trial. The analytical plan of the trial must state that these missing data / assessments can be explained when determining efficacy. For example, in most trials, the response of a subject PR-NE-PR can be confirmed as the efficacy.

It should be reported as symptomatic progression when the subject experienced an overall deterioration of his or her health, but with no objective evidence. Objective progression should be assessed even after treatment termination. Symptomatic deterioration is not an objective assessment of assessment: it is the reason for discontinuing treatment. The objective response of such subjects will be assessed by the target and non-target lesion conditions shown in Table 6 to 8.

Defined as early progression, early death and nonevaluable conditions are study exceptions and should be clearly described in each protocol (depending on the treatment interval and treatment cycle).

In some cases, it is difficult to identify local lesions from normal tissue. When evaluation of complete response is based on such a definition, we recommend biopsy before efficacy evaluation of local focal complete response. When some subjects with abnormal local lesion imaging findings are considered to represent lesion fibrosis or scar formation, FDG-PET is used as a similar assessment criterion to biopsy to confirm the efficacy of complete response. In such cases, the application of FDG-PET should be prospectively described in the protocol, while supported by reports of the specialist medical literature for this situation. However, it must be realized that the limitation of FDG-PET and biopsy itself (including their resolution and sensitivity) will lead to false positive results in complete remission evaluation.

**Table 6 Time point response: Subjects with target lesions (including or excluding non-target lesions)**

| Target focus | Non-target lesions | New lesions | Total relief |
|---|---|---|---|
| CR | CR | mistake | CR |
| CR | Non-CR / non-PD | mistake | PR |
| CR | Can't evaluate | mistake | PR |
| PR | Non-progressive or could not be fully assessed | mistake | PR |
| SD | Non-progressive or could not be fully assessed | mistake | SD |
| Not fully evaluated | Non-progression | mistake | NE |
| PD | Any situation | Yes or no | PD |
| Any situation | PD | Yes or no | PD |
| Any situation | Any situation | Yes | PD |

CR= complete remission

PR= partial remission

SD= stable disease

PD= progression disease

NE= Not assessable

**Table 7 Time point responses-Only subjects with non-target lesions**

| Non-target lesions | New lesions | Total relief |
|---|---|---|
| CR | mistake | CR |
| Non-CR or non-PD | mistake | Non-CR or non-PD |
| Not fully evaluated | mistake | Can't evaluate |
| Can not be clearly defined for the PD | Yes or no | PD |

Note: For non-target lesions, "non-CR/non-PD" refers to the therapeutic effect that is better than SD. Since SD is increasingly being used as an endpoint for evaluating therapeutic effects, a therapeutic effect for non-CR/non-PD has been defined to target situations where no lesions are measurable.

Treatment with ambiguous progressive findings (e. g. very small uncertain new lesions: preexisting cystic or neurotic lesions) can be continued until the next evaluation. If disease

progression is confirmed in the next assessment, the date of progression should be the date of prior suspected progression。

**Table 78 Optimal overall response for CR and PR efficacy**

| Total remission at the first time point | Total remission at the subsequent time point | Best total relief |
|---|---|---|
| CR | CR | CR |
| CR | PR | SD，PD or PR[a] |
| CR | SD | SD if SD lasts sufficient duration, otherwise PD |
| CR | PD | SD if SD lasts sufficient duration, otherwise PD |
| CR | NE | SD if SD lasts sufficient duration, otherwise NE |
| PR | CR | PR |
| PR | PR | PR |
| PR | SD | SD |
| PR | PD | SD if SD lasts sufficient duration, otherwise PD |
| PR | NE | SD if SD lasts sufficient duration, otherwise NE |
| NE | NE | NE |

Note: CR is complete response, PR is response, SD for stable disease, PD is progressive disease, and NE is not evaluable. Superscript "a": If CR truly occurs at the first time point and any disease appears at subsequent time points, even if the subject meets the PR criteria relative to baseline, the therapeutic response evaluation at subsequent time points will still be PD (because disease recurrence after CR). The best response depends on whether SD occurs within the shortest treatment interval. However, sometimes when the first evaluation is CR, but subsequent scans suggest that small lesions still appear, the subject's actual response at the first time point should be PR rather than CR. In this case, the initial CR judgment should be revised to PR, and the best response is PR.。

### 2.5. Frequency of tumor reevaluation

The frequency of tumor reevaluation during treatment is determined by the treatment regimen and should be consistent with the type and schedule of treatment. However, in phase trials where the benefit of treatment is unclear, follow-up every 6 to 8 weeks (designed at the end point of a cycle) is reasonable, and the length of the interval can be adjusted under special protocol or circumstances. The protocol should specify which tissue sites require baseline evaluation (usually those most likely to be closely associated to the metastatic lesion of the tumor type studied) and the frequency of evaluation repeats. Normally, target lesions and non-target lesions should be evaluated at each evaluation. In some optional cases, some

non-target lesions can be evaluated less frequently. For example, the bone scan should be repeated when the efficacy evaluation of the target disease is confirmed as CR or bone lesion progression is suspected.

After completion of treatment, the tumor re-evaluation depends on taking the response rate or the time to an event (progression / death) as the clinical trial endpoint. Time for an event (e. g. TTP / DFS / PFS) requires the routine repeat evaluation specified in the protocol. In particular, in randomized comparative trials, scheduled evaluations should be included in the schedule (e. g., 6 to 8 weeks during treatment, or 3 to 4 months after treatment) and should not be affected by other factors, such as treatment delay, dosing interval, and any other events that may lead to unbalanced treatment arm in the choice of disease evaluation time.

**2.6. Efficacy assessment / confirmation of remission period**

**2.6.1. Confirmation**

For non-randomized clinical studies with efficacy as the primary study endpoint, the efficacy of PR and CR must be confirmed to ensure that efficacy is not the result of misevaluation. This also allows for a reasonable interpretation of the results where historical data are available, but the efficacy in the historical data of these trials should also have been confirmed. However, in all other cases, such as randomized trials (or periods) or studies with disease stability or disease progression as the primary endpoint, efficacy confirmation is no longer required because this is of no value in the interpretation of trial results. Removing the requirement for efficacy confirmation, however, makes the central review even more important, especially in unblinded experimental studies.

In the case of SD, at least one measurement during the shortest time interval after the start of the trial (generally not less than 6 to 8 weeks) will meet the SD criteria specified in the protocol.

**2.6.2 Overall remission period**

The total remission period was from the time of measuring the first CR or PR (which was measured first) to the time of the first true record of disease recurrence or progression (using the minimum measurement recorded in the trial as a reference for disease progression). The time to total complete response was from time to first meeting CR criteria to time of first true recording of disease relapse or progression.

**2.6.3. Stable disease period**

Is the time from the start of treatment to disease progression (in the randomized trial, from the time of randomization), with the smallest sum in the trial as a reference (if the sum of baseline is the minimum, as the reference for PD calculation). The clinical relevance of

disease stabilization varies between studies and different diseases. If the proportion of the patients maintaining the shortest time stability period is used as the study endpoint, the protocol should specifically state the shortest time interval between the two measurements in the SD definition.

Note: Response, stabilization, and PFS were affected by the frequency of follow-up after baseline evaluation. Defining standard follow-up frequency is not within this guideline. The frequency of follow-up should consider many factors, such as disease type and stage, treatment cycle and standard norms. However, if inter-trial comparisons are required, the endpoint accuracy limitations of these measurements should be considered.

**2.7.PFS/TTP**

**2.7.1. II Phase of the clinical trial**

This guideline focuses on the use of objective remission as a research endpoint in phase clinical trials. In some cases, remission rates may not be optimal for evaluating the potential anticancer activity of new drugs / novel regimens. In these cases, PFS / PPF at the demarcation time points can be considered a suitable surrogate indicator for the original signal that provides the biological activity of new drugs. But it is clear that in a noncontrolled trial, these evaluations will be questioned, because seemingly valuable observations may be related to biological factors such as patient screening, rather than the role of pharmacological interventions. Therefore, phase clinical trials with these as study endpoints are best designed as randomized controls. But the clinical manifestations of some tumors is consistent (usually always poor) and non-randomized trials are reasonable. However, in these cases, the evidence of efficacy should be carefully documented when assessing the expected PFS or PPF due to the lack of positive controls.

## 6.3 Attachment 4: The EORTC QLQ-C30 scale and the EORTC-QLQ-CR29 scale

### 1）EORTC Quality of Life Measurement Scale QLQ-C30 (V3.0)

We would like to know something about you and your health, please answer all the questions for yourself. There is no "right" or "wrong" here, but only to draw circles on the number that best reflects your situation. The information you provide will be kept strictly confidential.

| | | Not have | some | match | extraordinary |
|---|---|---|---|---|---|
| 1 | Do you have difficulty engaging in some laborious activities, Like carrying a very heavy shopping bag or a suitcase? | 1 | 2 | 3 | 4 |
| 2 | Is long-distance walking so difficult for you? | 1 | 2 | 3 | 4 |
| 3 | Is it difficult for you to walk over a short distance outdoors？ | 1 | 2 | 3 | 4 |
| 4 | Do you need to stay in bed or in a chair during the day? | 1 | 2 | 3 | 4 |
| 5 | Do you need help while eating, dressing, bathing, or going to the bathroom? | 1 | 2 | 3 | 4 |

| | In the past one week： | Not have | some | match | extraordinary |
|---|---|---|---|---|---|
| 6 | Are you restricted in your work and daily activities？ | 1 | 2 | 3 | 4 |
| 7 | Are you restricted in engaging in your hobbies or leisure activities? | 1 | 2 | 3 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| 8 | Do you have the gas to promote? | 1 | 2 | 3 | 4 |
| 9 | Do you have any pain? | 1 | 2 | 3 | 4 |
| 10 | Do you need a break? | 1 | 2 | 3 | 4 |
| 11 | Do you have trouble sleeping? | 1 | 2 | 3 | 4 |
| 12 | Do you feel weak? | 1 | 2 | 3 | 4 |
| 13 | Do you lose your appetite (have no appetite)? | 1 | 2 | 3 | 4 |
| 14 | Do you feel sick? | 1 | 2 | 3 | 4 |
| 15 | Do you vomit? | 1 | 2 | 3 | 4 |
| 16 | Do you have constipation? | 1 | 2 | 3 | 4 |

| | In the past one week: | Not have | some | match | extraordinary |
|---|---|---|---|---|---|
| 17 | Do you have any diarrhea? | 1 | 2 | 3 | 4 |
| 18 | Do you feel tired? | 1 | 2 | 3 | 4 |
| 19 | Does the pain affect your daily activities? | 1 | 2 | 3 | 4 |
| 20 | Do you have difficulty concentrating on doing things, such as reading a newspaper or watching TV? | 1 | 2 | 3 | 4 |
| 21 | Do you feel nervous? | 1 | 2 | 3 | 4 |
| 22 | Do you feel worried? | 1 | 2 | 3 | 4 |
| 23 | Do you feel hot-tempered? | 1 | 2 | 3 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| 24 | Do you feel depressed (depressed)? | 1 | 2 | 3 | 4 |
| 25 | Do you feel difficulty remembering? | 1 | 2 | 3 | 4 |
| 26 | Does your physical condition or treatment affect your family life? | 1 | 2 | 3 | 4 |
| 27 | Does your physical condition or treatment affect your family activities? | 1 | 2 | 3 | 4 |
| 28 | Does your physical condition or treatment get you into financial difficulties? | 1 | 2 | 3 | 4 |

For the following questions, select a number best for you between 1 and 7 and circle.

29 How do you evaluate your overall health in the past week?

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| very bad | | | | | | beyond compare |

30 How do you evaluate your total quality of your life over the past week?

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| very bad | | | | | | beyond compare |

The EORTCQLQ-C30 scale, which the European Organization for Research on Cancer Treatment (EORTC: The European Organization for Research and Treatment of Cancer), launched the cross-cultural and cross-national QOL-C30 (Quality of Life Questionnare-Core 30) in 1993, Evaluation QOL from a multidimensional perspective, Can better reflect the QOL connotation, Has been applied to QOL measurements in cancer patients in multiple European countries and regions

1. Calculation of the entry score

The EORTC QLQ- -C30 (V3.0) is a core scale for all cancer patients with a total of 30 entries. Among them, items 29 and 30 are divided into seven levels, ranging from 1 to 7 points according to their answer options: other items are divided into four levels: from none, one point, more to many, directly rated from 1 to 4 points.

| dimension | Number of indicators | content |
|---|---|---|
| Physical function | 5 | Physical activity: carrying objects, walking, staying in bed for days and basic self-care conditions |
| Role function | 2 | Whether daily activities, work and hobbies are restricted |
| emotional function | 4 | Tension, worry, emotional control ability |
| cognitive function | 2 | Whether it is difficult to remember things, and whether you can concentrate on doing things |
| social function | 2 | Family life and social activities are hindered |
| Overall health status / quality of life | 2 | Self-rated overall health status and overall quality of life |
| physical symptom | 12 | Fatigue, nausea and vomiting, pain, dysphagia, sleep, disturbance, and decreased appetite |
| Health-related economic situation | 1 1 | The impact of the disease and the treatment on the patient's economy |

2. Calculation of the domain (dimension) score (coarse score)

For the convenience of statistical analysis and application, the scales are often divided into certain fields (domain). The domain is an aspect of the quality of life component, also known as dimensionality (dimension), when analyzed as an independent variable.

EORTC QLQ- -The 30 items of C30 (V3.0) can be divided into 15 areas, with 5 functional domains (body, role, cognitive, emotional, and social function), 3 symptom domains (fatigue, pain, nausea and vomiting), 1 general health / quality of life area, and 6 single items (each as one area). The score of the field is obtained by adding up and dividing it by the number of items included in the field (coarse RS, Raw Score), namely RS= （Q1+Q2+Q？）／n。

QLQ-C30（V3.0）Scoring method for each fields (coarse RS）

| Field (dimension) | proper | Number of entries | Score full distance（R） | scoring method |
|---|---|---|---|---|
| somatic function | functional form | 5 | 3 | (Q1+Q2+Q3+Q4+Q5)/5 |
| Role function | functional form | 2 | 3 | (Q6+Q7)/2 |
| emotional function | functional form | 4 | 3 | (Q21+Q22+Q23+Q24)/4 |
| cognitive function | functional form | 2 | 3 | (Q20+Q25)/2 |
| social function | functional form | 2 | 3 | (Q26+Q27)/2 |
| Total health status | | 2 | 6 | (Q29+Q30)/2 |
| tired | Symptomatic type | 3 | 3 | (Q10+Q12+Q18)/3 |
| nausea and vomiting | Symptomatic type | 2 | 3 | (Q14+Q15)/2 |
| pain | Symptomatic type | 2 | 3 | (Q9+Q19)/2 |
| anhelation | Symptomatic type | 1 | 3 | Q8 |
| lose sleep | Symptomatic type | 1 | 3 | Q11 |
| Loss of appetite | Symptomatic type | 1 | 3 | Q13 |
| astriction | Symptomatic type | 1 | 3 | Q16 |
| diarrhea | Symptomatic type | 1 | 3 | Q17 |
| economic hardship | Symptomatic type | 1 | 3 | Q28 |

3. Calculation of standard and chemical scores

In order to make the scores of each field compare with each other, the extreme differential method is further used for linear transformation to convert the coarse score into a standardized score (standard score, SS) within 0 100. In addition, the purpose is to change the direction of the score. Because QLQ-C30 scale, except item 29,30 are reverse entry (the greater the value, the worse the quality of life), and in the scoring rules: the

functional and overall health field score higher that functional condition and life quality is better, the higher the score for symptom areas indicates that the more symptoms or problems (worse quality of life). Therefore, the calibration time of the computing function field has to change the direction. Specifically, the following formula is calculated respectively (where R is the full score distance of each field or item).

Functional area: SS= [l- (RS-1) / R] 100

Symptom areas and general health status areas: SS=[（RS-1）/ R]×100

**2）The EORTC-QLQ-CR29 scale**

We would like to know something about you and your health, please answer all the questions for yourself. There is no "right" or "wrong" here, but only to draw circles on the number that best reflects your situation. The information you provide will be kept strictly confidential.

| | **In the past wee** | **Not have** | **some** | **match** | **extraordinary** |
|---|---|---|---|---|---|
| 31 | Have you urinate frequently during the day in the past week? | 1 | 2 | 3 | 4 |
| 32 | Have you urinate frequently during the day in the past week? | 1 | 2 | 3 | 4 |
| 33 | Have you involuntarily urinate or leaked in the past week? | 1 | 2 | 3 | 4 |
| 34 | Have you had any pain in urinating in the past week? | 1 | 2 | 3 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| 35 | Have you had any abdominal pain in the past week? | 1 | 2 | 3 | 4 |
| 36 | Have you had pain in the hip, anal area or rectum in the past week? | 1 | 2 | 3 | 4 |
| 37 | Have you filled your abdomen in the past week? | 1 | 2 | 3 | 4 |
| 38 | Have you had blood stools in the past week? | 1 | 2 | 3 | 4 |
| 39 | Did you have mucus in your stool in the past week? | 1 | 2 | 3 | 4 |
| 40 | What have you done in the past week? | 1 | 2 | 3 | 4 |
| 41 | Have you treated your hair loss in the past week? | 1 | 2 | 3 | 4 |
| 42 | Have you had any problems with your taste patterns in the past week? | 1 | 2 | 3 | 4 |
| 43 | Have you been worried about your future health in the past week? | 1 | 2 | 3 | 4 |
| 44 | Have you been worried about your weight in the past week? | 1 | 2 | 3 | 4 |
| 45 | Have you experienced less illness or treatment in the past week? | 1 | 2 | 3 | 4 |
| 46 | Have you felt that illness or treatment has reduced your feminine / masculine taste in the past week? | 1 | 2 | 3 | 4 |
| 47 | Have you been dissatisfied with your body in the past week? | 1 | 2 | 3 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| 48 | **Do you have a stoma pocket（colostomy or ileostomy）？ (select Yes/No)** | **Yes** | **No** | | |
| | **Question 48 for patients with ”yes”, please answer the following questions：** | **Not have** | **some** | **match** | **extraordinary** |
| 49 | In the past week, have you had an involuntary pocket exhaust or flatulence? | 1 | 2 | 3 | 4 |
| 50 | Have you leaked in your pocket in the past week? | 1 | 2 | 3 | 4 |
| 51 | Have you had pain in the skin around the fistula in the past week? | 1 | 2 | 3 | 4 |
| 52 | Have you changed your pockets frequently during the day in the past week? | 1 | 2 | 3 | 4 |
| 53 | Have you changed your pocket frequently at night in the past week? | 1 | 2 | 3 | 4 |
| 54 | Have you been embarrassed in the past week? | 1 | 2 | 3 | 4 |
| 55 | Have you had difficulty nursing the fistula in the past week? | 1 | 2 | 3 | 4 |
| | **Question 48 for patients with ”No”, please answer the following questions：：** | **Not have** | **some** | **match** | **extraordinary** |
| 49 | In the past week, have you had an involuntary pocket exhaust or flatulence? | 1 | 2 | 3 | 4 |
| 50 | Have you leaked in your pocket in the past week? | 1 | 2 | 3 | 4 |

| | | | | | |
|---|---|---|---|---|---|
| 51 | Have you had pain in the skin around the fistula in the past week? | 1 | 2 | 3 | 4 |
| 52 | Have you changed your pockets frequently during the day in the past week? | 1 | 2 | 3 | 4 |
| 53 | Have you changed your pocket frequently at night in the past week? | 1 | 2 | 3 | 4 |
| 54 | Have you been embarrassed in the past week? | 1 | 2 | 3 | 4 |
| | **In the past 4 weeks, men should answer questions 56、57** | **Not have** | **some** | **match** | **extraordinary** |
| 56 | How interested have you been in sex in the last 4 weeks? | 1 | 2 | 3 | 4 |
| 57 | Have you had difficulty achieving or maintaining an erection in the last 4 weeks? | 1 | 2 | 3 | 4 |
| | **In the past 4 weeks, women should answer questions 58、59** | **Not have** | **some** | **match** | **extraordinary** |
| 58 | How interested have you been in sex in the last 4 weeks? | 1 | 2 | 3 | 4 |
| 59 | Have you had any pain or discomfort during sexual intercourse in the last 4 weeks? | 1 | 2 | 3 | 4 |

The Colorectal Cancer Quality of Life Questionnaire (QLQ-CR29), a module of the Quality of Life Questionnaire-Core 30 (QLQ-C30), developed by the European Organization for Research and Treatment of Cancer to assess quality of life for specific aspects of colorectal cancer in cancer patients.

## 6.4Attachment 5: Anal Incontinence Wexner Scale Scale

一，Scale introduction

1. Evaluation method: conducted by doctors or test-experienced personnel.
2. Scale function: The Wexner incontinence scale was prepared by Wexner, Jorge and other researchers to assess the severity of patient defecation.
3. Applicable population: patients with fecal incontinence caused by various reasons.

二，operating guide

1. Content of the scale: The scale mainly includes four aspects of stool morphology, gastrointestinal incontinence, wearing pads and lifestyle, lasting for more than 4 weeks. There are 5 items, and each score is calculated according to Table 1.
2. Results analysis: the total score is the sum of all scores, the score range is 0-20 points, 0 points = normal, 20 points = complete incontinence.

**Table 1 The Wexnei' scoring scale of anal incontinence**

| variable | never | once in a while | sometimes | often | always | score |
|---|---|---|---|---|---|---|
| exhaust | 0 | 1 | 2 | 3 | 4 | |
| Loose stools | 0 | 1 | 2 | 3 | 4 | |
| formed stool | 0 | 1 | 2 | 3 | 4 | |
| Sanitary belt | 0 | 1 | 2 | 3 | 4 | |
| Lifestyle change | 0 | 1 | 2 | 3 | 4 | |
| total points | （ ）scores | | | | | |

三，Results and interpretation

Total score：（）scores。

Explanation: Wexner score scale introduces the analysis index is the total score, the score range is 0-20 points, 0 points = normal, 20 points = complete incontinence.