

# Clinical research protocol

Project Name:	mFOLFOX6 Combined with Citrus Flavonoid Tablets (Aimailang) as Neoadjuvant Therapy for Locally Advanced Rectal Cancer with High YWHAB Expression: A Prospective, Multi-center, Open-Label Randomized Controlled Phase II Clinical Trial
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**The Sixth Affiliated Hospital of Sun Yat-sen University**

Signature confirmation of the plan:

## Protocol Summary

Research Title	mFOLFOX6 Combined with Citrus Flavonoid Tablets (Aimailang) as Neoadjuvant Therapy for Locally Advanced Rectal Cancer with High YWHAB Expression: A Prospective, Multi-center, OpenLabel, Randomized Controlled Phase II Clinical Trial
Study Number	-
Research Group Leader Unit	The Sixth Affiliated Hospital of Sun Yat-sen University
Research Methods	Prospective, multicenter, open-label, randomized controlled, phase II clinical trial
Study Subjects	Patients with locally advanced rectal cancer with high expression of YWHAB
Objective of the study	<p><b>Main study objectives:</b></p> <ul style="list-style-type: none"> <li>● To evaluate neoadjuvant therapy with mFOLFOX6 combined with citrus flavonoid tablets (Aimailang) in patients with YWHAB high-expression locally advanced rectal cancer, and to compare the proportion of tumor downstaging (ypTNM 0-I stage) in each group of patients To provide a basis for the benefits of neoadjuvant chemotherapy regimens (mFOLFOX6 combined with citrusone tablets) in patients with YWHAB hyperexpression locally advanced rectal cancer.</li> </ul> <p><b>Secondary study objectives:</b></p> <ul style="list-style-type: none"> <li>● Evaluation of effectiveness: To evaluate the proportion of pathological complete response, 3-year Disease-Free survival (DFS), and Overall survival (Overall survival) in patients with YWHab-high expression locally advanced rectal cancer treated with the neoadjuvant regimen of mFOLFOX6 combined with citrus flavonoid tablets (Aimailang) And postoperative Tumor Regression Grading (TRG), etc.</li> <li>● Safety assessment: To evaluate the rate of treatment-related adverse</li> </ul>

	<p>reactions (grade 3 or above) in patients with YWHAB high-expression locally advanced rectal cancer treated with neoadjuvant regimens of mFOLFOX6 combined with citrus flavonoids tablets (Aimailang).</p>
Study endpoints	<p><b>Primary endpoints:</b></p> <p>The rate of tumor downstaging (ypTNM stage 0-I) after neoadjuvant chemotherapy in each treatment group</p> <p><b>Secondary endpoints:</b></p> <ul style="list-style-type: none"> <li>● 3-year disease-free survival (DFS)</li> <li>● Overall survival time (OS)</li> <li>● Postoperative tumor regression grading TRG</li> <li>● Rate of treatment-related adverse reactions (grade 3 and above)</li> </ul>
Inclusion criteria	<ol style="list-style-type: none"> <li>(1) The histopathological diagnosis was rectal adenocarcinoma. All other histological types were excluded, and the colonoscopy report or clinical physical examination suggested the presence of hemorrhoids.</li> <li>(2) With radiologically measurable or clinically assessable rectal tumor foci, the clinicopathological stage of the tumor is T2N+ or T3-4aNany, M0; Clinical staging is based on physical examination by a surgeon, enhanced CT of the chest and abdomen/pelvis, and pelvic MRI examination. If MRI is contraindicated, pelvic enhanced CT combined with transrectal ultrasound is used for assessment (9th Edition AJCC TNM staging, Appendix 1);</li> <li>(3) Pelvic MRI shows that the tumor is not adjacent (defined as distance &lt; 2 mm) to the mesorectal fascia (MRF negative).</li> <li>(4) Immunohistochemical staining of tissue specimens suggests rectal cancer patients with high expression of YWHAB;</li> <li>(5) The age at the time of obtaining the informed consent was 18-75 years old;</li> </ol>

	<p>(6) The Eastern Cancer Cooperative Physical Condition Score (ECOG) was 0-1 (refer to Appendix 3);</p> <p>(7) No previous systemic anti-tumor treatment for rectal cancer, including cytotoxic drugs, immune checkpoint inhibitor therapy, molecular targeted therapy, endocrine therapy, etc.</p> <p>(8) Have appropriate organ function based on the following laboratory test values obtained during the screening period: White blood cell count <math>\geq 3 \times 10^9/L</math>, neutrophil count <math>\geq 1.5 \times 10^9/L</math>, platelet count <math>\geq 75 \times 10^9/L</math>, serum total bilirubin <math>\leq 1.5 \times</math> upper limit of normal (UNL), aspartate aminotransferase or alanine aminotransferase <math>\leq 2.5 \times</math> UNL, serum creatinine <math>\leq 1.5 \times</math> UNL;</p> <p>(9) Female subjects of childbearing age must have a negative result of a serum pregnancy test within 3 days prior to the initiation of the study drug and be willing to use a medically approved high-efficiency contraceptive measure (such as an intrauterine device, contraceptive pill or condom) during the study period and within 3 months after the last administration of the study drug;</p> <p>(10) For male subjects whose partners were women of childbearing age, effective contraceptive methods were used during the study period and within 3 months after the last study administration;</p> <p>(11) With the consent of the subject himself/herself and having signed the informed consent form, the subject himself/herself is willing and capable of complying with the planned visits, study treatment, laboratory tests and other trial procedures.</p>
Exclusion criteria	<p>(1) Whole-body CT, MR, or PET-CT (including at least chest, whole abdomen, and pelvic) confirmed distant metastasis;</p> <p>(2) Patients with rectal adenocarcinoma confirmed as having mismatch repair protein deficiency (dMMR) by immunohistochemical testing,</p>

	<p>or as having microsatellite highly unstable type (MSI-H) by molecular testing.</p> <p>(3) Patients with complete intestinal obstruction, active bleeding or perforation and requiring emergency surgery;</p> <p>(4) Previous or concurrent presence of other active malignant tumors (excluding malignant tumors that have received curative treatment for more than 5 years without recurrence or carcinoma in situ that can be cured with adequate treatment);</p> <p>(5) Thrombotic or embolic events occurred within 12 months prior to enrollment in the study, such as cerebrovascular accidents (including transient ischemic attacks), pulmonary embolism, and deep vein thrombosis.</p> <p>(6) Within 12 months prior to enrollment: myocardial infarction, severe/unstable angina pectoris, NYHA grade 2 or above heart failure, clinically significant supraventricular or ventricular arrhythmias, and symptomatic congestive heart failure;</p> <p>(7) Systemic use of antibiotics for <math>\geq 7</math> days within 4 weeks prior to enrollment, or unexplained fever <math>&gt;38.5^{\circ}\text{C}</math> during the screening period/before the first administration (as determined by the investigator, fever due to tumor causes can be enrolled);</p> <p>(8) Having undergone major surgery such as laparotomy, thoracotomy, laparoscopic resection of organs, or severe trauma within 2 months prior to enrollment (the surgical incision should have healed completely before enrollment in this clinical trial);</p> <p>(9) Known presence of human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS-related disease);</p> <p>(10) Present with interstitial lung disease, non-infectious pneumonia, or uncontrolled systemic diseases (such as: diabetes, hypertension, pulmonary fibrosis, and acute pneumonia, etc.);</p>
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	<p>(11) Untreated active hepatitis (hepatitis B, defined as HBV-DNA <math>\geq</math> 500 IU/mL; hepatitis C, defined as HCV-RNA above the detection limit of analytical methods) or co-infection with hepatitis B and hepatitis C;</p> <p>(12) A history of known or suspected allergy to any related drug used in the study;</p> <p>(13) The investigator determined that there were other conditions that would disqualify the study (as stated in the informed consent form).</p>
Investigational drug	Oxaliplatin, calcium folinate, 5-fluorouracil, citrus flavonoids tablets (Aimailang)
Study treatment regimens	<p>This study was a prospective, multicenter, open-label, randomized controlled phase II clinical trial that included patients with preoperatively diagnosed YWHAB high-expression locally advanced (cT2N+ or cT3-4aNany, M0, and MRF negative) rectal cancer. Neoadjuvant chemotherapy was administered using mFOLFOX6 combined with citrus flavonoid tablets (Aimailang).</p> <p>mFOLFOX6 regimen combined with citrus flavonol tablets (Aimai Lang) <math>\times</math> 4-6 cycles before surgery, with 14 days as one treatment cycle. Oxaliplatin 85 mg/m<sup>2</sup> intravenous infusion for 180 minutes on day 1; Folinic acid 400 mg/m<sup>2</sup> intravenous infusion 120 minutes, day 1; 5-fluorouracil 2400 mg/m<sup>2</sup>, continuous intravenous infusion for 46 hours; With or without citrus flavonoid tablets (Aimailang) 500mg orally twice daily on days 1-14 of each 14-day cycle. This trial allows for dose adjustment and discontinuation of study treatment in patients with disease progression during neoadjuvant therapy, with direct surgical treatment or treatment in accordance with local guidelines. If a patient is unable to tolerate the planned six cycles of neoadjuvant therapy, surgery can also be performed ahead of schedule. Any patient who received other regimens of anti-cancer treatment before surgery</p>

	<p>was discontinued from study treatment and managed in accordance with the corresponding guidelines. Postoperative treatment was carried out in accordance with the clinical routine diagnosis and treatment norms "Chinese Colorectal Cancer Diagnosis and Treatment Norms (2025 Edition)" and the colorectal cancer diagnosis and treatment guidelines (2025 Edition) formulated by the Chinese Society of Clinical Oncology (CSCO). It should be noted that all enrolled control group patients were not allowed to take citrus flavonoids tablets (Aimailang) without authorization during the trial period. If oral administration of the drug was necessary for any reason, communication with the attending physician should be conducted and the attending physician should decide whether to use other drugs instead or terminate the trial for the patient.</p>
Determination of the sample size	<p>The primary endpoint of this study was the rate of tumor downstaging (ypTNM stage 0-I) in each treatment group. Based on our center 's previous data, the proportion of patients with locally advanced rectal cancer who received neoadjuvant chemotherapy with mFOLFOX6 downstaging (ypTNM 0-I) was about 35%, and the proportion of patients with YWHAB high expression rectal cancer (approximately one-third, n=86) who received neoadjuvant chemotherapy with mFOLFOX6 was about 25%. In patients with low YWHAB expression (about two-thirds, n=86), the tumor downstaging rate after receiving mFOLFOX6 neoadjuvant chemotherapy was approximately 43%. A previous single-center, prospective, open-label, small cohort exploration by our team found that the tumor downstaging rate in YWHab-high expression rectal cancer patients receiving mFOLFOX6 neoadjuvant chemotherapy was approximately 25.0% (a total of 20 cases, including 5 patients at ypTNM stage 0-I); The tumor downstaging rate in patients with YWHAB hyperexpression rectal cancer who received mFOLFOX6 combined with Aimailang neoadjuvant therapy was</p>

	<p>approximately 42.9% (28 cases, including 12 patients at ypTNM 0-I stage). The estimated tumor downgrading rate for YWHAB hyperexpression patients receiving neoadjuvant chemotherapy with mFOLFOX6 combined with citruxone tablets (Aimailang) was 40%, with a significance level of 0.05 for the unilateral test, a test efficacy of 0.8, a loss to follow-up rate of 10%, and an estimated sample size of 236 using PASS V15 software.</p>
The criteria for terminating the research treatment	<p>Subjects may discontinue treatment at any time for any reason, or the investigator may decide whether to discontinue treatment in the event of any adverse event.</p> <p>The termination of the study treatment does not represent withdrawal from the study. Since data on certain clinical events after treatment discontinuation may be very important to the study, this information must be collected until the subject's last planned visit, even if the subject has discontinued treatment.</p> <p>Treatment must be terminated if any of the following situations occur, but monitoring may continue in the study:</p> <ul style="list-style-type: none"> <li>● The subject requests to terminate the study drug treatment;</li> <li>● The researchers judged that it was in the best interest of the subjects to terminate the study treatment;</li> <li>● For patients who have received neoadjuvant therapy and whose imaging assessment suggests local disease progression or evidence of distant metastasis, it is recommended that they receive the standard treatment recommended by the current clinical guidelines after a multidisciplinary consultation (oncology, radiotherapy, radiology, colorectal surgery, hepatobiliary surgery, interventional surgery, ultrasound, etc.).</li> <li>● Pregnancy events occurred in the subjects during the study;</li> <li>● Any clinical adverse events, abnormal laboratory tests, or other medical conditions that could cause the subjects to no longer benefit from continuing the study treatment;</li> <li>● Overall deterioration of health status, unable to continue</li> </ul>

	<p>participating in the trial;</p> <ul style="list-style-type: none"> <li>● Significant program deviations such as disqualification and non-compliance of the subjects are found after enrollment;</li> <li>● Lost to follow-up;</li> <li>● Subject death;</li> <li>● Other reasons that the researchers believed could not continue to study the treatment.</li> </ul>
Criteria for withdrawing from the study	<p>(1) Subjects may withdraw from the trial at any time on their own initiative, or be requested to withdraw from the trial by the subject or the sponsor for safety or behavioral reasons or for failure to comply with the study visit time or procedure required by the protocol. Withdrawal criteria include:</p> <p>(2) The subjects withdrew their informed consent to participate in the study, were unwilling to return to the original research center for all research evaluations (including refusing surgical treatment after neoadjuvant therapy), and no longer agreed to any further contact or information collection with themselves or relevant personnel previously authorized by the subjects.</p> <p>(3) Lost to follow-up;</p> <p>(4) Subject death;</p> <p>(5) The sponsor terminated the study</p>
Evaluation of effectiveness	<p>(1) Tumor downstaging rate (ypTNM stage 0-I): Proportion of patients with locally advanced rectal cancer whose postoperative surgical specimens were pathologically staged at ypTNM stage 0-I after neoadjuvant chemotherapy with mFOLFOX6 or mFOLFOX6 combined with citrus flavonoids tablets (Aimailang).</p> <p>(2) Degree of pathological response: ① Complete response (CR) : All tumor target lesions disappear, no new lesions appear, and tumor markers remain normal for at least 4 weeks. ② Partial response (PR), a reduction</p>

	<p>of <math>\geq 30\%</math> in the sum of maximum diameters of tumor target lesions, maintained for at least 4 weeks. ③ Stable disease (SD), where the sum of the maximum diameters of tumor target lesions is reduced but does not reach PR, or increased but does not reach PD. ④ Progressive disease (PD), where the sum of the maximum diameters of tumor target lesions increases by at least 20%, or new lesions appear.</p> <p>(3) 3-year disease-free survival rate (DFS) : The proportion of patients who did not experience any of the following events from the time of inclusion in the study until the end of year 3: disease progression, surgical gross/microscopic tumor residue, local recurrence, distant metastasis, or death for any cause, whichever occurs first.</p> <p>(4) Overall survival time (OS) : The time from the start of inclusion in the study to death from any cause.</p> <p>(5) Postoperative tumor regression grade (TRG) : TRG0 refers to no residual tumor cells, TRG1 refers to a single cell or a small group of cells; TRG2 refers to residual cancer with a pro-connective tissue proliferative response; TRG3 refers to the minimal evidence of a tumor response.</p>
Safety evaluation	Treatment-related adverse reaction rate (grade 3 or above) : Adverse drug reactions of grade 3 or above determined to be related to drug treatment in anti-tumor drug/treatment clinical trials, excluding non-specific infusion reactions.
Planned trial date	March 2026 - March 2031

## 1. Research background

### (1) Research status at home and abroad

colorectal cancer (CRC) is the third most common malignant tumor worldwide. There are approximately over 1.8 million new cases each year, accounting for about 10% of all new malignant tumors, and the number of related deaths exceeds 910,000<sup>[1]</sup>. In recent years, the incidence and mortality rates of CRC in China have been increasing year by year. In 2020, the

incidence rate of CRC in China jumped to the second place among malignant tumors, with 550,000 new cases each year, and the mortality rate ranked fifth, with 280,000<sup>[2]</sup> new deaths in 2020. At present, the disease burden of CRC in China continues to increase, and the prevention and treatment of CRC still have a long way to go. The rectum terminates at the upper edge of the functional anal canal, defined as the palpable upper edge of the anal sphincter and the puborectalis muscle of the anorectal ring. Rectal cancer is a cancerous lesion that occurs in the rectum and is located below the virtual line from the sacral promontory to the upper margin of the union as determined by MRI. Determining the best treatment plan for patients with rectal cancer is a complex process. In addition to determining the purpose of rectal cancer surgery (i.e. curative or palliative), the possible functional outcomes after treatment must also be considered, including maintaining or restoring normal intestinal function, anal restraint, and preserving urogenital function. Especially for patients with low rectal cancer, achieving the goal of tumor cure and minimal impact on quality of life simultaneously is very challenging<sup>[3]</sup>. In addition, patients with rectal cancer have a higher risk of pelvic recurrence compared to colon cancer, and locally recurrent rectal cancer is associated<sup>[4-6]</sup> with a poor prognosis.

Currently, multimodal treatment for stage II or III rectal cancer typically includes preoperative fluoropyrimidine-based chemoradiotherapy, total mesorectal excision (TME), and postoperative adjuvant chemotherapy<sup>[7-8]</sup>. The FOWARC study conducted by our center confirmed that patients receiving mFOLFOX6 neoadjuvant chemotherapy had no significant differences in tumor downstaging rate, disease-free survival rate (DFS), local recurrence rate, and overall survival rate compared with standard preoperative chemoradiotherapy, but the incidence of postoperative anastomotic fistula was significantly reduced in the mFOLFOX6 neoadjuvant chemotherapy alone group. Anal function is better, which can effectively improve the long-term quality of life of patients. This indicates that neoadjuvant chemotherapy with mFOLFOX6 can partially and selectively replace preoperative chemoradiotherapy, effectively avoiding radiotherapy-related side effects without affecting the therapeutic effect, and the relevant research results have been cited<sup>[9-11]</sup> in the NCCN international guidelines. The study showed that tumor downstaging (ypTNM stage 0-I) was significantly associated <sup>[9-11]</sup>with better DFS in patients with locally advanced rectal cancer who received neoadjuvant therapy.

FOWARC results showed that the rate of tumor downstaging in patients with locally advanced

rectal cancer who received neoadjuvant chemotherapy with mFOLFOX6 was approximately 35%; This suggests that some patients are insensitive to mFOLFOX6 neoadjuvant chemotherapy. Identifying and screening out this group of patients who are resistant to mFOLFOX6 treatment is expected to further increase the tumor downgrading rate and improve prognosis in these patients through mFOLFOX6 chemotherapy combined with related drug targeted therapy.

Dysregulation of the Wnt/ $\beta$ -catenin signaling pathway is closely associated with the occurrence and development of various cancers, especially playing a significant role<sup>[12]</sup> in the tumorigenesis and progression of CRC. YWHA (14-3-3) family proteins are widely expressed in eukaryotic cells, Including seven subtypes beta (YWHAB) 14-3-3, 14-3-3 gamma (YWHAG), 14-3-3 epsilon (YWHAH), the 14-3-3 zeta (YWHAZ), 3 tau sigma (YWHAS) and 14-14-3-3 / theta (YWHAQ)<sup>[13]</sup>. These 14-3-3 isomers have highly conserved structures across different species and can form homodimers and heterodimers, acting as molecular linkers through protein-protein interactions<sup>[14]</sup>. As hubs of various signaling pathways, 14-3-3 proteins are involved in many cellular processes, including cell cycle regulation, protein transport, apoptosis, and neuronal plasticity<sup>[13-15]</sup>. More importantly, dysregulation of 14-3-3 proteins is involved in a variety of diseases, such as cancer, autoimmune diseases and metabolic disorders<sup>[13, 16]</sup>. Our previous basic research found that YWHAB (14-3-3 $\beta$ ) amplification is a key oncogenic somatic Copy Number Variations (CNV) driver gene and is associated with poor prognosis in patients with CRC, suggesting its potential role as a prognostic biomarker. In addition, experimental evidence from cell studies and mouse models suggests that YWHAB promotes chemotherapy resistance in CRC by enhancing glycolysis and nucleotide metabolism. Mechanistically, YWHAB interacts with  $\beta$ -TrCP, competes with  $\beta$ -catenin to bind to  $\beta$ -TrCP, inhibits  $\beta$  -TrCP-mediated  $\beta$ -catenin degradation<sup>[16]</sup>, thereby promoting the occurrence and development of CRC and chemotherapy resistance. In addition, through large-scale molecular docking and drug binding capacity screening, we found that FDA-approved drugs Hesperidin and Diosmin can specifically block the interaction between YWHAB and  $\beta$ -TrCP. It can significantly increase the sensitivity of YWHab-highly expressed CRC organoids and PDX to FOLFOX treatment. Therefore, our study deeply elucidates the mechanism of chemotherapy resistance in YWHab-highly expressed CRC, and YWHAB expression levels can serve as a

potential biomarker for predicting chemotherapy sensitivity in CRC patients and achieving precise chemotherapy. Blocking the YWHAB/ $\beta$ -TrCP/ $\beta$ -catenin signaling axis is expected to be an important means to increase chemosensitivity in patients with YWHab-high expression CRC.

Flavonoids are a large class of polyphenols<sup>[17]</sup> that are naturally present in plants. Previous studies have reported that flavonoids have multiple therapeutic effects, such as anti-inflammatory, antioxidant, and anti-hypercholesterolemia activities<sup>[18]</sup>. Hesperidin and diosamine are flavonoids commonly found in citrus fruits. Both have highly similar molecular structures and are known for their antioxidant properties, which help protect cells from free radical damage. Studies have shown that hesperidin and diosamine can support heart health<sup>[18-19]</sup> by enhancing vascular function and reducing inflammation, and have been proven beneficial for the brain, liver, eyes, etc. Hesperidin and diosamine have been reported to have anti-inflammatory, antioxidant, anti-tumor and antibacterial therapeutic potential<sup>[20-22]</sup>. Clinical trials and clinical practice have confirmed the therapeutic effects of hesperidin and diosamine in a variety of diseases, including cardiovascular diseases, neurological disorders, type 2 diabetes, venous ulcers, hemorrhoids, fatty liver disease associated with metabolic dysfunction, and cancer<sup>[23]</sup>. Previous studies<sup>[23]</sup> have shown that hesperidin can inhibit the protein expression level of nitric oxide synthase (iNOS), thereby reducing the production of nitrogen dioxide and prostaglandin E2(PGE2); Hesperidin is thus considered an inhibitor of cyclooxygenase-2 and iNOS, which may explain its anti-tumor and anti-inflammatory properties. A 2022 study identified the chemoprophylactic potential of hesperidin in 1, 2-dimethylhydrazine (DMH)-induced CRC, which prevents CRC<sup>[24]</sup> in vivo by modulating the Smad4 and activin A signaling pathways. Another study reported that hesperidin could effectively prevent the incidence<sup>[25]</sup> of azoxymethane-induced intestinal tumors in mice. In our previous study, we found through Microscale thermophoresis (MST) experiments that hesperidin has a strong binding affinity with the YWHAB protein, and both in vivo and in vitro experiments indicated that hesperidin and diosamine could effectively inhibit the interaction between YWHAB and  $\beta$ -TrCP; Animal experiments further suggested that compared with the FOLFOX regimen alone, the combination of citrus flavonoid tablets (Aimailang, each tablet containing 500mg of citrus flavonoid, 90% of which is diosamine and 10% is hesperidin) could further enhance the

inhibitory effect of FOLFOX on PDX tumors. The results of the in vitro and in vivo experiments fully confirm that FOLFOX combined with citrus flavonoid tablets (Aimalang) could be a potential treatment option for patients with YWHAB high-expression CRC, but this needs to be confirmed through further prospective clinical trials.

Although the effects of citrus flavonoids in treating multiple diseases have been confirmed in numerous clinical studies and clinical practices, most clinical trials use 300-600 mg of pure hesperidin as an oral supplement, but hesperidin has a low bioavailability due to its very low water solubility<sup>[21, 26, 27]</sup>. After a comprehensive consideration, we plan to conduct clinical trials using citrus flavonoid tablets (Aimai Lang) as a citrus flavonoid alternative. In clinical practice, citrus flavonoid tablets (Aimailang) are typically used to treat various symptoms related to venous and lymphatic insufficiency as well as acute attacks of hemorrhoids. In our previous basic research, diosamine, hesperidin, and citrus flavonoid tablets (Aimalang) were all effective in inhibiting the interaction between YWHAB and  $\beta$ -TrCP, thereby enhancing the tumor-suppressing effect of FOLFOX. And Aimai has been used in clinical practice, and its safety has been confirmed. Therefore, it is safe and reasonable to explore the neoadjuvant therapeutic effect of FOLFOX in combination with Aimailang as an alternative to hesperidin, both in terms of our previous basic research and safety.

A common strategy for improving tumor regression in patients with locally advanced rectal cancer is to enhance neoadjuvant therapy by adding systemic chemotherapy before chemoradiotherapy. Results from several Phase II clinical trials suggest<sup>[28-31]</sup> that administering some systemic adjuvant chemotherapy before chemoradiotherapy can enhance the anti-tumor response in patients with large primary tumors close to the mesorectal fascia. In these trials, the proportion of patients with pathological complete response was slightly improved when systemic adjuvant chemotherapy was administered before chemoradiotherapy. A 2004 German clinical trial, named CAO/ARO/AIO-94, established chemoradiotherapy with preoperative infusion of fluorouracil, total mesorectal excision, and postoperative fluorouracil chemotherapy as the standard combination therapy<sup>[32]</sup> for locally advanced rectal cancer. In 2015, the CAO/ARO/AIO-04 trial<sup>[33]</sup> added oxaliplatin to neoadjuvant chemoradiotherapy and adjuvant chemotherapy based on fluorouracil, significantly improving disease-free survival in patients with rectal cancer at clinical stage cT3-4 or cN1-2. The regimens established by

CAO/ARO/AIO-04 were also seen as a new treatment option for patients with locally advanced rectal cancer. The center has conducted a series of prospective studies in the field of adjuvant/neoadjuvant chemotherapy for locally advanced rectal cancer, such as the FOWARC study, and the related research results have been cited <sup>[9-11]</sup>multiple times in the NCCN guidelines. The mFOLFOX6 dual-drug regimen (5-fluorouracil/calcium folinate combined with oxaliplatin) is a high-intensity chemotherapy regimen and is one of the first-line chemotherapy regimens recommended by guidelines such as NCCN, ESMO, and CSCO for advanced colorectal cancer. But it remains unknown whether the mFOLFOX6 dual-drug regimen combined with Aimailang can improve the therapeutic effect to some extent in patients with YWHAB high-expression locally advanced rectal cancer, which needs to be confirmed through further clinical studies.

## **(2) The significance of the study**

This study intends to conduct a prospective, multicenter, open-label, randomized controlled phase II clinical trial: For patients with locally advanced (cT2N+ or cT3-4aNany, M0, and MRF negative) rectal cancer, immunohistochemical staining of YWHAB was performed on colonoscopy pathological biopsy specimens to screen out patients with locally advanced rectal cancer with high expression of YWHAB. Randomly assigned to the mFOLFOX6 treatment group or the mFOLFOX6 combined with citrus flavonoid tablets (Aimailang) treatment group for neoadjuvant therapy; After completing 4-6 cycles of neoadjuvant chemotherapy, the subjects underwent corresponding preoperative assessment by the attending physician and tumor resection by a professional colorectal surgery team. Postoperative treatment was carried out in accordance with the clinical routine diagnosis and treatment guidelines for colorectal cancer in China (2025 Edition) and the diagnosis and treatment guidelines for colorectal cancer formulated by the Chinese Society of Clinical Oncology (CSCO) (2025 Edition). By evaluating the efficacy of neoadjuvant treatment with the mFOLFOX6 combined with citrus flavonoid tablets (Aimailang) regimen in patients with locally advanced rectal cancer with high expression of YWHAB (proportion of patients achieving tumor downstaging in each treatment group, Proportion of patients with pathological complete response, three-year disease-free survival rate, overall survival time and tumor

regression grade TRG, etc.) and safety (drug-related adverse reactions, etc.) To evaluate the efficacy, safety and feasibility of neoadjuvant therapy with mFOLFOX6 combined with citrus flavonoid tablets (Aimailang) in patients with locally advanced (cT2N+ or cT3-4aNany, M0, and MRF negative) rectal cancer with high expression of YWHAB. This study, based on the team's previous research, animal experiments, clinical safety of citrus flavonoid tablets (Aimailang), and a small sample prospective exploration trial of mFOLFOX6 combined with citrus flavonoid tablets (Aimailang), is expected to improve the efficacy of patients with locally advanced rectal cancer with high expression of YWHAB and benefit more patients.

## **2 Study Design and Objectives**

### **2.1 Overall design of the study**

This study is a prospective, multicenter, open-access, randomized controlled phase II clinical trial of neoadjuvant treatment with mFOLFOX6 combined with citrus flavonoids tablets (Aimairang) for YWHab-high expression locally advanced (cT2N+ or cT3-4aNany, M0, and MRF negative) rectal cancer, and the study design meets ethical requirements.

### **2.2 Study Objectives**

#### **Main research objectives:**

To evaluate the proportion of patients achieving tumor downstaging (ypTNM 0-I) in patients with YWHAB high-expression locally advanced (cT2N+ or cT3-4aNany, M0, and MRF negative) rectal cancer treated neoadjuvant therapy with mFOLFOX6 combined with citrus flavonoids tablets (Aimailang), To provide evidence for the neoadjuvant effect of the citrus flavonoid tablets (Aimailang) enhanced mFOLFOX6 regimen in patients with YWHab-high expression locally advanced rectal cancer.

#### **Secondary study objectives:**

Evaluation of effectiveness: Evaluation of neoadjuvant treatment with mFOLFOX6 combined with citrus flavonoid tablets (Aimailang) regimen for YWHAB high expression locally advanced (cT2N+ or cT3-4aNany, M0) The proportion of pathological complete response, 3-year disease-free survival (DFS), overall survival time (OS), and tumor

regression grade (TRG) in patients with rectal cancer who were MRF-negative.

Safety assessment: To evaluate the treatment-related adverse reaction rate (grade 3 or above) of the neoadjuvant treatment regimen of mFOLFOX6 combined with citrus flavonoid tablets (Aimailang) in patients with locally advanced rectal cancer with high expression of YWHAB.

## 2.3 Study endpoints

### Primary endpoints and definitions:

This study primarily evaluated the proportion of patients achieving tumor downstaging (ypTNM 0-I stage) in patients with YWHAB high-expression locally advanced (cT2N+ or cT3-4aNany, M0, and MRF negative) rectal cancer treated with the neoadjuvant regimen of mFOLFOX6 combined with citrus flavonoids tablets (Aimailang). Tumor downstaging rate (ypTNM 0-I): The proportion of patients with locally advanced rectal cancer whose postoperative surgical specimens were pathologically staged at ypTNM 0-I after receiving neoadjuvant chemotherapy with mFOLFOX6 or mFOLFOX6 + citrus flavonoids (Aimailang).

### Secondary endpoints and definitions:

- (1) Pathological response is defined as follows: ① Complete response (CR) : all tumor target lesions disappear, no new lesions emerge, and tumor markers remain normal for at least 4 weeks. ② Partial response (PR), where the sum of the maximum diameters of tumor target lesions is reduced by  $\geq 30\%$  and maintained for at least 4 weeks. ③ Stable lesion (SD), where the sum of the maximum diameters of tumor target lesions is reduced to less than PR or increased to less than PD. ④ Disease progression (PD), where the sum of the maximum diameters of tumor target lesions increases by at least 20%, or new lesions appear.
- (2) 3-year disease-free survival (DFS) is the proportion of patients who did not experience any of the following events from the start of randomization to the end of year 3: disease progression, surgical gross/microscopic tumor residue, local recurrence, distant metastasis, or death for any cause, whichever occurs first.
- (3) Overall survival time (OS) is the time from randomization of a patient to death from

any cause.

- (4) Tumor regression grading TRG: TRG 0 means no residual tumor cells, TRG 1 means a single cell or a small group of cells; TRG 2 refers to residual cancer with a connective tissue proliferative response; TRG 3 refers to the minimal evidence of a tumor response.
- (5) Safety indicator, treatment-related adverse reaction rate (grade 3 or above) : In anti-tumor drug/treatment clinical trials, the drug adverse reaction determined to be related to the drug treatment is grade 3 or above, excluding non-specific infusion reactions.

### **3 Selection and withdrawal of subjects**

#### **3.1 Inclusion criteria:**

- (1) Histopathological diagnosis of rectal adenocarcinoma, exclusion of all other histological types, and colonoscopy report or clinical physical examination suggesting the presence of hemorrhoids;
- (2) With radiologically measurable or clinically assessable rectal tumor foci, the clinicopathological stage of the tumor is T2N+ or T3-4aNany, M0; Clinical staging is based on physical examination by a surgeon, enhanced CT of the chest and abdomen/pelvis, and pelvic MRI examination. If MRI is contraindicated, pelvic enhanced CT combined with transrectal ultrasound is used for assessment (9th Edition AJCC TNM staging, Appendix 1);
- (3) Pelvic MRI shows that the tumor is not adjacent (defined as distance < 2 mm) to the mesorectal fascia (MRF negative).
- (4) Immunohistochemical staining of tissue specimens suggests rectal cancer patients with high expression of YWHAB;
- (5) The age at the time of obtaining the informed consent form was 18 to 75 years old.
- (6) The Eastern Cancer Cooperative Physical Condition Score (ECOG) was 0-1 (refer to Appendix 3);
- (7) No previous systemic anti-tumor treatment for rectal cancer, including cytotoxic drugs, immune checkpoint inhibitor therapy, molecular targeted therapy, endocrine therapy, etc.

- (8) Have appropriate organ function based on the following laboratory test values obtained during the screening period: White blood cell count  $\geq 3 \times 10^9/L$ , neutrophil count  $\geq 1.5 \times 10^9/L$ , platelet count  $\geq 75 \times 10^9/L$ , serum total bilirubin  $\leq 1.5 \times$  upper limit of normal (UNL), aspartate aminotransferase or alanine aminotransferase  $\leq 2.5 \times$  UNL, serum creatinine  $\leq 1.5 \times$  UNL;
- (9) Female subjects of childbearing age must have a negative result of a serum pregnancy test within 3 days prior to the initiation of the study drug and be willing to use a medically approved high-efficiency contraceptive measure (such as an intrauterine device, contraceptive pill or condom) during the study period and within 3 months after the last administration of the study drug;
- (10) For male subjects whose partners were women of childbearing age, effective contraceptive methods were used during the study period and within 3 months after the last study administration;
- (11) With the consent of the subject himself/herself and having signed the informed consent form, the subject himself/herself is willing and capable of complying with the planned visits, study treatment, laboratory tests and other trial procedures.

### **3.2 Exclusion criteria:**

- (1) Whole-body CT, MR, or PET-CT(including at least chest, whole abdomen, and pelvic) confirmed distant metastasis;
- (2) Patients with rectal adenocarcinoma confirmed as having mismatch repair protein deficiency (dMMR) by immunohistochemical testing, or as having microsatellite highly unstable type (MSI-H) by molecular testing.
- (3) Patients with complete intestinal obstruction, active bleeding or perforation and requiring emergency surgery;
- (4) Previous or concurrent presence of other active malignant tumors (excluding malignant tumors that have received curative treatment for more than 5 years without recurrence or carcinoma in situ that can be cured with adequate treatment);
- (5) Thrombotic or embolic events such as cerebrovascular accident (including transient ischemic attack), pulmonary embolism, deep vein thrombosis occurred within 12 months

prior to enrollment in the study;

- (6) Within 12 months prior to enrollment: myocardial infarction, severe/unstable angina pectoris, NYHA grade 2 or above heart failure, clinically significant supraventricular or ventricular arrhythmias, and symptomatic congestive heart failure;
- (7) Systemic use of antibiotics for  $\geq 7$  days within 4 weeks prior to enrollment, or unexplained fever  $>38.5^{\circ}\text{C}$  during the screening period/before the first administration (as determined by the investigator, fever due to tumor causes can be enrolled);
- (8) Having undergone major surgery such as laparotomy, thoracotomy, laparoscopic resection of organs, or severe trauma within 2 months prior to enrollment (the surgical incision should have healed completely before enrollment in this clinical trial);
- (9) It is known that there is human immunodeficiency virus (HIV) infection or AIDS-related diseases.
- (10) Present with interstitial lung disease, non-infectious pneumonia, or uncontrolled systemic diseases (such as: diabetes, hypertension, pulmonary fibrosis, and acute pneumonia, etc.);
- (11) Untreated active hepatitis (hepatitis B, defined as HBV-DNA  $\geq 500$  IU/mL; hepatitis C, defined as HCV-RNA above the detection limit of analytical methods) or co-infection with hepatitis B and hepatitis C;
- (12) A history of known or suspected allergy to any related drug used in the study;
- (13) The investigator determined that there were other conditions that would disqualify the study (as stated in the informed consent form).

### **3.3 Exit criteria:**

A subject may withdraw from the trial at any time on their own initiative, or may be requested to withdraw from the trial by the subject or the sponsor for safety or behavioral reasons or for failure to comply with the study visit time or procedure required by the protocol.

Withdrawal criteria include:

- (1) Subjects withdraw their informed consent to participate in the study and are unwilling to return to the original research center for all study evaluations (including refusal to undergo surgery after neoadjuvant);
- (2) For patients who, after neoadjuvant therapy, were assessed by imaging as having local

disease progression or evidence of distant metastasis, a multidisciplinary consultation (oncology, radiation oncology, radiology, colorectal surgery, hepatobiliary surgery, interventional surgery, ultrasound, etc.) was recommended to receive the standard treatment recommended by the current clinical guidelines.

- (3) Clinical adverse events, abnormal laboratory tests, or complications occurred, and the investigator considered that continued participation in the study was not in the best interest of the subjects;
- (4) Overall deterioration of health status, unable to continue participating in the trial;
- (5) Pregnancy events occurred in the subjects during the study;
- (6) Significant protocol deviations such as disqualification and non-compliance were found in the subjects after enrollment;
- (7) Lost to follow-up;
- (8) Subject death;
- (9) Other circumstances where researchers consider it necessary to withdraw from the study, such as significant protocol violations, etc.

## **4. Research and treatment**

**4.1 Study treatment grouping** (Introduce grouping methods. If random grouping is used, describe the method that generates the random sequence allocation: elaborate on the randomization method used, and if stratification is used, elaborate on the stratification factors and the allocation of cases; Hidden random allocation: Describe the methods used to perform random allocation, such as central randomization, sealed, opaque envelope method, etc. Blinding and unblinding: Who blinds after pre-measures such as subjects, healthcare providers, outcome assessors, data analysts, how blinding is carried out, under what circumstances unblinding can be done, and procedures for emergency unblinding during the study process)

This study used block randomization, with independent statisticians generating random sequences using SAS software, with a block length of 4, to ensure randomness and objectivity in the grouping process. The randomly assigned sequences were completed before the start of the trial and kept confidential and safeguarded by a third party not involved in the clinical

implementation. Subjects were grouped using a random number table method in this study. Random sequences were generated by independent statisticians and kept by independent third parties unrelated to the study's implementation. Grouping information was enclosed in sequentially numbered opaque sealed envelopes, which were opened by independent personnel in the order of enrollment to ensure the independence and unpredictability of the grouping process. Researchers have access to the grouping information of subjects only after they are enrolled. Although this study was an open-access design, researchers and subjects were informed of the grouping during the intervention implementation, strict allocation concealment was implemented before grouping to minimize the risk of selective bias. This study was a prospective, open-label, double-arm, phase II clinical trial without blinding.

This study was a prospective, multicenter, open-access, randomized controlled, phase II clinical trial with the mFOLFOX6 regimen neoadjuvant therapy group and the mFOLFOX6 regimen combined with citrus flavonoids tablets (Aimailang) neoadjuvant therapy group. This study plans to collect endoscopic pathological biopsy specimens from patients with locally advanced rectal cancer (cT2N+ or cT3-4aNany, M0, and MRF negative) for YWHAB immunohistochemical detection to screen out patients with locally advanced rectal cancer with high expression of YWHAB. The patients were randomly assigned to either the mFOLFOX6 treatment group or the mFOLFOX6 combined with citrus flavonoid tablets (Aimailang) treatment group for neoadjuvant therapy. The mFOLFOX6 regimen was 4-6 cycles preoperatively, with one treatment cycle every 14 days: intravenous infusion of oxaliplatin 85 mg/m<sup>2</sup> for 180 minutes on day 1; Folinic acid 400 mg/m<sup>2</sup> intravenous infusion for 120 minutes, day 1; 5-fluorouracil 2400 mg/m<sup>2</sup>, continuous intravenous infusion for 46 hours. Citrus Flavonoids Tablets (Aimailang) treatment regimen: Citrus flavonoids tablets (Aimailang) 500mg orally twice daily, administered on days 1-14 of each 14-day cycle.

This trial allows for dose adjustment and discontinuation of study treatment in patients with disease progression during neoadjuvant therapy, with direct surgical treatment or treatment in accordance with local guidelines. Early surgery can also be performed if the patient is unable to tolerate the planned six cycles of neoadjuvant therapy. Any patient who received other anti-cancer regimens before surgery was discontinued from study treatment and managed in accordance with local guidelines. Postoperative treatment in both groups was determined by the

investigators, including continued treatment with the mFOLFOX6 regimen combined with citrus flavonoids tablets (Aimai Lang). It should be noted that all enrolled patients in the control group were not allowed to take citrus flavonoids (Aimailang) without authorization during the trial period. If oral administration of the drug was required for any reason, they should communicate with their attending physician and the physician should decide whether to use other drugs instead or terminate the trial for the patient.

## 4.2 Dosage adjustment of the study drug

### 4.2.1 Treatment protocol discontinuation or dose adjustment procedures

During the study treatment of the subjects, adverse events will be continuously monitored and the subjects will be instructed to inform the attending physician of any and all adverse events in a timely manner. In the event of a less severe toxic reaction, the investigator may adjust the dose or postpone the administration if, after consultation with the medical monitor or sponsor, it is believed that dose adjustment or postponement is beneficial to the safety of the subject. In the event of any interruption in administration, the time to resume administration may be delayed by up to 14 days to allow the subject to recover from the toxicity. If it is still not possible to resume to the standard required by the study after 14 days, discontinue the trial.

### 4.2.2 Meet the criteria for initiating the next treatment cycle

The study drug may be administered only if the test results on the first day before administration of the next treatment cycle confirm that all the criteria listed in the table below are met.

Table 1.1 Meets the criteria for initiating the next treatment cycle	
Hemoglobin values	$\geq 90\text{g/L}$
Neutrophil count	$\geq 1.5 \times 10^9/\text{L}$
Platelet count	$\geq 75 \times 10^9/\text{L}$
Serum total	$\leq 1.5 \times \text{UNL}$

bilirubin	
Aspartate transferase	$\leq 3 \times \text{UNL}$
Alanine transferase	$\leq 3 \times \text{UNL}$
Serum creatinine	$\leq 1.5 \times \text{UNL}$
diarrhea	An increase of less than 4 times per day compared to baseline
Oral mucositis	Only mucosal erythema, no patchy ulcers or pseudomembrane formation
Sensory nerve disorder	Abnormal or dull sensations that do not affect daily activities
<p><i>Note: When an adverse reaction other than those mentioned above is found that causes a delay in the start of the next treatment cycle, the investigator will determine whether the next treatment cycle can be initiated when the adverse reaction is alleviated or recovered.</i></p>	

#### 4.2.3 Study the drug dosage levels

If a dose reduction is medically required, the dose levels of oxaliplatin and 5-fluorouracil may be adjusted according to the criteria listed in Table 1.2. Each adjustment can only reduce the dose level by one, and the total number of dose level adjustments throughout the study period may not exceed two. The fixed dose of folinate is 400 mg/m<sup>2</sup>, or 7.5mg/kg (3-week protocol). All dose level adjustments should be documented for a clear reason.

Table 1.2 Study drug dose levels			
Drug names	Starting dose (mg/m <sup>2</sup> )	Dose level -1 (mg/m <sup>2</sup> )	Dose level -2 (mg/m <sup>2</sup> )
Oxaliplatin	85	65	50
5-FU	2400	2300	2200
Citrus Flavonoids Tablets (Aimai Lang)	Oral administration, 500mg Bid	Oral administration, 500mg Qd	Oral administration, 250mg Qd

#### 4.2.4 Dose adjustment criteria

Dose adjustment was made based on the severity classification (NCI CTCAE version 6.0)

and duration of adverse events that occurred after administration in the previous treatment cycle. In the event of severe hematological and liver adverse events (excluding liver function impairment due to disease progression), the dosages of oxaliplatin, 5-FU, and citrus flavonoid tablets (Aimailang) should be reduced simultaneously; In cases of severe diarrhea and mucosa-associated toxicity (excluding vomiting and alopecia), the doses of 5-FU and citrus flavonoids (Aimailang) should be reduced simultaneously. See Table 1.3 for details.

<b>Table 1.3 Dose Adjustments for Selected Adverse Events</b>		
Adverse events	Severity grading (NCI CTCAE version 6.0)	Study drug dose adjustment
Granulocytopenia	Grade 4 ( $< 0.5 \times 10^9/L$ )	Reduce one dose level
Granulocytic fever	Grade 3 or above (ANC $< 1.0 \times 10^9/L$ , body temperature $\geq 38.5^\circ\text{C}$ )	Reduce the dose level by one
Thrombocytopenia	Grade 3 and above ( $< 50 \times 10^9/L$ )	Reduce the dose level by one
Aspartate transferase	Grade 3 and above ( $> 5 \times \text{ULN}$ )	Reduce one dose level
Aspartate transferase	Grade 3 and above ( $> 5 \times \text{ULN}$ )	Reduce one dose level
Serum total bilirubin	Grade 3 and above ( $> 3 \times \text{ULN}$ )	Reduce one dose level
Note: If the severity of an adverse event does not meet the dose reduction criteria but does not meet the administration criteria by the time the next treatment cycle begins and recurs $\geq 2$ times, the dose should be reduced by one level for the corresponding drug in accordance with the above requirements.		

Oxaliplatin can cause characteristic neurotoxic reactions, mainly manifested as sensory nerve disorders. When the above serious adverse events occur, the dose of oxaliplatin should be reduced alone. The adjustment criteria are detailed in Table 1.4.

<b>Table 1.4 Dose adjustments for oxaliplatin-related neurotoxicity</b>		
Adverse events	Occurs	Study the dose adjustment of the drug

Grade 1: Abnormal sensation or dullness of sensation that will completely subside within one week	Any one of them occurs	No adjustment required
Grade 2: Abnormal sensation or dullness of sensation that does not completely subside over 2 treatment weeks	Any one of them occurs	Reduce one dose level
Grade 3: Abnormal sensation or dullness of sensation with dysfunction	First occurrence	Discontinue the drug and reduce the dose level by 1 if recovery is possible in the next cycle of treatment
	Failure to return to a grade 2 reaction after drug withdrawal	Permanent discontinuation

### 4.3 Combination and concomitant medications

#### 4.3.1 Prohibited concurrent and concomitant medications

Other anti-tumor treatments, including: chemotherapy, molecular targeted therapy, hormone therapy, immunotherapy, biotherapy, non-palliative radiotherapy, and immunomodulators (including but not limited to: interferon, interleukin-2, etc.) are not permitted during the study period.

#### 4.3.2 Other anti-tumor treatments and investigational medications

Other anti-tumor treatments not specified in this study protocol will not be permitted when the subject is undergoing the study treatment. No other systemic anti-tumor treatment will be allowed.

## 5. Study procedures

### 5.1 Screening Period

After patients sign the informed consent form, they enter the screening period. Endoscopic pathological biopsy specimens of patients with locally advanced (cT2N+ or cT3-4aNany, M0, and MRF negative) rectal cancer are collected for YWHAB immunohistochemical testing to screen out patients with locally advanced rectal cancer with high expression of YWHAB for inclusion in the study. Laboratory tests and imaging evaluations conducted for routine clinical diagnosis and treatment before signing the informed consent can be used if within the specified window period.

The following screening should be completed within 28 days before the initiation of the investigational drug treatment:

- Obtain the informed consent form signed by the subject;
- Collect demographic information: name, gender, date of birth, height, weight, etc.
- Collecting adverse events: Record adverse events starting from signing the informed consent form;
- Tumor diagnosis: Pathological confirmation date, pathological grade, clinical imaging stage (TNM), clinical stage, etc.
- History of tumor treatment;
- Imaging: Enhanced chest, abdominal and pelvic CT+ transrectal ultrasound or enhanced chest, abdominal and pelvic CT+ enhanced pelvic MRI;
- Colonoscopy pathological biopsy specimens were subjected to YWHAB immunohistochemical testing.
- (Not mandatory) Determine codons 12 and 13 of exon 2 KRAS, codons 59 and 61 of exon 3, codons 117 and 146 of exon 4, codons 12 and 13 of exon 2 NRAS, and codons 59 and 61 of exon 3 in tumor tissue Codons 117 and 146 of exon 4 and BRAF V600E gene status;
- MMR/MSI detection of tumor tissues
- The following screening should be completed within 7 days before the initiation of investigational drug treatment:
- Weight and ECOG score;
- Vital signs: pulse, respiratory rate, body temperature and blood pressure;
- Comprehensive physical examination: General condition, head and face, skin, lymph

nodes, eyes, ear, nose and throat, mouth, respiratory system, cardiovascular system, abdomen, reproductive - urinary system, musculoskeletal system, nervous system and mental state, etc.

- Blood routine: Red blood cell count, hemoglobin, platelet count, white blood cell count, neutrophil count and lymphocyte classification count;
- Urine routine: white blood cell count, red blood cell count, urine protein; If urine protein is  $\geq 2+$ , a 24-hour urine protein quantification test is required;
- Blood biochemistry: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase ( $\gamma$ -GT), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (AKP), blood urea nitrogen (BUN) or urea (preferably blood urea nitrogen), total protein (TP), albumin (ALB), creatinine (Cr), blood glucose (GLU)  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cl^-$ ;
- Tumor-associated markers;
- Coagulation function: Activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen (FIB), international normalized ratio (INR);
- 12-lead electrocardiogram: Attention should be paid to QT, QTc and P-R intervals. If there are any abnormalities, additional relevant tests should be conducted as determined by the researcher;
- Echocardiography: At least an assessment of left ventricular ejection fraction (LVEF) should be included;
- Pregnancy test: For women of childbearing age, using a serum pregnancy test.

## 5.2 Treatment period

The treatment period starts from the first administration of the drug to the end of the study treatment. The first study dose should be administered as close as possible to the time when screening checks confirm eligibility for inclusion or exclusion. All tests and evaluations (except imaging tests) should be completed within 3 days prior to the first administration. For laboratory tests on day 1 of cycle 1 (blood routine, urine routine, fecal occult blood, blood biochemistry, coagulation function and thyroid function) and electrocardiogram tests, if the

corresponding baseline laboratory tests were conducted within 7 days prior to the first administration, they do not need to be repeated.

The following evaluations should be completed before each study treatment dosing:

Weight and ECOG score;

Vital signs;

- Physical examination;
- Blood routine: Red blood cell count, hemoglobin, platelet count, white blood cell count, neutrophil count, and lymphocyte classification count;
- Blood biochemistry: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase ( $\gamma$ -GT), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (AKP), blood urea nitrogen (BUN) or urea (preferably blood urea nitrogen), total protein (TP), albumin (ALB), creatinine (Cr), blood glucose (GLU) K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>;
- Urine routine: white blood cells, red blood cells, urine protein; If urine protein is  $\geq 2+$ , a 24-hour urine protein quantification test is required;
- Coagulation function: Activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen (FIB), international normalized ratio (INR);
- Tumor-associated markers;
- 12-lead electrocardiogram: Attention should be paid to QT, QTc and P-R intervals. If there are any abnormalities, additional relevant tests should be conducted as determined by the researcher;
- Record adverse events;
- Imaging assessment: Imaging assessment was conducted after 6 and 12 cycles of mFOLFOX6 regimen adjuvant chemotherapy, including enhanced chest, abdominal and pelvic CT and pelvic MRI. Unplanned imaging tests may be performed in any subject when disease progression (such as symptom deterioration) is suspected during treatment.

### **5.3 Treatment End/Exit Study visit**

Relevant evaluations and examinations are required when the subjects end the study treatment/withdraw from the study. It should be carried out as follows:

- Weight and ECOG score;
- Vital signs: pulse, respiratory rate, body temperature and blood pressure;
- Comprehensive physical examination: General condition, head and face, skin, lymph nodes, eyes, ear, nose and throat, mouth, respiratory system, cardiovascular system, abdomen, reproductive - urinary system, musculoskeletal system, nervous system and mental state, etc.
- Blood routine: red blood cell count, hemoglobin, platelet count, white blood cell count, neutrophil count and lymphocyte classification count;
- Urine routine: white blood cell count, red blood cell count, urine protein; If urine protein is  $\geq 2+$ , a 24-hour urine protein quantification test is required;
- Blood biochemistry: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase ( $\gamma$ -GT), total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (AKP), blood urea nitrogen (BUN) or urea (preferably blood urea nitrogen), total protein (TP), albumin (ALB), creatinine (Cr), blood glucose (GLU)  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$  +  $Cl^-$ ;
- Tumor-associated markers;
- Coagulation function: Activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen (FIB), international normalized ratio (INR);
- 12-lead electrocardiogram: Attention should be paid to QT, QTc and P-R intervals. If there are any abnormalities, additional relevant tests should be conducted as determined by the researcher;
- Record adverse events.

#### **5.4 Follow-up period**

Survival follow-up period until the subject's death, loss to follow-up, withdrawal of informed consent and refusal to continue providing information, or termination of the study by the sponsor. During this period, visits were made every 3 months through effective means such

as telephone follow-ups to collect survival information and subsequent anti-tumor treatment information (if the subject initiates a new anti-tumor treatment, the treatment plan and start and end times should be recorded).

For subjects without radiological evidence of disease progression, radiological assessment should continue at the efficacy evaluation frequency specified in this study until disease progression, death, loss to follow-up, withdrawal of informed consent and refusal to continue providing information, initiation of other anti-tumor treatments, or sponsor termination of the study. Radiological evidence of disease progression in such subjects should be obtained as much as possible.

## **6 Evaluation of efficacy**

### **6.1 Validity parameters:**

The primary efficacy measure of this study was the proportion of patients achieving tumor downstaging (ypTNM stage 0-I) in each treatment group. The secondary efficacy measures were the proportion of patients achieving pathological complete response, 3-year disease-free survival (DFS), overall survival time (OS), and tumor regression grade (TRG).

### **6.2 Criteria for evaluation of efficacy:**

This study primarily evaluated the proportion of patients achieving tumor downstaging (ypTNM 0-I stage) in patients with YWHAB high-expression locally advanced (cT2N+ or cT3-4aNany, M0, and MRF negative) rectal cancer treated with the neoadjuvant regimen of mFOLFOX6 combined with citrus flavonoids tablets (Aimailang).

- (1) Tumor downstaging rate (ypTNM 0-I): The proportion of patients with locally advanced rectal cancer whose postoperative surgical specimens were pathologically staged at ypTNM 0-I after receiving neoadjuvant chemotherapy with mFOLFOX6 or mFOLFOX6 combined with citrus flavonoids (Aimailang).
- (2) The degree of pathological response is defined as follows: ① Complete response (CR) : All tumor target lesions disappear, no new lesions emerge, and tumor markers remain normal for at least 4 weeks. ② Partial response (PR), where the sum of the maximum diameters of tumor target lesions is reduced by  $\geq 30\%$  and maintained for at least 4 weeks.

- ③ Stable lesion (SD), where the sum of the maximum diameters of tumor target lesions is reduced to less than PR or increased to less than PD. ④ Disease progression (PD), where the sum of the maximum diameters of tumor target lesions increases by at least 20%, or new lesions appear. Subjects whose primary study endpoints were not collected were included in the denominator of this ratio and not in the numerator.
- (3) Clinical deterioration in the absence of conclusive evidence of disease progression in accordance with (RECIST version 1.1) does not count as disease progression when determining disease-free progression-free time (DFS). For deceased subjects whose progression was not previously reported, it was considered that progression occurred on the date of death. Subjects who neither had disease progression nor died will be deleted on the date of their last evaluable tumor evaluation. The independent central imaging service provider that uses an independent central imaging review to confirm the subject will receive all images from the research center and determine the subject's disease progression according to RECIST 1.1 criteria.
- (4) Survival record and assessment: Disease and survival of subjects were followed up every 3 months after the end of treatment or exit from the group by means of telephone follow-up, etc., until death, loss to follow-up, withdrawal of informed consent and refusal to continue providing information, or sponsor termination of the study.
- (5) Tumor regression grading TRG: TRG 0 refers to no residual tumor cells, TRG 1 refers to a single cell or a small group of cells; TRG 2 refers to residual cancer with a connective tissue proliferative response; TRG 3 refers to the minimal evidence of a tumor response.

## 7. Safety evaluation

### 7.1 Safety Parameters

The safety parameters for this study included clinical symptoms, vital signs, physical examination, and laboratory tests (blood routine, blood biochemistry, and coagulation function, etc.). Reference: National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (Version 6.0) (NCI CTCAE version 6.0) ([https://ctep.cancer.gov/protocoldevelopment/electronic\\_applications/ctc.htm](https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm)) is observed to evaluate the adverse events, Including type, incidence, severity, occurrence and end time,

whether it was a serious adverse event, relevance to the study treatment, and outcome.

## **7.2 Definition of adverse events**

An adverse event is any adverse medical event that occurs to a patient or a clinical study subject after the administration of a drug, which does not necessarily have a causal relationship with the treatment. Therefore, an adverse event can be any adverse and other unintended signs (including abnormal laboratory findings) and symptoms, or a disease that is temporally related to the use of a medical product, whether or not considered to be related to the investigational drug, including at least the following situations:

- Exacerbation of pre-existing (prior to entering clinical trials) medical conditions/diseases (including exacerbation of symptoms, signs, or abnormal laboratory tests);
- Any newly occurring adverse medical condition (including symptoms, signs, or newly diagnosed disease);
- Abnormal clinically significant laboratory test values or results.

## **7.3 Definition of a serious adverse event**

A serious adverse event is any adverse medical condition that occurs at any dose and meets one of the following criteria:

- Fatal (resulting in death; note: Death is a consequence, not an event);
- Life-threatening (" life-threatening "means that the patient is immediately at risk of death at the time of the event, not the assumption that it would have caused death if the event had been more serious);
- It leads to the patient's hospitalization or an extension of the hospital stay;
- Resulting in lifelong or severe disability/functional defect;
- Resulting in congenital malformations or birth defects;
- It is of medical significance or requires intervention to prevent the occurrence of any of these consequences.

Tumor progression or deterioration that occurs during the study (including the emergence of new metastases and deaths resulting from disease progression) should be included as part of the efficacy evaluation and should not be reported as an adverse event or

serious adverse event.

#### **7.4 Definition of a serious adverse event**

Disease progression is defined as the deterioration of the subject's condition caused by the indication of the study. Including imaging progression and progression of clinical symptoms and signs. The presence of new metastases relative to the primary tumor, or the progression of existing metastases, is considered disease progression. Events resulting from symptoms and signs of disease progression that are life-threatening, require hospitalization or extended hospital stay, or result in permanent or severe disability, dysfunction affecting work ability, congenital abnormalities, or birth defects are not reported as serious adverse events. Deaths resulting from symptoms and signs of disease progression are reported as SAEs.

#### **7.5 Classification of adverse events and serious adverse events**

##### **7.5.1 Criteria for determining the severity of adverse events:**

The severity of adverse events will be graded using NCI CTCAE version 6.0 and reported in detail as required by CRF. In the event of an adverse event not included in NCI CTCAE version 6.0, the following five-level scoring system will be used:

- Mild: No clinical symptoms or only mild clinical symptoms; Only clinical or laboratory test abnormalities; No treatment is required.
- Moderate: Requires smaller, local, or non-invasive treatment; Limited use of tools and other daily activities that are age-appropriate.
- Severe; Seriously ill or having medically serious symptoms but not life-threatening for the time being; Resulting in hospitalization or an extension of hospital stay; Resulting in disability; Limitations in taking care of oneself in daily life.
- Life-threatening; Life-threatening immediately, requiring urgent treatment
- Death: A death associated with an adverse event.

##### **7.5.2 Determination of the relationship between adverse events and study treatment**

The relationship between adverse events and treatment should be evaluated using the following criteria:

- Likely related (the first three must be present) : This category refers to adverse events that are considered to be related to the investigational drug with a high degree of certainty. An adverse event can be considered "likely related" if the following criteria are met:
  1. The occurrence of an adverse event has a reasonable temporal correlation with the application of the drug.
  2. Known patient disease status, environmental or toxic factors, or other treatments used by the patient do not reasonably account for adverse events.
  3. Adverse reactions disappear or are alleviated after the dose is stopped or reduced. (But there are important exceptions to certain drug-related adverse reactions that do not disappear even after discontinuation; such as: (1) myelosuppression, (2) tardive dyskinesia)
  4. Adverse events are in line with the pattern of suspected drug reactions.
  5. When used again, adverse events reappeared.
- Possibly related (the first two must be present) : This category refers to adverse events that are unlikely to be related to the administration of the investigational drug but whose association cannot be definitely ruled out. An adverse event may be considered "possibly related" if the following criteria are met:
  1. The occurrence of an adverse event has a reasonable temporal correlation with the application of the drug.
  2. Adverse reactions may be caused by the patient's disease status, environmental or toxic factors, or other concomitant treatments used by the patient.
  3. Adverse events are consistent with the reaction pattern of the suspected drug.
- May not be related (the first two must be present) : This category applies to adverse events that meet the following criteria:
  1. The occurrence of adverse events does not have a reasonable temporal correlation with the application of the drug.
  2. Adverse events were clearly caused by the patient's disease status, environmental or toxic factors, or other concomitant treatments used by the patient.
  3. Adverse events do not conform to the response pattern of the suspected drug.
  4. When the drug was administered again, the adverse event did not occur or did not

worsen.

- Unrelated: This category refers to an adverse event that is clearly determined to be caused solely by external factors (disease, environment, etc.) and does not meet the criteria for drug relevance under "possibly unrelated", "possibly related", or "very likely related".

### **7.5.3 Abnormalities in laboratory tests**

Laboratory test results will be recorded on the laboratory test data page in the CRF. The study monitor continuously reviews the CRF records. Any laboratory test anomalies that meet SAE standards should be immediately reported using the SAE report form and also recorded as AE in the CRF.

Laboratory test anomalies do not need to be reported as AE on the AE page of the CRF unless they meet any of the following criteria:

- Accompanied by clinical symptoms;
- Need to change the study medication (e.g. : dose adjustment, temporary interruption or permanent discontinuation of administration)
- It is necessary to change the accompanying treatment (for example: adding the corresponding treatment, interrupting the accompanying treatment or administration, no longer continuing or making other changes);
- The researchers consider it to be of significant medical importance (as they decide based on scientific medical judgment whether the independently occurring laboratory test anomaly can be classified as an AE).

## **7.6 Follow-up and reporting of adverse events**

### **7.6.1 Collection and follow-up of adverse events/serious adverse events:**

The collection of adverse events began when the subjects signed the informed consent form and ended 30 days after the last study administration.

The collection period for serious adverse events began with the signing of the informed consent form by the subjects. For serious adverse events not related to the study drug, it was up to 90 days after the last study administration or the initiation of a new anti-tumor treatment

(whichever was reached first); After that, only serious adverse events related to the study drug were collected.

Adverse events/serious adverse events should be followed up until the event disappears, resolves to baseline level or  $\leq$  grade 1, reaches a stable state, or is reasonably explained (such as loss to follow-up, death) for the best possible outcome. The investigator should ask about the adverse events/serious adverse events that occurred after the previous visit at each visit and provide follow-up information in a timely manner as requested by the sponsor.

#### **7.6.2 Reporting of serious adverse events:**

During the study period, regardless of the treatment a patient received, any serious adverse event or significant abnormal laboratory test results that occurred, the investigator must complete the adverse event reporting Form and the CFDA's serious Adverse Event Reporting Form and report to the designated contact person within 24 hours of being informed:

Serious adverse event reporting contact: GCP Office, Sixth Hospital Affiliated to Sun Yat-sen University

Research Group Leader Unit Contact: Hu Tuo (Sun Yat-sen University Sixth Affiliated Hospital)

Email: hutuo3@mail.sysu.edu.cn

Address: 26, Yuancun 2nd Cross Road, Tianhe District, Guangzhou

The person in charge of serious adverse event reporting shall, within one working day after receiving the serious adverse event report from the investigator, fax the serious adverse event reporting form signed by the investigator to the unit in charge. It must be carried out in accordance with the international council for harmonization (ICH) guidelines for the management of safety data in clinical trials, definitions and standards for rapid reporting.

#### **7.6.3 Reporting of non-serious adverse events:**

In addition to specific adverse events, other non-serious adverse events should also be recorded by researchers on the adverse events page of the CRF and in retrieval format. The collection of adverse events for each patient was within 30 days from the date of signing the informed consent form to the end of the last administration. The following are the minimum

requirements for recording on the adverse event reporting form: identity of the subject, drug, duration of the adverse event, start date of the event, and causality.

#### **7.6.4 Follow-up of abnormal laboratory tests**

If an unexplained laboratory test anomaly occurs and the anomaly is clinically relevant, the test should be re-conducted immediately and followed up until the test value returns to normal range and/or a reasonable explanation for the anomaly is found. If there is a clear explanation, it should be recorded on the CRF.

#### **7.6.5. Pregnancy report**

Pregnancy should be strictly avoided during the study. If a female subject becomes pregnant during the study period, the study treatment must be immediately discontinued and the investigator must be informed immediately. Pregnancies that occur within six months of the completion of the study treatment must also be reported to the investigator.

All male subjects were required to take effective contraceptive measures during the study and six months after the study was completed. If a male patient's partner becomes pregnant during the study and within 6 months after the completion of treatment, it should be reported to the investigator and the sponsor. The investigator should report all pregnancy cases to the sponsor within 24 hours using the clinical Trial pregnancy report form.

Pregnancy events should be followed up until 30 days after the end of pregnancy. Pregnancy events include spontaneous abortion or induced abortion, details of delivery, birth defects or congenital malformations of the newborn, malformations and abnormalities of stillbirth, and complications of the mother and the newborn. Reporting time, visitation reference for serious adverse events.

Researchers should advise patients about the risks of continuing the pregnancy and the possible effects on the fetus. And patients should continue to be monitored until the end of pregnancy. Information regarding the outcome of the pregnancy must be provided to the sponsor.

## **8 Safety evaluation**

### **8.1 Analyze the population**

- Informed Consent Set (ICS) : Subjects who signed the informed consent form were included in this analysis set.
- intention to treatment (ITT) : According to the principle of intention-to-treat analysis, including all patients who were randomized into groups, the analysis was conducted according to the groups assigned during randomization. This analysis set was the primary analysis set for the efficacy endpoints.
- Full Analysis Set (FAS) : The full analysis set was determined in accordance with the Intent To Treat (ITT) principle, consisting of all subjects who had been enrolled and received at least one study drug treatment. Based on the analysis of the full analysis set, subjects will participate in the analysis according to the group assigned to their enrollment (regardless of the drug treatment they actually received);
- Per-Protocol Set (PPS) : patients in the ITT set who did not experience significant protocol deviations that would affect efficacy assessment. The PP population was used for sensitivity analysis of primary efficacy endpoints and key secondary efficacy endpoints.
- Safety Set (SS) : It will include all subjects who have received at least one study drug treatment. The safety set is the primary analysis population used for the safety analysis.

### **8.2 General Analysis**

Measurement data were aggregated using mean, standard deviation, median, maximum, minimum; Count data were aggregated by frequency and percentage; Time-event data were used to estimate survival rates and plot survival curves using Kaplan-Meier, and if necessary, plot survival curves and estimate overall 95% confidence intervals of median time.

### **8.3 Therapeutic Effect Analysis and Statistical Methods**

Mean and standard deviation were used for statistical analysis of the proportion of patients achieving tumor downstaging (ypTNM stage 0-I), the proportion of participants achieving pathological complete response, and the tumor regression grade TRG in each treatment group. For three-year disease-free survival and overall survival time, survival curves

were plotted using the Kaplan-Meier method, and 95% confidence intervals for median time were calculated using the Brookmeyer-Crowley method. The Log-rank method was used to compare the differences between groups and to estimate the hazard ratio (HR) for each endpoint based on the Cox proportional model, and to explore the analysis of the impact of factors other than treatment on the differences at each endpoint. In addition, the number and percentage of cases with endpoint events, the number and percentage of cases with deletions, will be calculated and classified by causes of deletions.

#### **8.4 Safety analysis and statistical methods**

The safety analysis will be based on the safety set. The safety analysis is limited to descriptive statistical summaries, including but not limited to the following aspects. The specific analysis will be described in the statistical analysis plan:

- Summary of adverse events (all-cause and treatment-related);
- Incidence and severity of adverse events (all-cause and treatment-related);
- Summary of details of serious adverse events;
- Analysis of adverse event correlations;
- Abnormalities in laboratory indicators, vital signs, and electrocardiogram data.

The safety analysis will be based on descriptive statistical summaries and safety sets.

This study will encode adverse events using the Standard Medical Terminology Set for Pharmacy Administration (MedDRA). This study will list all adverse events that occurred during treatment, drug-related adverse events, serious adverse events, and drug-related serious adverse events in a table in accordance with the NCI CTCAE version 6.0 standard, using the worst classification. Laboratory test parameters during the study period were summarized in accordance with the NCI CTCAE version 6.0 standard, using the worst-case classification.

#### **8.5 Sample size determination**

The primary endpoint of this study was the rate of tumor downstaging (ypTNM stage 0-I) in each treatment group. Based on our center 's previous data, the proportion of patients with locally advanced rectal cancer who received neoadjuvant chemotherapy with mFOLFOX6 downstaging (ypTNM 0-I) was about 35%, and the proportion of patients

with YWHAB high expression rectal cancer (approximately one-third, n=86) who received neoadjuvant chemotherapy with mFOLFOX6 was about 25%. In patients with low YWHAB expression (approximately two-thirds, n=86), the tumor downstaging rate after receiving mFOLFOX6 neoadjuvant chemotherapy was about 42.9%. A previous single-center, prospective, open-label, small cohort exploration by our team found that the tumor downstaging rate of YWHAB-high expression rectal cancer patients receiving mFOLFOX6 neoadjuvant chemotherapy was approximately 25.0% (total 20 cases, including 5 patients at ypTNM stage 0-I); The tumor downstaging rate in patients with YWHAB hyperexpression rectal cancer who received mFOLFOX6 combined with Aimailang neoadjuvant therapy was approximately 42.9% (28 cases, including 12 patients at ypTNM 0-I stage). The estimated tumor downgrading rate for YWHAB hyperexpression patients receiving neoadjuvant chemotherapy with mFOLFOX6 combined with citrusone tablets (Aimailang) was 40%, with a significance level of 0.05 for the unilateral test, a test efficacy of 0.8, a loss to follow-up rate of 10%, and an estimated sample size of 236 using PASS V15 software.

## **9. Ethical considerations**

### **9.1 Local regulations/Declaration of Helsinki**

The researcher must ensure that the study is conducted in full compliance with the principles of the Helsinki Declaration or the requirements of the laws and regulations of the place where the study is conducted in order to achieve maximum protection for the individual. The study must fully comply with the principles set out in the ICH tripartite guideline "GCP Operating Guidelines (January 1997)" or comply with local regulations to provide greater protection for the subjects. In other countries with GCP, researchers will strictly follow the relevant provisions.

### **9.2 Good Clinical Practice for Drugs**

This research will be carried out in accordance with the ethical principles reflected in the Good Clinical Practice for Drugs as defined by ICH. This study will be carried out in accordance with the study protocol. The study protocol and any amendments will be subject to

informed consent from the institutional review board /independent ethics committee (IRB/IEC) before the study commences. All serious violations must be reported immediately to the sponsor institution and the principal investigator. A serious violation is an act that violates the GCP principles and requirements related to the study or protocol and is likely to affect, or seriously affect, the safety, physical or mental health of the study subjects or the scientific value of the study. Participants in this study should be qualified through education, training and experience in the relevant work. This study must not employ individuals who have been sanctioned, or who have engaged in scientific misconduct or deception. Rules and regulations must be implemented to ensure treatment in all aspects of the study.

### **9.3 Institutional Review Committee/Independent Ethics Committee**

Prior to the start of the study, the researcher must obtain a written and dated approval/supportive opinion document from IRB/IEC regarding the study protocol, informed consent form, and other written information provided to the subjects. The researcher or sponsor should also provide IRB/IEC with a copy of the researcher's manual or product instruction, as well as the information to be provided to the subjects and any updates.

Researchers or sponsors should provide relevant reports, updates and other information to IRB/IEC in accordance with regulatory requirements or institutional procedures.

### **9.4 Informed Consent**

The investigator must ensure that the subject or an acceptable legal representative (for subjects who are unable to sign the informed consent form in person) is clear and fully informed of the purpose of the trial, the potential risks, and other critical questions related to the clinical trial in which the subject wishes to participate. Before participating in this clinical study, each subject or legal representative should voluntarily sign a written informed consent form, including informed consent for any screening procedures to be conducted to determine the inclusion criteria of the subjects. The investigator or the designated person must inform the subjects that they have the right to refuse to participate in the study or to withdraw from the study at any time for any reason.

Subject rights, safety and health are the most important matters that researchers should

consider, and should take precedence over the scientific and social value of the study.

## **10、 Data collection, management and quality assurance**

The data from this study will be recorded via CRF, and the research center will transcribe it from the original paper file to the CRF. The study monitor will verify and cross-check the CRF(original document verification) against the records of the GCP control researchers to ensure the accuracy and reliability of the data collection. All observed patients at each participating center will undergo the original document check. Difference reports will be generated accordingly and provided to the research center for the researchers to analyze. In addition, CRF data will be subject to continuous review to ensure medical and scientific reasonableness.

## **11、 Case report form**

Researchers should ensure that the data reported to sponsors in CRF and all required reports are accurate, complete, and timely.

## **12 Confidentiality of trial documentation and subject records**

Researchers must ensure the anonymity of subjects and prevent unauthorized parties from knowing their identities. In CRF or other documents submitted to the sponsor, subjects should not be identified by their names but by identification codes. The investigator should keep a record of subject enrollment showing the code, name and address. Researchers should keep certain documents strictly confidential at all times, such as the written informed consent form signed by the subject and the subject enrollment record.

## **13 Conditions for modification of the study protocol**

Modifications to the research protocol can only be made after consultation between the appropriate representative of the sponsor and the investigator. Modifications to the study protocol should be drafted by the sponsor's representative and evaluated and approved in advance by another qualified sponsor. All modifications to the study protocol must be submitted to the corresponding ethics committee for information or approval in accordance with local procedures and regulatory requirements, and approval must be obtained before any changes can be

implemented.

## 14、Conditions for terminating the study

The sponsor and the investigator reserve the right to terminate the trial at any time. If necessary, both parties may, after review and consultation, arrange for the implementation of this procedure based on the specific circumstances of the study. When terminating the study, the researcher will give full consideration to protecting the interests of the subjects.

## References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. **Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.** *CA Cancer J Clin* 2021; 71 (3) : 209-249. The doi: 10.3322 / CAAC. PMID: 21660 33538338
2. LATEST GLOBAL CANCER DATA: CANCER BURDEN RISES TO 19.3 MILLION NEW CASES AND 10.0 MILLION CANCER DEATHS IN 2020 QUESTIONS AND ANSWERS (Q&A). Retrieved Dec 16, 2020, from <https://www.iarc.fr/faq/latest-global-cancer-data-2020-qa/>
3. Baxter NN, Garcia-Aguilar J. **Organ preservation for rectal cancer.** *J Clin Oncol* 2007; 25 (8) : 1014-1020. The doi: 10.1200 / jco 2006.09.7840 PMID: 17350952
4. Rajput A, Bullard Dunn K. **Surgical management of rectal cancer.** *Semin Oncol* 2007; 34 (3) : 241-249. The doi: 10.1053 / j.s eminoncol 2007.03.005 PMID: 17560986
5. Weiser MR, Landmann RG, Wong WD, Shia J, Guillem JG, Temple LK, et al. **Surgical salvage of recurrent rectal cancer after transanal excision.** *Dis Colon Rectum* 2005; 48(6):1169-1175.doi:10.1007/s10350-004-0930-3 PMID:15793645
6. Wiig JN, Larsen SG, Giercksky KE. **Operative treatment of locally recurrent rectal cancer.** *Recent Results Cancer Res* 2005; 165:136-147.doi:10.1007/3-540-27449-9\_15 PMID: 15865028
7. Morino M, Risio M, Bach S, Beets-Tan R, Bujko K, Panis Y, et al. **Early rectal cancer: the European Association for Endoscopic Surgery (EAES) clinical consensus conference.** *Surg Endosc* 2015; 29(4):755-773.doi:10.1007/s00464-015-4067-3 PMID:25609317
8. Benson AB, Venook AP, al-Hawary MM, Cederquist L, Chen YJ, Ciombor KK, et al. **Rectal Cancer, Version 2.2018 NCCN Clinical Practice Guidelines in Oncology.** *J Natl Compr Canc Netw* 2018; 16 (7) : 874-901. The doi: 10.6004 / JNCCN. 2018.0061 PMID: 30006429
9. Deng Y, Chi P, Lan P, et al. **Modified FOLFOX6 With or Without Radiation Versus Fluorouracil and Leucovorin With Radiation in Neoadjuvant Treatment of Locally Advanced Rectal Cancer: Initial Results of the Chinese FOWARC Multicenter, Open-Label, Randomized Three-Arm Phase III Trial.** *J Clin Oncol.* 2016; 34 (27) : 3300-3307. The doi: 10.1200 / JCO 2016.66.6198
10. Deng Y, Chi P, Lan P, et al. **Neoadjuvant Modified FOLFOX6 With or Without Radiation Versus Fluorouracil Plus Radiation for Locally Advanced Rectal Cancer: Fin**

- al Results of the Chinese FOWARC Trial.** *J Clin Oncol.* 2019; 37(34):3223-3233. doi:10.1200/JCO.18.02309
11. Zhang J, Chi P, Shi L, et al. **Neoadjuvant Modified Infusional Fluorouracil, Leucovorin, and Oxaliplatin With or Without Radiation Versus Fluorouracil Plus Radiation for Locally Advanced Rectal Cancer: Updated Results of the FOWARC Study After a Median Follow-Up of 10 Years.** *J Clin Oncol.* Published online December 13, 2024. doi:10.1200/JCO-24-01676
  12. Pair FS, Yacoubian TA. **14-3-3 Proteins: Novel Pharmacological Targets in Neurodegenerative Diseases.** *Trends Pharmacol Sci* 2021; 42(4):226-238. doi:10.1016/j.topi.2021.01.001 PMID:33518287
  13. Mhawech P. **14-3-3 proteins--an update.** *Cell Res* 2005; 15(4):228-236. doi:10.1038/j.cr.7290291 PMID:15857577
  14. Ormanecy M, Thuleau P, Mazars C, Cotellet V. **CDPKs and 14-3-3 Proteins: Emerging Duo in Signaling.** *Trends Plant Sci* 2017; 22 (3) : 263-272. The doi: 10.1016/j.t. the plants. The 2016.11.007 PMID: 28065409
  15. Hermeking H. **The 14-3-3 cancer connection.** *Nat Rev Cancer* 2003; 3(12):931-943. doi:10.1038/nrc1230 PMID:14737123
  16. Lu Y, Xie S, Zhang W, Zhang C, Gao C, Sun Q, et al. **Twa1/Gid8 is a  $\beta$ -catenin nuclear retention factor in Wnt signaling and colorectal tumorigenesis.** *Cell Res* 2017; 27 (12) : 1422-1440. The doi: 10.1038/cr.2017.107 PMID: 28829046
  17. Gentile D, Fornai M, Colucci R, Pellegrini C, Tirotta E, Benvenuti L, et al. **The flavonoid compound apigenin prevents colonic inflammation and motor dysfunctions associated with high fat diet-induced obesity.** *PLoS One* 2018; 13 (4) : e0195502. Doi: 10.1371/journal.pone. PMID: 0195502 29641549
  18. Middleton E, Jr., Kandaswami C, Theoharides TC. **The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer.** *Pharmacol Rev* 2000; 52(4):673-751 PMID:11121513
  19. Haidari F, Heybar H, Jalali MT, Ahmadi Engali K, Helli B, Shirbeigi E. **Hesperidin supplementation modulates inflammatory responses following myocardial infarction.** *J Am Coll Nutr* 2015; 34 (3) : 205-211. The doi: 10.1080/07315724.2014.891269 PMID: 25757593
  20. Li C, Schluesener H. **Health-promoting effects of the citrus flavanone hesperidin.** *Crit Rev Food Sci Nutr* 2017; 57 (3) : 613-631. The doi: 10.1080/10408398.2014.906382 PMID: 25675136
  21. Wei D, Ci X, Chu X, Wei M, Hua S, Deng X. **Hesperidin suppresses ovalbumin-induced airway inflammation in a mouse allergic asthma model.** *Inflammation* 2012; 35 (1):114-121. doi:10.1007/s10753-011-9295-7 PMID:21287361
  22. Ji Z, Deng W, Chen D, Liu Z, Shen Y, Dai J, et al. **Recent understanding of the mechanisms of the biological activities of hesperidin and hesperetin and their therapeutic effects on diseases.** *Heliyon* 2024; (5) : 10 e26862. Doi: 10.1016/j.heliyon.2024.E26862 PMID: 38486739
  23. Sakata K, Hirose Y, Qiao Z, Tanaka T, Mori H. **Inhibition of inducible isoforms of cyclooxygenase and nitric oxide synthase by flavonoid hesperidin in mouse macrophage cell line.** *Cancer Lett* 2003; 199(2):139-145. doi:10.1016/s0304-3835(03)00386-0 PMID:12

24. El-Deek SEM, Abd-Elghaffar SKH, Hna RS, Mohamed HG, El-Deek HEM. **Effect of Hesperidin against Induced Colon Cancer in Rats: Impact of Smad4 and Activin A Signaling Pathway.** *Nutr Cancer* 2022; 74 (2) : 697-714. The doi: 10.1080/01635581.2021.1907424 PMID: 33818196
25. Saiprasad G, Chitra P, Manikandan R, Sudhandiran G. **Hesperidin alleviates oxidative stress and downregulates the expressions of proliferative and inflammatory markers in azoxymethane-induced experimental colon carcinogenesis in mice.** *Inflamm Res* 2013; 62(4):425-440.doi:10.1007/s00011-013-0595-2 PMID:23377175
26. Jiao Q, Xu L, Jiang L, Jiang Y, Zhang J, Liu B. **Metabolism study of hesperetin and hesperidin in rats by UHPLC-LTQ-Orbitrap MS (n).** *Xenobiotica* 2020; 50 (11) : 1311-1322. The doi: 10.1080/00498254.2019.1567956 PMID: 30654682
27. Li YM, Li XM, Li GM, Du WC, Zhang J, Li WX, et al. **In vivo pharmacokinetics of hesperidin are affected by treatment with glucosidase-like BglA protein isolated from yeasts.** *J Agric Food Chem* 2008; 56 (14) : 5550-5557. The doi: 10.1021 / jf800105c PMID: 18570429
28. Brierley JD, Cummings BJ, Wong CS, Keane TJ, O'Sullivan B, Catton CN, et al. **Adenocarcinoma of the rectum treated by radical external radiation therapy.** *Int J Radiat Oncol Biol Phys* 1995; 31(2):255-259.doi:10.1016/0360-3016(94)e0102-p PMID:7836077
29. Francois Y, Nemoz CJ, Baulieux J, Vignal J, Grandjean JP, Partensky C, et al. **Influence of the interval between preoperative radiation therapy and surgery on downstaging and on the rate of sphincter-sparing surgery for rectal cancer: the Lyon R90-01 randomized trial.** *J Clin Oncol* 1999; 17 (8) : 2396. Doi: 10.1200 / jco 1999.17.8.2396 PMID: 10561302
30. Habr-Gama A, Perez RO, Proscurshim I, Nunes Dos Santos RM, Kiss D, Gama-Rodrigues J, et al. **Interval between surgery and neoadjuvant chemoradiation therapy for distal rectal cancer: does delayed surgery have an impact on outcome?** *Int J Radiat Oncol Biol Phys* 2008; 71 (4) : 1181-1188. The doi: 10.1016 / j.ijrobp. 2007.11.035 PMID: 18234443
31. Kerr SF, Norton S, Glynne-Jones R. **Delaying surgery after neoadjuvant chemoradiotherapy for rectal cancer may reduce postoperative morbidity without compromising prognosis.** *Br J Surg* 2008; 95 (12) : 1534-1540. The doi: 10.1002 / BJS. PMID: 18942057
32. Sauer R, Becker H, Hohenberger W, Rodel C, Wittekind C, Fietkau R et al. **Preoperative versus postoperative chemoradiotherapy for rectal cancer.** *N Engl J Med* 2004; 351 (17) : 1731-1740. The doi: 10.1056 / NEJMoa040694 PMID: 15496622
33. Rodel C, Graeven U, Fietkau R, Hohenberger W, Hothorn T, Arnold D et al. **Oxaliplatin added to fluorouracil-based preoperative chemoradiotherapy and postoperative chemotherapy of locally advanced rectal cancer (the German CAO/ARO/AIO-04 study): final results of the multicentre, open-label, randomised, phase 3 trial.** *Lancet Oncol* 2015; 16(8):979-989.doi:10.1016/s1470-2045(15)00159-x PMID:26189067

## Appendix

### Appendix 1. The 9th edition of the AJCC/UICC Rectal Cancer TNM Staging System - Definitions of T, N, and M

#### Primary tumor (T)

<b>Tx</b>	The primary tumor cannot be evaluated
<b>T0</b>	No evidence of primary tumor
<b>Tis</b>	Carcinoma in situ: Confined within the epithelium or invading the lamina propria of the mucosa
<b>T1</b>	The tumor invades the submucosa
<b>T2</b>	The tumor invaded the muscular layer
<b>T3</b>	The tumor penetrates the muscular layer and reaches the paracolonial tissue
<b>T4a</b>	Tumor penetrates the visceral layer of the peritoneum
<b>T4b</b>	The tumor directly invades or adheres to other organs or structures

#### Regional lymph nodes (N)

<b>Nx</b>	Regional lymph nodes cannot be evaluated
<b>N0</b>	No regional lymph node metastasis
<b>N1</b>	1 to 3 regional lymph node metastases
<b>N1a</b>	There is one regional lymph node metastasis
<b>N1b</b>	There are 2 to 3 regional lymph node metastases
<b>N1c</b>	There is tumor implantation in subserosal, mesenteric, non-peritoneal covering colon tissue, and no regional lymph node metastasis
<b>N2</b>	There are more than four regional lymph node metastases
<b>N2a</b>	There are 4 to 6 regional lymph node metastases
<b>N2b</b>	7 and more regional lymph node metastases

#### Distant metastasis (M)

<b>Mx</b>	Distant metastasis cannot be evaluated
<b>M0</b>	No distant metastasis
<b>M1</b>	There is distant metastasis
<b>M1a</b>	Distant metastasis is confined to a single organ or site (e.g. : liver, lung, ovary,

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	or non-regional lymph nodes)
<b>M1b</b>	Distant metastasis is distributed in more than one organ or site or peritoneal metastasis
<b>M1c</b>	Metastasis to the peritoneal surface (including peritoneal metastasis alone or with metastasis to other sites or organs)

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**Appendix 2. 9th Edition AJCC/UICC Rectal Cancer TNM Staging System -  
Anatomical staging/Prognostic groups**

<b>Staging</b>	<b>T</b>	<b>N</b>	<b>M</b>
<b>0</b>	Tis	N0	M0
<b>I</b>	T1	N0	M0
	T2	N0	M0
<b>IIA</b>	T3	N0	M0
<b>IIB</b>	T4a	N0	M0
<b>IIC</b>	T4b	N0	M0
<b>IIIA</b>	T1-2	N1/N1c	M0
	T1	N2a	M0
<b>IIIB</b>	T3-4a	N1	M0
	T2-3	N2a	M0
	T1-2	N2b	M0
<b>IIIC</b>	T4a	N2a	M0
	T3-4a	N2b	M0
	T4b	N1-2	M0
<b>IVA</b>	Any T	Any N	M1a
<b>IVB</b>	Any T	Any N	M1b
<b>IVC</b>	Any T	Any N	M1c

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### Appendix 3. ECOG Performance Rating Sheet

Scoring	Physical condition
0	Full normal activity ability, capable of performing all pre-illness activities without restriction.
1	Physical activity is restricted, but one can move freely and engage in light physical activities, such as general household chores or office work.
2	Able to take care of oneself but unable to do any work activities, and able to get up and move around at least 50% of waking hours.
3	Only partially self-sufficient in daily life, lying in bed or sitting for more than 50% of waking hours.
4	Completely unable to take care of oneself, completely lying in bed or sitting.
5	Death.

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