# Study Protocol

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| **Official title** | An Open-Label, Two-Arm, Phase II Clinical Trial of Neoadjuvant mFOLFOX6 Combined with Citrus Flavonoid Tablets (Alvenor) for Locally Advanced Rectal Cancer with High YWHAB Expression |
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## The Sixth Affiliated Hospital of Sun Yat-sen University

## 1. Background

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

Colorectal cancer (CRC) is the third most common malignant tumor globally, with over 1.8 million new cases annually, accounting for about 10% of all new malignant tumors, and causing more than 910,000 deaths [1]. In recent years, the incidence and mortality of CRC in China have been on the rise. By 2020, CRC had become the second most common malignant tumor in China, with 550,000 new cases annually and a mortality rate ranking fifth, with 280,000 new deaths in 2020 [2]. The disease burden of CRC in China is continuously increasing, and the prevention and control efforts for CRC remain a significant challenge. The rectum ends at the upper edge of the functional anal canal, defined as the palpable upper edge of the puborectalis muscle of the anal sphincter and the anorectal ring. Rectal cancer refers to cancerous lesions that occur in the rectum, located below the virtual line from the sacral promontory to the upper edge of the symphysis, as determined by MRI. Determining the optimal treatment plan for rectal cancer patients is a complex process. Besides deciding whether the surgery is curative or palliative, it is also necessary to consider potential functional outcomes after treatment, including maintaining or restoring normal bowel function, anal control, and preserving urinary and reproductive functions. For patients with low-lying rectal cancer, achieving tumor cure while minimizing the impact on quality of life is particularly challenging [3]. Moreover, compared to colon cancer, rectal cancer patients have a higher risk of pelvic recurrence, and local recurrence of rectal cancer is associated with a poorer prognosis [4-6].

Currently, the multimodal treatment for stage II or III rectal cancer typically includes preoperative chemoradiotherapy with fluorouracil-based regimens, total mesorectal excision (TME), and postoperative adjuvant chemotherapy[7-8]. The FOWARC study conducted by our center has demonstrated that, compared to standard preoperative chemoradiotherapy, patients who received mFOLFOX6 neoadjuvant chemotherapy showed no significant differences in tumor downstaging, disease-free survival (DFS), local recurrence rate, or overall

survival. However, the incidence of postoperative anastomotic leakage was significantly lower in patients receiving only mFOLFOX6 neoadjuvant chemotherapy, with better anal function and improved long-term quality of life. This suggests that mFOLFOX6 neoadjuvant chemotherapy can partially replace preoperative chemoradiotherapy, effectively avoiding radiotherapy-related side effects without compromising treatment efficacy. The findings have been cited in the NCCN international guidelines[9-11]. Studies have shown that tumor downstaging (ypTNM 0-I stage) is significantly associated with better DFS in locally advanced rectal cancer patients who receive neoadjuvant therapy[9-11]. The FOWARC study found that about 35% of locally advanced rectal cancer patients who received mFOLFOX6 neoadjuvant chemotherapy had tumor downstaging. This indicates that some patients may be less sensitive to mFOLFOX6 neoadjuvant chemotherapy. Identifying and screening out these patients who are resistant to mFOLFOX6 treatment, and further improving their tumor downstaging rate and prognosis through mFOLFOX6 chemotherapy combined with targeted therapy, holds promise.

The dysregulation of the Wnt/β-catenin signaling pathway is closely linked to the development and progression of various cancers, particularly in colorectal cancer (CRC) [12]. The YWHA (14-3-3) family of proteins is widely expressed in eukaryotic cells, including seven subtypes: 14-3-3β (YWHAB), 14-3-3γ (YWHAG), 14-3-3ε(YWHAH), 14-3-3ζ (YWHAZ), 14-3-3σ (YWHAS) and 14-3τ/θ(YWHAQ)[13].. These 14-3-3 isoforms exhibit highly conserved structures across different species and can form both homodimers and heterodimers, acting as molecular connectors through protein-protein interactions [14]. As the central hub of various signaling pathways, 14-3-3 proteins are involved in numerous cellular processes, including cell cycle regulation, protein transport, apoptosis, and neuronal plasticity [13-15]. More importantly, dysregulation of 14-3-3 proteins is associated with multiple diseases, such as cancer, autoimmune disorders, and metabolic disorders [13,16]. Our preliminary research has identified that the amplification of YWHAB (14-3-3β) is a key

driver of copy number variations (CNVs) in cancer cells and is associated with poor outcomes in CRC patients, suggesting its potential as a biomarker for prognosis. Furthermore, experimental evidence from cell studies and mouse models indicates that YWHAB enhances glycolysis and nucleotide metabolism, thereby promoting chemotherapy resistance in colorectal cancer (CRC). Mechanistically, YWHAB interacts with β-TrCP and competitively binds to β-catenin, inhibiting the degradation of β-catenin mediated by β-TrCP, thus promoting the development and progression of CRC and its chemotherapy resistance. Additionally, through large-scale molecular docking and drug binding affinity screening, we discovered that FDA-approved drugs hesperidin and diosmin can specifically block the interaction between YWHAB and β-TrCP, significantly enhancing the sensitivity of YWHAB high-expressing CRC organoids and patient-derived xenografts (PDX) to FOLFOX treatment. Therefore, our research has provided a deeper understanding of the chemotherapy resistance mechanism in YWHAB high-expressing CRC. The expression level of YWHAB can serve as a potential biomarker for predicting chemotherapy sensitivity in CRC patients and for achieving precise chemotherapy. Blocking the YWHAB/β-TrCP/β-catenin signaling axis is expected to be an important approach to improve the chemotherapy sensitivity of YWHAB high-expressing CRC patients.

Flavonoids are a major class of polyphenolic compounds naturally found in plants[17]. Previous studies have reported that flavonoids have multiple therapeutic effects, such as anti-inflammatory, antioxidant, and anti-cholesterol activity[18]. Naringin and dasiglucoside are common flavonoids found in citrus fruits, with highly similar molecular structures, known for their antioxidant properties, which help protect cells from damage by free radicals. Research has shown that naringin and dasiglucoside can support heart health by enhancing vascular function and reducing inflammation[18-19], and they have been proven beneficial to the brain, liver, and eyes. It has been reported that naringin and dasiglucoside have anti-inflammatory, antioxidant, anti-tumor, and antibacterial

therapeutic potential[20-22]. Clinical trials and practices have confirmed the therapeutic effects of naringin and dasiglucoside on various diseases, including cardiovascular disease, neurological disorders, type 2 diabetes, venous ulcers, hemorrhoids, fatty liver disease associated with metabolic dysfunction, and cancer[23]. Previous studies [23] have found that naringin can inhibit the protein expression level of nitric oxide synthase (iNOS), thereby reducing the production of nitrogen dioxide and prostaglandin E2 (PGE2); therefore, naringin is considered an inhibitor of cyclooxygenase-2 and iNOS, which may explain its anti-tumor and anti-inflammatory properties. A study in 2022 found that naringin has chemopreventive potential in colorectal cancer (CRC) induced by 1,2-dimethylhydrazine (DMH), and naringin prevents CRC in vivo by regulating the Smad4 and activin A signaling pathways[24]. Another study reported that hesperidin can effectively prevent the incidence of intestinal tumors in mice induced by azoxymerthane [25]. In our preliminary research, we discovered through microscale thermophoresis (MST) experiments that hesperidin has a strong binding affinity with YWHAB protein. Both in vitro and in vivo experiments have shown that hesperidin and docetaxel can effectively inhibit the interaction between YWHAB and β-TrCP. Animal experiments further suggest that, compared to the FOLFOX regimen alone, combining citrus flavonoid tablets (Aimalang, each tablet containing 500mg of citrus flavonoids, 90% docetaxel, and 10% hesperidin) with the FOLFOX regimen can enhance the inhibitory effect of FOLFOX on PDX tumors. These in vitro and in vivo results fully confirm that the combination of FOLFOX and citrus flavonoid tablets (Aimalang) can serve as a potential treatment option for patients with high YWHAB expression CRC, although this needs to be further confirmed through prospective clinical trials.

Although the efficacy of citrus flavonoid tablets in treating various diseases has been confirmed through extensive clinical research and practice, most clinical trials use 300-600 mg of pure hesperidin for oral supplementation. However, due to its extremely low water solubility, hesperidin has a relatively low bioavailability [21,26,27]. After comprehensive consideration, we plan to use

citrus flavonoid tablets (Aimalang) as an alternative to citrus flavonoids in our clinical trials. In clinical practice, citrus flavonoid tablets (Aimalang) are commonly used to treat various symptoms associated with venous and lymphatic insufficiency, as well as acute hemorrhoids. Our preliminary studies have shown that docetaxel, hesperidin, and citrus flavonoid tablets (Aimalang) can effectively inhibit the interaction between YWHAB and β-TrCP, thereby enhancing the anti-tumor effects of FOLFOX. Furthermore, Aimalang has been clinically applied, and its safety has been confirmed. Therefore, based on our preliminary research and safety findings, using Aimalang as an alternative to hesperidin to explore the neoadjuvant treatment effects of FOLFOX combined with Aimalang in locally advanced rectal cancer with high YWHAB expression is both safe and reasonable.

A common strategy to improve tumor regression in patients with locally advanced rectal cancer is to enhance neoadjuvant therapy by adding systemic chemotherapy before radiotherapy and chemotherapy. Several Phase II clinical trials have shown that [28-31] systemic adjuvant chemotherapy before radiotherapy and chemotherapy can enhance the anti-tumor response in patients with large primary tumors near the mesorectal fascia. In these trials, the proportion of patients achieving pathological complete remission slightly improved after systemic adjuvant chemotherapy before radiotherapy and chemotherapy. In 2004, a German clinical trial named CAO/ARO/AIO-94 established preoperative infusion of fluorouracil as the standard combination therapy for locally advanced rectal cancer [32]. In 2015, the CAO/ARO/AIO-04 trial [33] added oxaliplatin to neoadjuvant radiotherapy and chemotherapy based on fluorouracil, significantly improving the disease-free survival rate in patients with cT3-4 or cN1-2 locally advanced rectal cancer. The protocol established by CAO/ARO/AIO-04 is also considered a new treatment option for patients with locally advanced rectal cancer. Our 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 relevant research findings have

been frequently cited in NCCN guidelines [9-11]. The mFOLFOX6 double-drug regimen (5-fluorouracil and leucovorin calcium in combination with oxaliplatin) is a highly potent chemotherapy regimen recommended by guidelines such as NCCN, EMSO, and CSCO for the first-line treatment of advanced colorectal cancer. However, it remains unclear whether the mFOLFOX6 double-drug regimen combined with Apatinib can enhance the treatment outcomes for patients with YWHAB high-expressing locally advanced rectal cancer. This requires further clinical research to confirm.

**(2) Significance of the study**

This study aims to conduct a prospective, open-label, double-arm, Phase II clinical trial. For patients with locally advanced rectal cancer, YWHAB immunohistochemical staining will be performed on colonoscopy biopsy specimens to identify patients with high YWHAB expression. These patients will then be randomly assigned to either the mFOLFOX6 treatment group or the mFOLFOX6 combined with Citrus Flavonoid Tablets (Aimalang) treatment group for neoadjuvant therapy. After completing 4-6 cycles of neoadjuvant chemotherapy, patients will undergo preoperative assessments by their attending physicians and have their tumors surgically removed by a specialized colorectal surgery team. Postoperatively, they will receive 6-8 cycles of adjuvant chemotherapy. The effectiveness and safety of the mFOLFOX6 combined with Citrus Flavonoid Tablets (Aimalang) regimen in neoadjuvant therapy for YWHAB high-expression locally advanced rectal cancer patients will be evaluated. This includes assessing the proportion of patients achieving tumor downstaging, the proportion of patients achieving pathological complete remission, the three-year disease-free survival rate, overall survival time, and tumor regression grade (TRG). The study is based on the team's previous basic research, animal experiments, and the clinical safety of Citrus Flavonoid Tablets (Aimalang), aiming to improve the efficacy of YWHAB high-expression locally advanced rectal cancer patients and benefit more patients.

## 2. Research design and purpose

### 2.1 Overall design of the study

This study is a prospective, open, double-arm, phase II clinical trial to explore the mFOLFOX6 combined with citrus flavonoid tablets (Aimalang) regimen as neoadjuvant therapy for YWHAB high expression locally advanced rectal cancer. The study design is in accordance with ethical requirements.

### 2.2 Research purpose

**Main research objectives:**

To evaluate the proportion of patients achieving tumor downstaging (ypTNM 0-I stage) in YWHAB high-expressing locally advanced rectal cancer treated with mFOLFOX6 combined with Citrus Flavonoid Tablets (Aimalang) as a neoadjuvant therapy, to provide evidence that Citrus Flavonoid Tablets (Aimalang) enhances the effectiveness of the mFOLFOX6 regimen in this patient population.

**Secondary study objectives:**

Efficacy evaluation: The proportion of pathological complete remission, 3-year disease-free survival (DFS), overall survival time (OS) and tumor regression grade TRG were evaluated in patients with YWHAB high expression locally advanced rectal cancer treated with mFOLFOX6 combined with citrus flavonoid tablets (Aimalang) as neoadjuvant therapy.

Safety evaluation: The treatment-related adverse reaction rate (grade 3 or above) of mFOLFOX6 combined with citrus flavonoid tablets (Aimalang) regimen for neoadjuvant treatment of YWHAB high expression local advanced rectal cancer patients was evaluated.

### 2.3 Study endpoints

**Main endpoints and definitions:**

This study primarily evaluates the proportion of patients with YWHAB

high expression locally advanced rectal cancer who achieve tumor downstaging (ypTNM 0-I stage) after neoadjuvant therapy with mFOLFOX6 combined with Citrus Flavonoid Tablets (Aimianlang). The tumor downstaging rate (ypTNM 0-I stage) refers to the percentage of patients with locally advanced rectal cancer who, after receiving neoadjuvant chemotherapy with mFOLFOX6 or mFOLFOX6 + Citrus Flavonoid Tablets (Aimianlang), have their postoperative pathological stage of the surgical specimen classified as ypTNM 0-I stage.

**Secondary endpoints and definitions:**

(1) The degree of pathological remission is defined as follows: ① Complete Remission (CR): All tumor target lesions have disappeared, no new lesions have appeared, and tumor markers are normal, lasting at least 4 weeks. ② Partial Response (PR): The sum of the maximum diameters of tumor target lesions has decreased by at least 30%, lasting at least 4 weeks. ③ Stable Disease (SD): The sum of the maximum diameters of tumor target lesions has not decreased by more than PR, or increased by less than PD. ④ Disease Progression (PD): The sum of the maximum diameters of tumor target lesions has increased by at least 20%, or new lesions have appeared.

(2) The 3-year disease-free survival rate (DFS) is the proportion of patients who have not experienced 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 reason, whichever occurs first.

(3) Overall survival time (OS) is the time from randomization to death for any reason.

(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 means residual cancer with a proliferative response to connective tissue; TRG 3 means minimal evidence of tumor response.

(5) Safety indicators, treatment-related adverse reaction rate (grade III and

above): Antitumor drugs/therapy In clinical trials, drug adverse reactions determined to be related to drug therapy at grade III and above, excluding nonspecific infusion reactions.

## 3. Selection and withdrawal of subjects

### 3.1 Inclusion criteria:

(1) Histopathology was diagnosed as rectal adenocarcinoma, all other histological types were excluded, and the colonoscopy report or clinical physical examination suggested the presence of hemorrhoids;

(2) The clinical pathological stage of the tumor is T3-4 or N+ and M0; 8th edition AJCC TNM staging, Appendix 1)

(3) Immunohistochemical staining of tissue samples showed that YWHAB was highly expressed in rectal cancer patients;

(4) The age of obtaining informed consent is 18-75 years old;

(5) Eastern United States Cooperative Group Physical Status Score (ECOG) was 0-1 points (refer to Appendix 3);

(6) No previous systemic anti-tumor treatment for rectal cancer, including cytotoxic drugs, immune checkpoint inhibitors, molecular targeted therapy, endocrine therapy, etc.;

(7) Based on the following laboratory test values obtained during the screening period, appropriate organ function is present: white blood cell count ≥ 3 x 109/L, neutrophil count ≥ 1.5 x 109/L, platelet count ≥ 75 x 109/L, serum total bilirubin ≤ 1.5 x upper limit of normal (UNL), aspartate aminotransferase or alanine aminotransferase ≤ 2.5 x UNL, serum creatinine ≤ 1.5 x UNL;

(8) Women of childbearing age must have a negative serum pregnancy test within 3 days prior to the start of study medication and be willing to use a medically recognized effective contraceptive method (e.g., IUD, pill, or condom) during the study and for 3 months after the last dose of study medication;

(9) For male subjects whose partner is a woman of reproductive age, effective contraception was used during the study and for 3 months after the last study dose;

(10) The subject has given his/her own consent and signed an informed consent form, and the subject is willing and able to comply with the planned visits, research treatment, laboratory tests and other trial procedures.

**3.2 Exclusion criteria:**

(1) Whole body CT, MR or PET-CT (at least including chest, whole abdomen and pelvis) confirmed distant metastasis;

(2) Whole genome testing for DPD enzyme deficiency or 7/7 homozygotes for UGT1A1*28 locus in patients;

(3) The patient has complete intestinal obstruction, active bleeding or perforation and requires emergency surgery;

(4) Previous or concurrent presence of other active malignancies (excluding malignancies that have been treated curatively and have not recurred for more than 5 years or carcinoma in situ that can be cured by adequate treatment);

(5) Having had a thrombotic or embolic event, such as a cerebrovascular accident (including transient ischemic attack), pulmonary embolism, or deep vein thrombosis, within 12 months prior to enrollment;

(6) In the 12 months prior to enrollment, the following conditions occurred: myocardial infarction, severe/unstable angina, NYHA class II or higher heart failure, clinically significant supraventricular or ventricular arrhythmias, and symptomatic congestive heart failure;

(7) Systemic use of antibiotics for more than 7 days within 4 weeks prior to enrollment, or unexplained fever>38.5°C during screening/ prior to first dose (fever due to tumor can be enrolled by investigator);

(8) Received major surgery or severe trauma, such as cesarean section, thoracotomy, or organ resection through laparoscopic surgery, within 2

months prior to enrollment (surgical incisions should be completely healed before enrollment in this clinical trial);

(9) Known to have human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS)-related diseases;

(10) The presence of interstitial lung disease, non-infectious pneumonia or uncontrolled systemic diseases (e.g., diabetes, hypertension, pulmonary fibrosis and acute pneumonia);

(11) Untreated active hepatitis (Hepatitis B, defined as HBV-DNA ≥ 500 IU/mL; Hepatitis C, defined as HCV-RNA above the detection limit of the assay) or co-infection with hepatitis B and C;

(12) A known or suspected history of allergy to any relevant drug used in the study;

(13) Patients who the researchers thought were not suitable for the study.

**3.3 Exit criteria:**

Participants may withdraw from the trial at any time, or be asked to withdraw by the investigator or sponsor for safety or behavioral reasons or because they are unable to comply with the study visit time or steps required by the protocol. Withdrawal criteria include:

(1) The subject withdrew the informed consent to participate in the study and refused further follow-up;

(2) Clinical adverse events, laboratory abnormalities or concurrent diseases occur and the subject considers it not in the best interest of the subject to continue to participate in the study;

(3) A general deterioration of health status, making it impossible to continue participation in the trial;

(4) Pregnancy events in subjects during the study;

(5) Significant deviations from the protocol, such as unqualified or non-compliance of subjects, were found after enrollment;

(6) Disappearance;

(7) Subject death;

(8) The researchers identified other situations in which withdrawal from the study was necessary, such as significant protocol violations.

## 4. Research treatment

### 4.1 Study treatment grouping

This study is a prospective, open-label, double-arm, Phase II clinical trial that includes a neoadjuvant treatment group using the mFOLFOX6 regimen and a neoadjuvant treatment group combining the mFOLFOX6 regimen with Citrus Flavonoid Tablets (Aimalang). The study aims to enroll patients with locally advanced rectal cancer who are in clinical stage I, II, or III. Endoscopic biopsy specimens will be collected for YWHAB immunohistochemical testing to identify patients with high YWHAB expression in locally advanced rectal cancer. These patients will then be randomly assigned to either the mFOLFOX6 treatment group or the mFOLFOX6 combined with Citrus Flavonoid Tablets (Aimalang) treatment group for neoadjuvant therapy. The mFOLFOX6 regimen involves 4-6 cycles before surgery and 6-8 cycles after surgery, with each cycle lasting 14 days: on day 1, 85 mg/m² of oxaliplatin is administered intravenously over 180 minutes; on day 1, 400 mg/m² of leucovorin is administered intravenously over 120 minutes; and on day 1, 2400 mg/m² of 5-fluorouracil is administered continuously intravenously for 46 hours. The Citrus Flavonoid Tablets (Aimalang) treatment regimen involves taking 500mg of Citrus Flavonoid Tablets (Aimalang) orally three times daily, with doses given on days 1-14 of each 14-day cycle.

This trial allows for dose adjustments. Patients who experience disease progression during neoadjuvant therapy may stop the study treatment and directly undergo surgery or follow local guidelines. If a patient cannot tolerate the planned 6 cycles of neoadjuvant therapy, early surgery can be considered. Any patient who has received another anticancer regimen before surgery will stop the study treatment and be managed according to local guidelines. The postoperative

treatment for both groups is determined by the investigator, which may include continuing the mFOLFOX6 regimen combined with Citrus Flavonoid Tablets (Aimalang). It is important to note that all patients in the control group are prohibited from taking Citrus Flavonoid Tablets (Aimalang) without authorization during the trial. If a patient needs to take this medication for any reason, they should consult their primary physician, who will decide whether to use an alternative or discontinue the patient's participation in the trial.

## 4.2 Dose adjustment of the investigational drug

### 4.2.1 Protocol for discontinuation or dose adjustment of treatment

During the study treatment, adverse events will be continuously monitored, and participants will be instructed to promptly inform their attending physician of any adverse events. In cases of mild toxicity, if the investigator, after consultation with the medical monitor or sponsor, deems that dose adjustment or delayed administration is beneficial to the participant's safety, the dose may be adjusted or administration may be postponed. If a dose interruption occurs, the resumption of medication can be delayed by up to 14 days to allow the participant to recover from the toxicity. If the participant has not recovered to the study-specified starting dose within 14 days, the trial will be terminated.

### 4.2.2 Compliance with the criteria for starting the next treatment cycle

The study drug can be administered only after the results of the examination on the first day before the next treatment cycle confirm that all the criteria listed in the table below are met.

| **Table 1.1 Conforms to the criteria for starting the next treatment cycle** | |
|---|---|
| Hemoglobin values | ≥ 90g/L |
| Neutrophil | ≥ 1.5×109/L |

| | |
|---|---|
| count | |
| platelet count | ≥ 75×109/L |
| serum total bilirubin | ≤ 1.5×UNL |
| Aspartate transferase | ≤ 3×UNL |
| Alanine transferase | ≤ 3×UNL |
| Serum creatinine | ≤ 1.5×UNL |
| diarrhoea | Compared with baseline, the number of bowel movements per day increased by less than 4 times |
| Oral mucositis | There was only mucosal erythema without patchy ulceration or pseudomembrane formation |
| Sensory nerve disorder | Feelings of abnormality or dullness that do not interfere with daily activities |
| *Note: If an adverse reaction other than the above is found that causes a delay in the start of the next treatment cycle, the investigator will determine whether the administration of the next treatment cycle can be started when the adverse reaction is reduced or recovered.* | |

**4.2.3 Study drug dose levels**

If a reduction in drug dosage is required in medical practice, the dose levels of oxaliplatin and 5-fluorouracil can be adjusted according to the criteria listed in Table 1.2. Each adjustment should only reduce one dose level, and the total number of dose level adjustments throughout the study period should not exceed two. The fixed dose of suberythrocitrin is 400 mg/m² or 7.5 mg/kg (for the 3-week regimen). All dose level adjustments must be clearly documented with the reasons for the adjustment.

| Table 1.2 Study drug dose levels | | | |
|---|---|---|---|
| Name of drug | Initial dose ($mg/m^2$) | Dose level-1 ($mg/m^2$) | Dose level-2 ($mg/m^2$) |
| oxaliplatin | 85 | 65 | 50 |
| 5-FU | 2400 | 2300 | 2200 |
| Citrus flavonoid tablets (Aimalang) | Take orally, 500mg Tid | Take orally, 500mg twice daily | Take orally, 500mg Qd |

#### 4.2.4 Dose adjustment criteria

Dose adjustments should be made based on the severity and duration of adverse events following the previous treatment cycle, as classified by the NCI CTCAE version 6.0. If severe hematological or liver-related adverse events occur (excluding liver function impairment due to disease progression), the doses of oxaliplatin, 5-FU, and Citrus Flavonoid Tablets (Aimalang) should be reduced simultaneously. If severe diarrhea or mucosal toxicity (excluding vomiting and hair loss) occurs, the doses of 5-FU and Citrus Flavonoid Tablets (Aimalang) should also be reduced simultaneously. See Table 1.3 for details.

| Table 1.3 Dose adjustment for selected adverse events | | |
|---|---|---|
| adverse event | Severe disease classification (NCI CTCAE version 6.0) | Study dose adjustment |
| granulocytopenia | Grade 4 (<0.5 x 109/L) | Reduce the dose level |
| Febrile granulocytopenia | Grade 3 or above (ANC <1.0 x 109/L, body temperature ≥ 38.5℃) | Reduce the dose level |
| thrombocytopenia | Grade 3 or above (<50 x 109/L) | Reduce the dose level |
| Aspartate transferase | Grade 3 or higher (>5 x ULN) | Reduce the dose |

| | | |
|---|---|---|
| | | level |
| Aspartate transferase | Grade 3 or higher (>5 x ULN) | Reduce the dose level |
| serum total bilirubin | Grade 3 or higher (>3 x ULN) | Reduce the dose level |
| Note: If the severity of an adverse event does not meet the dose reduction criteria, but the time of administration at the beginning of the next treatment cycle is still not in accordance with the administration criteria, and it occurs repeatedly for more than 2 times, the corresponding drug should be reduced by one dose level according to the above requirements. | | |

Oxaliplatin can cause characteristic neurotoxic reactions, mainly manifested as sensory nerve disorders. When the above serious adverse events occur, the oxaliplatin dose should be reduced separately, and the standard adjustment is shown in Table 1.4.

**Table 1.4 Dose adjustment for cisplatin-related neurotoxicity**

| Adverse event | Happen | Study dose adjustment of drugs |
|---|---|---|
| Grade 1: abnormal or dull sensation, which can completely subside within one week | Any one of them happen | No adjustment required |
| Grade 2: abnormal or dull sensation, which does not completely subside during the 2 treatment cycles | Any one of them happen | Reduce the dose level |
| Level 3: Abnormal or dull sensation with associated dysfunction | First time happened | If the next cycle of treatment can be resumed after discontinuation, |

| | | |
|---|---|---|
| | | reduce the dose level by 1 |
| | The reaction failed to recover to grade 2 after discontinuation of the drug | Permanent discontinuation of medication |

### 4.3 Combination and concomitant drugs

#### 4.3.1 Prohibited combination and concomitant drugs

No other anti-tumor treatment is allowed during the study, including: chemotherapy, molecular targeted therapy, hormone therapy, immunotherapy, biological therapy, non-palliative radiotherapy and immunomodulators (including but not limited to: interferon, interleukin-2, etc.).

#### 4.3.2 Other anti-tumor treatment and experimental drugs

During the study treatment, the subject will not be allowed to receive other anti-tumor treatments not specifically specified in this protocol. Other systemic anti-tumor treatments will not be allowed.

## 5. Research process

### 5.1, Screening period

After the patient signs the informed consent form, they enter the screening phase. Endoscopic biopsy specimens from patients with locally advanced rectal cancer are collected for YWHAB immunohistochemical testing to identify those with high YWHAB expression, and these patients are selected for inclusion in the study. Before signing the informed consent, patients undergo routine laboratory tests and imaging assessments as required by clinical practice. If the relevant data is available within the specified time window, it can be utilized.

The following screening should be completed within 28 days prior to the start of study drug therapy:

- Obtain informed consent signed by the subject;

- Collect demographic data: name, sex, date of birth, height, weight, etc.;
- Collect adverse events: record adverse events from the time of signing informed consent;
- Tumor diagnosis: date of pathological confirmation, pathological grading, clinical imaging staging (TNM), clinical staging, etc.;
- History of cancer treatment;
- Imaging examination: enhanced CT of chest, abdomen and pelvis;
- The YWHAB immunohistochemical detection was performed on the endoscopic biopsy specimens.
- (Non-essential) Specify the KRAS exon 2 codons 12 and 13, exon 3 codons 59 and 61, exon 4 codons 117 and 146, NRAS exon 2 codons 12 and 13, exon 3 codons 59 and 61, exon 4 codons 117 and 146, and BRAF V600E gene status;
- (Mandatory) MMR/MSI detection of tumor tissue;
- The following screening should be completed within 7 days prior to the start of study drug therapy:
- Weight and ECOG score;
- Life 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, oral cavity, respiratory system, cardiovascular system, abdomen, reproductive and urinary system, musculoskeletal, nervous system and mental state;
- Complete blood count: red blood cell count, hemoglobin, platelet count, white blood cell count, neutrophil count and lymphocyte classification count;
- Routine urine: white blood cells, red blood cells, protein in urine; if protein in urine is greater than or equal to 2+,24-hour urine protein quantification should be added;
- Blood biochemical: ALT, AST, γ-GT, TBIL, DBIL, AKP, BUN or urea (preferably BUN), TP, ALB, Cr, GLU, $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Cl^-$;

- Tumor 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 abnormalities, other relevant tests should be done according to the investigator's judgment;
- Echocardiography: at least the assessment of left ventricular ejection fraction (LVEF) should be included;
- Pregnancy test: suitable for women of childbearing age, using serum pregnancy test.

**5.2, treatment period**

The treatment period begins with the first dose of the study drug and ends when the study treatment is discontinued. The first study medication should be administered as close as possible to the completion of the screening period to confirm eligibility. All examinations and assessments, except for imaging studies, should be completed within three days before the first dose. For the laboratory tests (complete blood count, urinalysis, fecal occult blood test, biochemical blood tests, coagulation function, and thyroid function) and electrocardiogram on the first day of the first cycle, if baseline laboratory tests were conducted within seven days before the first dose, they do not need to be repeated.

The following assessments should be completed prior to each study treatment administration:

Weight and ECOG score;

vital sign ；

- check-up ；
- Complete blood count: red blood cell count, hemoglobin, platelet count, white blood cell count, neutrophil count and lymphocyte classification

count;

- Blood biochemical: ALT, AST, γ-GT, TBIL, DBIL, AKP, BUN or urea (preferably BUN), TP, ALB, Cr, GLU, K+, Na+, Ca2+, Mg2+, Cl-;
- Routine urine: white blood cells, red blood cells, protein in urine; if protein in urine is greater than or equal to 2+,24-hour urine protein quantification should be added;
- Coagulation function: activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen (FIB), international normalized ratio (INR);
- Tumor markers;
- 12. Lead electrocardiogram: attention should be paid to QT, QTc and P-R intervals. If there are abnormalities, other relevant tests should be done according to the investigator's judgment;
- Document adverse events;
- Imaging evaluation: Imaging evaluation was performed after 6 cycles and 12 cycles of mFOLFOX6 regimen adjuvant chemotherapy, including chest, abdominal and pelvic enhanced CT and pelvic MRI. Any subject suspected of disease progression during treatment (e.g., symptom deterioration) could undergo unplanned imaging evaluation.

### 5.3 End of treatment/exit from study visit

When the subject has completed the study treatment/exited the study, relevant assessments and examinations are required. They should be performed as follows:

- Weight and ECOG score;
- Life 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, oral cavity, respiratory system, cardiovascular system, abdomen, reproductive and urinary system, musculoskeletal, nervous system and mental state;
- Complete blood count: red blood cell count, hemoglobin, platelet count,

white blood cell count, neutrophil count and lymphocyte classification count;

- Routine urine: white blood cells, red blood cells, proteinuria; if proteinuria is greater than or equal to 2+,24-hour urine protein quantification should be added;
- Blood biochemical: ALT, AST, γ-GT, TBIL, DBIL, AKP, BUN or urea (preferably BUN), TP, ALB, Cr, GLU, K+, Na+, Ca2+, Mg2+, Cl−;
- Tumor 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 abnormalities, other relevant tests should be done according to the investigator's judgment;
- Document adverse events.

**5.4, follow-up period**

The survival follow-up period ends when the subject dies, is lost to follow-up, withdraws from informed consent and refuses to continue providing information, or the sponsor terminates the study. During this period, a visit is conducted every three months through telephone or other effective methods to collect survival information and details about subsequent anti-tumor treatments (if the subject starts new anti-tumor treatment, the treatment plan and its duration should be recorded).

For subjects without imaging evidence of disease progression, imaging assessments should continue to be conducted at the frequency specified in this study until the subject experiences disease progression, death, loss to follow-up, withdrawal of informed consent and refusal to continue providing information, initiation of other anti-tumor treatments, or termination of the study by the sponsor. Every effort should be made to obtain imaging evidence of disease

progression in these subjects.

## 6. Effectiveness evaluation

### 6.1 Effectiveness parameters:

The main efficacy indicator of this study was the proportion of patients in each treatment group who achieved tumor downstaging (ypTNM 0-I stage), and the secondary efficacy indicators were the proportion of patients with complete pathological remission, 3-year disease-free survival (DFS), overall survival time (OS) and tumor regression grade TRG.

### 6.2 Evaluation criteria of effectiveness:

This study evaluated the proportion of patients with YWHAB high expression locally advanced rectal cancer who achieved tumor downstaging (ypTNM 0-I stage) in neoadjuvant treatment with mFOLFOX6 combined with citrus flavonoid tablets (Aimalang).

(1) Downstaging rate of tumor (ypTNM 0-I stage): the proportion of patients with locally advanced rectal cancer who received mFOLFOX6 or mFOLFOX6 combined with citrus flavonoid tablets (Aimalang) as neoadjuvant chemotherapy, and the proportion of patients with postoperative pathological stage of ypTNM 0-I stage in surgical specimens.

(2) The degree of pathological remission is defined as follows: ① Complete Remission (CR): All tumor target lesions have disappeared, no new lesions have appeared, and tumor markers are normal, lasting at least 4 weeks. ② Partial Response (PR): The sum of the maximum diameters of tumor target lesions has decreased by at least 30%, lasting at least 4 weeks. ③ Stable Disease (SD): The sum of the maximum diameters of tumor target lesions has not decreased by more than PR, or increased by less than PD. ④ Disease Progression (PD): The sum of the maximum diameters of tumor target lesions has increased by at least 20%, or new lesions have appeared.

Subjects who did not meet the primary endpoint criteria are included in the denominator but not in the numerator.

(3) When determining the disease-free survival (DFS), clinical deterioration without clear evidence of disease progression according to the RECIST 1.1 criteria is not considered a disease progression. For subjects who died without prior reported disease progression, it is considered that the disease progressed at the time of death. Subjects who have neither experienced disease progression nor died will be censored at their last evaluable tumor evaluation date. Independent central imaging review will be conducted to confirm the independent central imaging service provider of the subject, which will receive all images from the research center and assess the subject's disease progression according to the RECIST 1.1 criteria.

(4) Survival record and evaluation: the subject will be followed up every 3 months on disease and survival after the end of treatment or withdrawal from the group, by telephone follow-up, until death, loss to follow-up, withdrawal of informed consent and refusal to continue to provide information or termination of the study by the sponsor.

(5) Tumor regression grading TRG: TRG 0 means no residual tumor cells, TRG 1 means a single cell or small group of cells; TRG 2 means residual cancer with a proliferative response to connective tissue; TRG 3 means minimal evidence of tumor response.

## 7. Safety evaluation

### 7.1 Safety parameters

The safety parameters of this study include clinical symptoms, vital signs, physical examination, and laboratory tests (such as complete blood count, biochemical tests, and coagulation function). The study evaluates adverse events according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE version 6.0) (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm),

including the type, incidence, severity, onset and resolution time, whether they are serious, their relevance to the study treatment, and their outcomes.

### 7.2 Definition of adverse events

Adverse events refer to any adverse medical outcomes experienced by patients or clinical trial participants after taking the medication, which may not necessarily be causally linked to the treatment. Therefore, adverse events can include any adverse and other unexpected physical signs (including abnormal laboratory findings) and symptoms, as well as diseases that are temporally associated with the use of the medical product, regardless of whether they are considered related to the study drug. These events must at least include the following:

- Worsening of the original (pre-clinical) medical condition/disease (including worsening of symptoms, signs or laboratory abnormalities);
- Any new medical condition (including symptoms, signs or newly diagnosed disease);
- Abnormal clinically significant laboratory test values or results.

### 7.3 Definition of serious adverse events

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

- Fatality (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 that death would be caused by a more serious event);
- Resulting in hospitalization or prolonged hospital stay;
- Causing lifelong or severe disability/functioning impairment;
- Causing birth defects or birth defects;
- Of medical significance or requiring intervention to prevent any of these outcomes.

Tumor progression or deterioration (including the appearance of new metastases and death due to disease progression) that occurs during the course of the study should be part of the efficacy evaluation and should not be reported as an adverse event or serious adverse event.

### 7.4 Definition of serious adverse events

Disease progression is defined as the deterioration of a subject's condition due to the study's indications. This includes both imaging and clinical symptom and sign advancements. The appearance of new metastases relative to the primary tumor or the progression of existing metastases is considered disease progression. Life-threatening symptoms and signs resulting from disease progression, requiring hospitalization or extended hospital stays, or leading to permanent or severe disability affecting work capacity, are not reported as serious adverse events. However, deaths caused by symptoms and signs due to disease progression are reported as Serious Adverse Events (SAE).

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

#### 7.5.1 Criteria for judging 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 on the CRF. If an adverse event is not included in NCI CTCAE version 6.0, the following five-level classification will be used:

- Mild: no clinical symptoms or mild clinical symptoms; only clinical or laboratory abnormalities; no treatment is required.
- Moderate: Requires minor, localized or non-invasive treatment; limited use of tools and other daily activities consistent with age.
- Severe; serious or medically severe symptoms that are not life-threatening at the time of presentation; hospitalization or prolonged hospital stay; disability; and limited ability to perform activities of daily living.

- Life-threatening; immediate life danger and urgent medical treatment is required
- Death: Death associated with adverse events.

### 7.5.2 Judgment of the relationship between adverse events and study treatment

The following criteria should be used to assess the relationship between adverse events and treatment:

- It is likely to be relevant (must have the first three): This category refers to adverse events for which there is a high degree of certainty that they are related to the investigational drug. An adverse event may be considered "likely to be relevant" if the following criteria are met:
    1. The occurrence of adverse events is associated with the use of drugs in a reasonable time.
    2. The known patient disease status, environmental or toxic factors or other treatments used by the patient do not reasonably explain the adverse event.
    3. After the dose is stopped or reduced, the adverse reactions will disappear or be alleviated. (However, there are some important exceptions, some drug-related adverse reactions will not disappear even after the drug is stopped; such as: (1) bone marrow suppression, (2) delayed motor dysfunction)
    4. The adverse events were consistent with the pattern of suspected drug reactions.
    5. When used again, the adverse event reappeared.
- May be related (must have the first two): This category refers to adverse events that are unlikely to be related to the administration of the investigational drug but cannot be definitively excluded from association. An adverse event may be considered "may be related" if the following criteria are met:

1. The occurrence of adverse events is associated with the use of drugs in a reasonable time.
2. Adverse reactions may be caused by the patient's disease status, environment or toxic factors or other concomitant treatments used by the patient.
3. The adverse events were consistent with the pattern of suspected drug reactions.

- May be irrelevant (must have the first two): This category applies to adverse events that meet the following criteria:
  1. The occurrence of adverse events is not reasonably time-related to drug use.
  2. Adverse events were clearly caused by the patient's disease status, environment or toxic factors or other concomitant treatments used by the patient.
  3. The adverse events did not conform to the pattern of suspected drug reactions.
  4. When the drug was used again, the adverse events did not occur or worsen.
- Irrelevant: This category refers to adverse events that are clearly and definitively judged to be caused only by external factors (disease, environment, etc.) and do not meet the criteria for drug-relatedness under "probably irrelevant", "probably related" or "likely related".

### 7.5.3. Abnormal laboratory examination

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

Laboratory abnormalities 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;
- Changes in study medication are required (e.g., dose adjustment, temporary interruption or permanent discontinuation of administration)
- Changes in concomitant therapy are required (e.g., add corresponding therapy, discontinue concomitant therapy or administration, discontinue or make other changes);
- The researchers considered it of important medical significance (the researchers decided whether the independent laboratory test abnormalities could be classified as AE based on scientific medical judgment).

## 7.6 Follow-up and reporting of adverse events

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

The collection period of adverse events starts from the date of signing the informed consent form by the subject and ends 30 days after the last study drug.

The collection period of serious adverse events begins with the signing of informed consent by the subject. For serious adverse events unrelated to the study drug, the collection period is 90 days after the last study drug or the start of new anti-tumor treatment (whichever comes first); after that, only serious adverse events related to the study drug will be collected.

Follow-up for adverse events or serious adverse events should continue until the event resolves, is alleviated to baseline levels or ≤1 level, reaches a stable state, or is reasonably explained (such as loss to follow-up or death). The best possible outcome should be sought. Researchers should inquire about any adverse events or serious adverse events that have occurred since the last visit during each follow-up visit and provide timely follow-up information as requested by the sponsor.

### 7.6.2 Reporting of serious adverse events:

During the study, regardless of the treatment received by the patient, any serious adverse events or serious laboratory test results that occur must be reported by the investigator to the CFDA Serious Adverse Event Report Form

and the adverse event report form, and reported to the designated contact within 24 hours of learning:

Contact person for serious adverse event reporting: GCP Office, Sixth Affiliated Hospital of Sun Yat-sen University

Contact person of the research group leader: Hu Tuo (The Sixth Affiliated Hospital of Sun Yat-sen University)

Email: hutuo3@mail.sysu.edu.cn

Address: No.26, Erheng Road, Yuancun, Tianhe District, Guangzhou

The contact person for serious adverse event reporting should, within one working day of receiving a report from a researcher, fax the signed form to the lead unit. The report must adhere to the guidelines and standards set by the International Council for Harmonization (ICH) for the management of safety data in clinical trials and the definition of 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 event page of the CRF in a retrieval format. The collection period for adverse events of each patient is from the date of signing the informed consent form to 30 days after the last medication administration. The minimum requirements for recording on the adverse event report form include: the subject's identity, the drug, the duration of the adverse event, the start date of the event, and the causality.

**7.6.4 Follow-up of laboratory abnormalities**

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

### 7.6.5. Pregnancy reporting

Pregnancy should be strictly avoided during the study. If a female subject becomes pregnant during the study, the study treatment must be discontinued immediately and the investigator must be notified immediately. Pregnancy occurring within 6 months after completion of study treatment must also be reported to the investigator.

All male participants must use effective contraception throughout the study and for 6 months after the study ends. If a male patient's partner becomes pregnant during the study or within 6 months after the treatment ends, they should report this to the investigator and the sponsor. The investigator must 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 or induced abortion, delivery details, birth defects or congenital malformations of the newborn, deformities and abnormalities of stillbirths, and complications of the mother and newborn. The time of reporting and visit should be referred to serious adverse events.

The investigator should advise the patient of the risks and possible effects on the fetus of continuing the pregnancy and should continue to monitor the patient until the end of the pregnancy. Information on the outcome of the pregnancy must be provided to the sponsor.

## 8. Safety evaluation

### 8.1 Analysis of population

- Informed Consent Set ICS: This analysis set will include all subjects who have signed an informed consent form.
- Intention-to-treat (ITT) analysis set: According to the principle of intention-to-treat analysis, all patients who were randomly assigned to the group at the time of randomization were analyzed according to the group into which they were assigned. This analysis set is the main analysis set for

efficacy endpoints.

- The Full Analysis Set (FAS) is defined as the group of all participants who were enrolled and received at least one dose of the study drug, following the intention to treat (ITT) principle. In analyses based on the FAS, participants are included according to their assigned group at enrollment, regardless of the actual treatment they received;
- In line with the protocol analysis set (Per-Protocol Set, PPS), ITT patients who did not experience significant protocol deviations that affected the efficacy assessment were included. The PP population was used for sensitivity analysis of the primary and key secondary efficacy endpoints.
- Safety set (Safety Set, SS): Will include all subjects who have received at least one study drug treatment. The safety set is the primary population for safety analysis.

**8.2 General analysis**

The mean, standard deviation, median, maximum and minimum values were used to summarize the measurement data; the frequency and percentage were used to summarize the count data; Kaplan-Meier survival rate was used to estimate the survival rate and draw the survival curve for time-event data, and the overall 95% confidence interval of median time was estimated when necessary.

**8.3 Efficacy analysis and statistical methods**

For the proportion of patients in each treatment group who achieved tumor downstaging (ypTNM 0-I stage), the proportion of participants with pathological complete remission, and the tumor regression grading (TRG), statistical analysis was conducted using mean values and standard deviations. For the three-year disease-free survival rate and overall survival time, Kaplan-Meier curves were plotted, and the 95% confidence interval for the median time was calculated using the Brookmeyer Crowley method. The Log-rank test was used to compare differences between groups, and the hazard ratios (HR) for each endpoint were

estimated using the Cox proportional hazards model, exploring the impact of factors other than treatment on these endpoints. Additionally, the number and percentage of cases that occurred at each endpoint, as well as the number and percentage of censored cases, were calculated and categorized by censoring reason.

### 8.4 Safety analysis and statistical methods

The security analysis will be based on the security set. The security analysis is limited to descriptive statistical summaries, including but not limited to the following aspects, which 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 correlation;
- Abnormal occurrence of laboratory indicators, vital signs and electrocardiogram data.

The security analysis will be based on descriptive statistical summaries and based on the security set.

This study will use the standard medical terminology set (MedDRA) of pharmaceutical management to code adverse events. The study will use tables to list all adverse events, drug-related adverse events, serious adverse events, and drug-related serious adverse events that occurred during treatment, according to the NCI CTCAE version 6.0 standard and using the worst grading. Additionally, the study will summarize the laboratory test parameters during the research period according to the NCI CTCAE version 6.0 standard and using the worst grading.

### 8.5 Sample size determination

This study primarily focuses on the rate of tumor downstaging (ypTNM 0-I stage) in each treatment group. Based on current data, after

neoadjuvant chemotherapy with mFOLFOX6, approximately 35% of locally advanced rectal cancer patients achieve tumor downstaging (ypTNM 0-I stage). Among these, about one-third (n=90) of YWHAB high-expressing locally advanced rectal cancer patients achieved a tumor downstaging rate of about 25%, while about two-thirds (n=90) of YWHAB low-expressing locally advanced rectal cancer patients achieved a tumor downstaging rate of about 43%. It is estimated that the tumor downstaging rate for YWHAB high-expressing patients receiving mFOLFOX6 combined with citrus flavonoid tablets (Aimalang) neoadjuvant chemotherapy is 40%, with a significance level of 0.05, an efficiency of 0.8, and a dropout rate of 10%. The sample size was estimated to be 236 using PASS V15 software.

## Ethical considerations

### 9.1 Local regulations/Declaration of Helsinki

Researchers must ensure that their studies fully comply with the principles of the Declaration of Helsinki or the laws and regulations of the research location to maximize the protection of individuals. The studies must fully adhere to the principles outlined in the ICH guidelines "GCP Operational Guidelines (January 1997)" or meet local regulatory requirements to provide greater protection for participants. In other countries with GCP, researchers will strictly follow the relevant regulations.

### 9.2 Good Clinical Practice for drugs

This study will be conducted in accordance with the ethical principles outlined in the Good Clinical Practice (GCP) as defined by the International Council for Harmonisation (ICH). The study will follow the protocol, which, along with any amendments and informed consent forms from participants, will be reviewed and approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) prior to the study's commencement. Any

serious violations must be immediately reported to the sponsor and the principal investigator. Serious violations refer to actions that contravene the principles and requirements of GCP related to the study or protocol, potentially affecting or severely impacting the safety, physical or mental health, or scientific value of the study subjects. Participants in this study must have the necessary qualifications through education, training, and relevant work experience. This study will not involve individuals who have been subject to sanctions or are suspected of scientific misconduct or deception. To ensure compliance with all regulations and to guarantee the treatment of all aspects of the study, strict adherence to the rules and regulations is required.

### 9.3 Institutional Review Board/Independent Ethics Committee

Before the research begins, researchers must obtain written and dated approval or supporting opinion documents from the IRB/IEC for the study protocol, informed consent form, and other written information provided to participants. Researchers or sponsors should also provide the IRB/IEC with a copy of the investigator's brochure or product manual, as well as any updates to the information to be provided to participants.

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

### 9.4 Informed consent

Researchers must ensure that the subjects or their legal representatives (for those unable to sign the informed consent form in person) are fully aware and understand the purpose of the trial, potential risks, and other key issues related to the clinical trial they are volunteering for. Before participating in this clinical study, each subject or their legal representative must voluntarily sign a written informed consent form, including any screening procedures to determine eligibility criteria. Researchers or designated personnel must inform the subjects

of their right to refuse participation or withdraw from the study at any time for any reason.

The rights, safety and health of the subjects are the most important matters that researchers should consider, and should take precedence over the scientific and social values of the research.

## 10、Data collection, management and quality assurance

The data from this study will be recorded using CRFs, which will be transcribed from paper original documents to the CRFs by the research center. The study monitors will verify and cross-check the CRFs against the GCP-compliant records of the researchers (original document verification) to ensure the accuracy and reliability of the data collection. All observed patients at each participating center will undergo original document verification. A discrepancy report will be generated and provided to the research center for analysis by the researchers. Additionally, the CRF data will continue to be reviewed to ensure its medical and scientific validity.

## 11、Case report form

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

## 12. Confidentiality of test documents and subject records

Researchers must ensure the anonymity of participants and prevent unauthorized parties from learning their identities. In CRFs or other documents submitted to the sponsor, participants should be identified by an identification code rather than their names. Researchers should maintain a record of participant enrollment that includes the code, name, and address. Certain documents, such as the written informed consent form signed by the participant and the enrollment records, should always be kept strictly confidential.

## 13. Conditions for modification of research plan

Only after consultations between the sponsor's appropriate representative and the researchers can the research protocol be modified. Any modifications to the protocol must be drafted by the sponsor's representative and reviewed and approved by another qualified individual from the sponsor. All modifications to the protocol must be submitted to the relevant ethics committee for information or approval, in accordance with local procedures and regulations, and any changes must be approved before implementation.

## 14、Conditions for termination of the study

The sponsor and the investigator reserve the right to terminate the trial at any time. If necessary, the parties may review and negotiate and arrange for the implementation of this procedure based on the specific circumstances of the study. When the study is terminated, the investigator will give full consideration to the protection of the interests of the subjects.

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