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Raltitrexed, S-1 and fruquintinib (RSF) in the treatment of refractory metastatic colorectal cancer: study protocol for a multicenter, prospective, single-arm, phase II trial

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Abstract

Background Metastatic colorectal cancer (mCRC) remains a significant clinical challenge, particularly for patients who have failed standard first- and second-line therapies. Despite advancements in targeted therapies, options for third-line treatments are limited, with current regimens such as regorafenib, fruquintinib, and TAS-102 demonstrating modest efficacy. The RS regimen, combining raltitrexed and S-1, has shown improved objective response rates (ORR) and progression-free survival (PFS) compared to standard therapies. Fruquintinib, a vascular endothelial growth factor receptor (VEGFR) inhibitor, has also demonstrated efficacy in heavily pretreated mCRC patients, including those resistant to prior anti-VEGF therapies. Combining these agents in the RSF regimen leverages complementary mechanisms of action to address resistance and improve outcomes.

Methods This multicenter, prospective, single-arm, open-label Phase II clinical trial evaluates the efficacy and safety of the RSF regimen in mCRC patients who have failed first- and second-line therapies. Eligible patients will receive S-1 orally (14 days), raltitrexed intravenously (day 1), and fruquintinib orally (14 days) in a 21-day cycle. The primary endpoint is ORR, assessed using RECIST v1.1 criteria. Secondary endpoints include PFS, overall survival (OS), disease control rate (DCR), and quality of life (QoL). Safety will be monitored per NCI-CTCAE v4.0 criteria.

Discussion The RSF regimen represents a novel approach to third-line treatment in mCRC, integrating chemotherapy and targeted therapy to enhance tumor response while managing toxicity. By leveraging complementary mechanisms of action, this study aims to optimize therapeutic outcomes in heavily pretreated patients. Further clinical research is essential to validate efficacy, safety, and potential biomarkers for patient selection.

Trial registration ClinicalTrials.gov identifier: NCT06427005, registered on 19 June 2024.

Keywords Metastatic colorectal cancer, S-1, Raltitrexed, Fruquintinib, RSF regimen, VEGFR inhibitor, Third-line therapy, RECIST v1.1

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Background

Colorectal cancer (CRC) ranks as the third most commonly diagnosed cancer worldwide and the second leading cause of cancer-related deaths, with over 1.9 million new cases and 935,000 deaths estimated in 2020 [1]. Despite significant advancements in systemic therapies, approximately 30–40% of CRC patients progress to unresectable advanced stages [2]. Among these, metastatic CRC (mCRC) poses a particularly daunting challenge, as effective options for third-line or subsequent treatments remain limited [3].

Despite the rapid advancements in immunotherapy for various malignancies, systemic chemotherapy combined with targeted agents remains the cornerstone of treatment for metastatic colorectal cancer (mCRC), particularly for patients with proficient mismatch repair (pMMR) or microsatellite stable (MSS) tumors [4, 5]. However, as the disease progresses, therapeutic options become increasingly limited. Regorafenib, fruquintinib, and TAS-102 currently represent the standard of care for third-line therapy [6–9]. While these agents have shown modest benefits, with objective response rates (ORRs) below 5% and median overall survival (OS) ranging from 6 to 9 months, their adverse event profiles pose significant challenges [6, 10]. Notably, fruquintinib and regorafenib frequently cause hand-foot skin reaction (HFSR), which severely impacts patients' quality of life. Grade 3 or worse HFSR occurred in 17% of regorafenib-treated [9] and 10.8% of fruquintinib-treated patients [11]. However, dose reductions can often improve tolerability, enabling patients to continue treatment while mitigating these side effects [12, 13]. In clinical practice, physicians often adjust dosing or intervals to manage toxicities like HFSR and hypertension, despite limited supporting data. However, such modifications may reduce efficacy, potentially lowering ORRs and OS benefits. Further research is needed to optimize dosing regimens that balance efficacy and tolerability.

S-1, a fluoropyrimidine derivative, inhibits dihydropyrimidine dehydrogenase (DPD) to enhance the bioavailability of 5-FU, overcoming one mechanism of chemotherapy resistance. Its efficacy has been demonstrated in advanced and metastatic colorectal cancer (mCRC), both as monotherapy and in combination regimens like SOX and IRIS [14–16]. Raltitrexed directly inhibits thymidylate synthase (TS), a key enzyme in DNA synthesis, and has shown efficacy in 5-FU-resistant CRC [17–19]. Our team developed the RS regimen, combining S-1 and raltitrexed, which achieved an ORR of 13.0% as a third-line treatment for refractory mCRC [19], with external validation confirming its efficacy [18]. To further improve therapeutic outcomes, bevacizumab was introduced into the RS regimen, forming the RSA regimen. A Phase II study conducted at West China Hospital demonstrated that the RSA regimen achieved an improved ORR of 15.9%, median progression-free survival (mPFS) of 3.7 months, and mOS of 12.2 months [20], surpassing standard third-line therapies like regorafenib and fruquintinib.

Despite these advancements, the need for further improvement in outcomes for refractory mCRC patients remains. This is particularly critical given that the majority of patients now receive bevacizumab during both first- and second-line treatments (commonly referred to as bevacizumab cross-line therapy), leaving limited options for effective anti-angiogenic strategies in later lines of therapy. Fruquintinib, a highly selective VEGFR inhibitor, has been shown to significantly extend PFS and OS in heavily pretreated mCRC patients, including those previously treated with bevacizumab. The FRESCO and FRESCO-2 trials validated fruquintinib's efficacy internationally, supporting its inclusion in global treatment strategies for mCRC [6, 11].

Herein, we designed a prospective, single-arm, multicenter, open-label Phase II clinical trial to evaluate the efficacy and safety of combining S-1, raltitrexed, and fruquintinib (RSF regimen) in mCRC patients who failed standard first-line and second-line treatments. This study aims to leverage the complementary mechanisms of these agents—S-1 enhancing 5-FU activity, raltitrexed targeting TS, and fruquintinib inhibiting VEGFR-mediated angiogenesis—to provide an effective and tolerable therapeutic option for this challenging patient population.

Methods and analysis

Study design

This study was approved by the Biomedical Ethics Committee of West China Hospital, Sichuan University (Approval No. 2023-71). The trial is registered on the National Clinical Trial Registry (NCT06427005) (Table 1). The participated centers can be found in supplementary Appendix 1. The trial protocol and this manuscript were developed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (Fig. 1).

This study is a multicenter, prospective, single-arm, open-label Phase II exploratory clinical trial. The primary objective is to evaluate the objective response rate (ORR) of the S-1 + raltitrexed + fruquintinib (RSF) regimen in metastatic colorectal cancer (mCRC) patients who have failed standard first- and second-line therapies. Secondary objectives include progression-free survival (PFS), overall survival (OS), disease control rate (DCR), safety, and quality of life (QoL). Exploratory objectives include the identification of predictive biomarkers of efficacy.

Table 1 World health organization trial registration data set

Data Category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT06427005
Date of registration in primary registry	19 June 2023
Secondary identifying numbers	2025-01-19
Source(s) of monetary or material support	Beijing Xisike Clinical Oncology Research Foundation
Primary sponsor	West China Hospital
Contact for public queries	Weibing Leng, M.D. Phone: +86-28-85422408 FAX:+86-28-85423203 Email: lengweibing@wchscu.cn
Contact for scientific queries	Meng Qiu, MD. Colorectal Cancer Center, Sichuan University West China Hospital. No.37 Guoxue Lane, Wuhou District, Chengdu, Sichuan, China.
Public title	Fruquintinib Plus S-1 and Raltitrexed (RSF) for mCRC
Scientific title	Raltitrexed, S-1 and Fruquintinib (RSF) in the Treatment of Refractory Metastatic Colorectal Cancer: a Multicenter, Prospective, Single-arm, Phase II Trial
Countries of recruitment	China
Health condition(s) or problem(s) studied	metastatic colorectal cancer (mCRC)
Interventional Model	Single Group Assignment
Intervention(s)	Experimental: RSF treatment arm Participants received Fruquintinib (5 mg daily for 14 days followed by a 7-day break), oral S-1 (80–120 mg daily for 14 days, followed by a 7-day break), and raltitrexed (3 mg/m^2 on day 1, with a maximum dose of 5 mg) every 3 weeks.
Masking	None (Open Label)
Key inclusion criteria	1. Age ≥ 18 years, any gender. 2. Patients with metastatic colorectal adenocarcinoma confirmed by pathological histology or cytology. 3. Expected survival time ≥ 12 weeks. 4. ECOG score of 0–2. 5. Previously treated for metastatic colorectal cancer with fluoropyrimidine (allowing intravenous and/or oral fluoropyrimidine formulations, excluding DPD enzyme inhibitors), irinotecan, and oxaliplatin chemotherapy, which failed (treatment failure defined as intolerable adverse reactions, disease progression during treatment, or disease progression within 6 months after completing adjuvant chemotherapy); regardless of prior use of targeted drugs such as cetuximab or bevacizumab. 6. Patients must have an interval of at least 2 weeks since the last chemotherapy (at least 1 week for oral chemotherapy drugs) or more than 4 weeks since the end of radiotherapy, with the study's observable lesions located outside the radiotherapy target area. 7. According to RECIST 1.1 criteria, at least one measurable tumor lesion with a maximum diameter ≥ 1 cm as determined by spiral CT scan. Laboratory test results within 1 week before enrollment must meet the following criteria: (1) Hemoglobin ≥ 90 g/L; Platelets (PLT) ≥ 75 × 10^9/L; (2) White blood cells (WBC) ≥ 3.0 × 10^9/L; Neutrophils (ANC) ≥ 1.5 × 10^9/L; (3) Serum creatinine (Cr) ≤ 1.5 × upper limit of normal (ULN); (4) Total bilirubin (TBI) ≤ 1.5 × ULN; Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 × ULN (≤ 5 × ULN if there is liver metastasis). 8. No prior use of raltitrexed or S-1 (or DPD enzyme inhibitors) in the treatment of colorectal cancer. 9. Signed informed consent.

Table 1 (continued)

Data Category	Information
Key exclusion criteria	1. Patients unable to take oral medications. 2. Patients who have previously been treated with small molecule TKI drugs. 3. Patients with severe hepatic or renal insufficiency, or a recent history of myocardial infarction (within 3 months). 4. Patients with a history of other malignancies within the past five years, except for cured cervical carcinoma in situ and basal cell carcinoma of the skin. 5. Patients with a history of inflammatory bowel disease or extensive colonic resection, ≥ 50% or extensive small bowel resection with chronic diarrhea, or intestinal obstruction. 6. Patients with severe uncontrolled internal medical conditions or acute infections (fever > 38 °C due to infection). 7. Patients with symptomatic brain or leptomeningeal metastases (unless the patient has been treated for brain or leptomeningeal metastases > 6 months, with negative imaging results within 4 weeks before study entry, and has stable clinical symptoms related to brain or leptomeningeal metastases at study entry). 8. Patients with clinically significant, uncontrolled pleural effusion or ascites despite clinical intervention. 9. Pregnant or breastfeeding women, or patients of reproductive potential (males or females not in menopause for less than 1 year) unwilling to use contraception. 10. Patients known to be allergic to raltitrexed, S-1, and Fruquintinib or any of their components. 11. Patients deemed unsuitable for participation in this clinical trial by the investigator.
Study type	Multicenter, Prospective, Single-arm, Phase II Trial
Date of first enrolment	Feb 2023
Target sample size	66
Recruitment status	Recruiting
Primary outcome(s)	objective response rates (ORR)
Key secondary outcomes	progression-free survival (PFS), overall survival (OS), disease control rate (DCR), and quality of life (QoL)

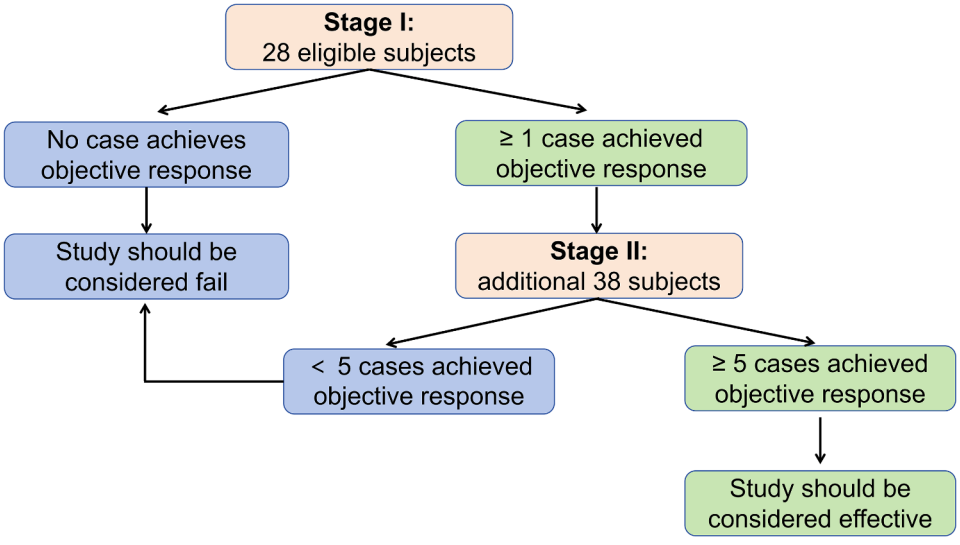

Fig. 1 The SPIRIT flow diagram of this trial. MSS, microsatellite stable; RECIST, Response Evaluation Criteria in Solid Tumors; NCI-CTC, National Cancer Institute Common Toxicity Criteria

Study population

The study population consists of patients with MSS/pMMR mCRC who experienced disease progression or intolerable toxicity after standard first- and second-line treatment. The selection of patients is based on the following inclusion and exclusion criteria:

Inclusion criteria

1. Age ≥ 18 years, any gender.
2. Patients with metastatic colorectal adenocarcinoma confirmed by pathological histology or cytology.
3. Expected survival time ≥ 12 weeks.
4. ECOG score of 0–2.
5. Previously treated for metastatic colorectal cancer with fluoropyrimidine (allowing intravenous and/or oral fluoropyrimidine formulations, excluding DPD enzyme inhibitors), irinotecan, and oxaliplatin chemotherapy, which failed (treatment failure defined as intolerable adverse reactions, disease progression during treatment, or disease progression

within 6 months after completing adjuvant chemotherapy); regardless of prior use of targeted drugs such as cetuximab or bevacizumab.
6. Patients must have an interval of at least 2 weeks since the last chemotherapy (at least 1 week for oral chemotherapy drugs) or more than 4 weeks since the end of radiotherapy, with the study's observable lesions located outside the radiotherapy target area.
7. According to RECIST 1.1 criteria, at least one measurable tumor lesion with a maximum diameter ≥ 1 cm as determined by spiral CT scan.

   Laboratory test results within 1 week before enrollment must meet the following criteria:

   (1) Hemoglobin ≥ 90 g/L; Platelets (PLT) ≥ 75 × 10^9/L;
   (2) White blood cells (WBC) ≥ 3.0 × 10^9/L; Neutrophils (ANC) ≥ 1.5 × 10^9/L;
   (3) Serum creatinine (Cr) ≤ 1.5 × upper limit of normal (ULN);
   (4) Total bilirubin (TBI) ≤ 1.5 × ULN; Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 × ULN (≤ 5 × ULN if there is liver metastasis).

8. No prior use of raltitrexed or S-1 (or DPD enzyme inhibitors) in the treatment of colorectal cancer.
9. Signed informed consent.

Exclusion criteria

1. Patients unable to take oral medications.
2. Patients who have previously been treated with small molecule TKI drugs.
3. Patients with severe hepatic or renal insufficiency, or a recent history of myocardial infarction (within 3 months).
4. Patients with a history of other malignancies within the past five years, except for cured cervical carcinoma in situ and basal cell carcinoma of the skin.
5. Patients with a history of inflammatory bowel disease or extensive colonic resection, ≥ 50% or extensive small bowel resection with chronic diarrhea, or intestinal obstruction.
6. Patients with severe uncontrolled internal medical conditions or acute infections (fever > 38 °C due to infection).
7. Patients with symptomatic brain or leptomeningeal metastases (unless the patient has been treated for brain or leptomeningeal metastases > 6 months, with negative imaging results within 4 weeks before study entry, and has stable clinical symptoms related to brain or leptomeningeal metastases at study entry).
8. Patients with clinically significant, uncontrolled pleural effusion or ascites despite clinical intervention.
9. Pregnant or breastfeeding women, or patients of reproductive potential (males or females not in menopause for less than 1 year) unwilling to use contraception.
10. Patients known to be allergic to raltitrexed, S-1, and Fruquintinib or any of their components.
11. Patients deemed unsuitable for participation in this clinical trial by the investigator.

Sample size calculation

The sample size was determined using Simon's two-stage optimal design to evaluate the efficacy of the RSF regimen (Fig. 2). This design minimizes the number of patients required to test the hypothesis while ensuring statistical rigor. The null hypothesis (H0) assumes that the ORR of the RSF regimen is 4%, based on historical data from the FRESCO-2 trial [6], where fruquintinib monotherapy achieved an ORR of approximately 4%. The alternative hypothesis (H1) assumes an ORR of 15%, supported by clinical results from the RSA regimen developed by our team [20]. With a one-sided alpha error of 0.10 and a beta error of 0.10 (power = 90%), the first stage requires enrolling 28 patients, and if fewer than 1 patient achieves an objective response, the study will be terminated early for futility. If the first stage is successful, an additional 38 patients will be enrolled in the second stage, for a total of 66 patients, and if fewer than 5 patients achieve an objective response across both stages, the regimen will be deemed ineffective. This approach balances ethical considerations by minimizing patient exposure to potentially ineffective treatments while maintaining the statistical power to detect a significant improvement in ORR. The chosen sample size also accounts for potential dropout rates, ensuring sufficient evaluable patients to draw robust and meaningful conclusions.

Interventions

Treatment

Eligible patients will receive the RSF regimen as follows: Raltitrexed will be administered intravenously at 3.0 mg/m² over 15 min on day 1 of each cycle. S-1 will be administered orally twice daily after meals on days 1–14 of each 21-day cycle, with the dosage determined based on body surface area (BSA): <1.25 m²: 80 mg/day, 1.25–1.5 m²: 100 mg/day, and ≥ 1.5 m²: 120 mg/day. Fruquintinib will be administered orally at 5 mg once daily on days 1–14, followed by a 7-day rest period. Patients will continue treatment until disease progression, unacceptable toxicity, or withdrawal of consent, with the total treatment duration varying depending on individual patient responses and tolerability. Although there are no absolute

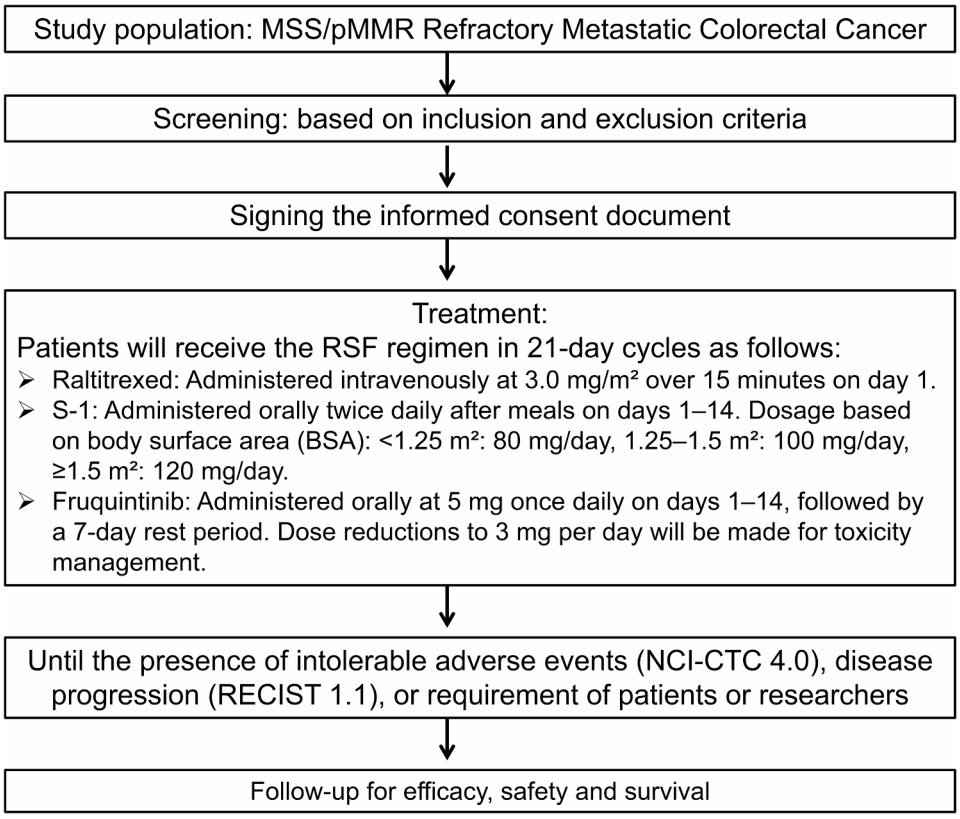

Fig. 2 Simon's two-stage design

contraindications with other treatments, caution will be exercised when co-administering drugs that may cause overlapping toxicities, including cardiotoxicity, hepatotoxicity, nephrotoxicity, myelosuppression, and neurotoxicity. Antitumor treatments outside the study protocol are prohibited; however, best supportive care for symptom management will be permitted based on investigator discretion and standard treatment guidelines.

Patients will maintain a diary card to log medication schedules and record any adverse reactions. Any reported discomfort will prompt immediate clinical evaluation and intervention. Concomitant medications and treatments administered within 30 days before and during the study will be documented in the case report form (CRF) in compliance with Good Clinical Practice (GCP).

Toxicity monitoring and dose adjustments

Toxicity will be assessed before each treatment cycle through patient history, physical examination, and laboratory evaluations, including complete blood counts, liver function tests, and renal function tests. Adverse events (AEs) will be graded according to the NCI-CTCAE v4.0 criteria. Table 2 provides an overview of a patient's journey during the trial.

Dose adjustments will be tailored based on the type and severity of observed toxicities. For chemotherapy-related toxicities (e.g., myelosuppression, nausea, vomiting, diarrhea), simultaneous dose reductions for S-1 and raltitrexed will be implemented. For severe hematologic toxicities (e.g., grade ≥ 3 neutropenia or thrombocytopenia), S-1 and raltitrexed will each be reduced by one dose level, with treatment delayed until recovery if toxicity persists. Minimum dose levels for S-1 are 60 mg/day and for raltitrexed are 2.0 mg/m². For fruquintinib-related toxicities (e.g., hand-foot syndrome, hypertension, fatigue, liver dysfunction), the dose of fruquintinib will be reduced to 3 mg/day for grade ≥ 3 toxicities, with further reduction to 2 mg/day if necessary. Treatment pauses may be required for sustained toxicities, and resumption will depend on recovery. For other difficult-to-classify toxicities, dose adjustments will be guided by the investigator's clinical judgment to ensure patient safety and treatment continuity. Discontinuation of the study regimen will be considered if toxicities persist despite two dose reductions, if intolerable adverse events recur, or if patients withdraw or show disease progression. Supportive care measures will include antiemetics for nausea and vomiting, antihypertensives for elevated blood pressure, specialized skin care for hand-foot syndrome, and general measures for fatigue and other symptoms.

Table 2 Schedule of patient visits and assessments

	Screening Period		Treatment Period (21 days per cycle)					Post-treatment		
			First Cycle (C1)			Second Cycle and Subsequent Cycles (C2-Cx)		Post-treatment/Exit		
	Within 4 weeks pre-dosing	Within 1 weeks pre-dosing	D1	D8 (± 3days)	D14 (± 3days)	D1 (± 3days)	D14 (± 3days)	End of treatment/Exit (14 ± 3 days)	Safety Follow-up (30 days/visit)	Survival Follow-up (3 months/visit)
Informed consent	×									
Complication check	×									
Criteria Check	×		×							
Performance status check	×		×	×	×	×	×			
Medical history and physical examination [a]	×		×	×	×	×	×	×	×	×
Height, weight, BSA	×		×	×		×				
Echocardiogram (ECG)	×					×				
Vital sign	×		×	×	×	×	×			
Laboratory Examination										
Haematology [b]		×		×	×	×	×	×	×	
Blood Chemisty [c]		×		×	×	×	×	×	×	
Coagulation Test [d]		×		×	×	×	×	×	×	
Tumor Marker		×				×			×	
Myocardial Enzyme Spectrum Test		×						×		
Virology [e]	×		For HBV positve subject, monitor the viral load every 2 cycle					×		
Pregnancy Test [f]	×								×	
Efficacy Evalution										
Tumer Imaging Examination	×		Once every 6 weeks (± 7days)					×		
Adverse Events/Concomitant Medications										
Adverse Events [g]	Monitored continuously from signing the informed consent form until at 30 days after the last dose									
Concomitant Medications										
Follow-up Survival										

Table 2 (continued)

	Screening Period		Treatment Period (21 days per cycle)					Post-treatment		
			First Cycle (C1)			Second Cycle and Subsequent Cycles (C2-Cx)		Post-treatment/Exit		
	Within 4 weeks pre-dosing	Within 1 weeks pre-dosing	D1	D8 (± 3days)	D14 (± 3days)	D1 (± 3days)	D14 (± 3days)	End of treatment/Exit (14 ± 3 days)	Safety Follow-up (30 days/visit)	Survival Follow-up (3 months/ visit)
Disease Progression Survival Status								Once every 3 months(± 7 days) imaging evaluation until disease progression or initiation of other tumor treatments (non-imaging PD patients)		

[a] To be performed at screening. An eye examination should be performed immediately/as soon as possible for any patients experiencing a decrease or loss of vision during treatment

[b] Haematology to include: haemoglobin, platelets, white blood count (WBC), neutrophils, lymphocytes, and blast count/percentage. May be ± 3 days prior to the start of each cycle

[c] Biochemistry to include: albumin, bilirubin, alanine transferase/aspartate aminotransferase, alkaline phosphatase, calcium, creatinine, potassium, glomerular fltration rate, total protein, urea, amylase or lipase, sodium, triglycerides, and cholesterol. May be ± 3 days prior to the start of each cycle

[d] Coagulation to include prothrombin time (PT) *or* PT ratio *or* international normalised ratio (INR) and activated partial thromboplastin time (APTT) *or* APPT ratio

[e] Virology to include hepatitis B, hepatitis C, and HIV

[f] For women of childbearing potential, to be performed within 14 days of frst dose of study drug, at the start of each cycle of treatment and at the end of treatment visit

[g] Serious adverse events collected from the point of consent, adverse events from the start of trial treatment

Efficacy assessment

Baseline imaging will be performed within 4 weeks prior to starting the RSF regimen to document measurable disease and establish baseline parameters. This imaging will include contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) of the chest, abdomen, and pelvis, and will be assessed according to RECIST v1.1 criteria. The same imaging modality will be used consistently throughout the study for each participant. Follow-up imaging will occur every two treatment cycles (approximately every six weeks), with adjustments allowed based on clinical judgment. Progressive disease will be defined by radiological evidence, with biomarker changes, such as carcinoembryonic antigen (CEA) levels, used as supportive data but not as sole criteria. Patients showing clinical benefit may continue RSF treatment despite RECIST v1.1-defined progression, as determined by the investigator and patient preference. Tumor response classifications include complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). This imaging schedule aims to ensure thorough monitoring of treatment response and disease dynamics. For patients with disease progression, survival status should be assessed every 3 months after treatment discontinuation. Survival data will be calculated. The imaging Examination timeline is presented in Table 2.

Outcome measures

Primary outcome

The primary outcome of this study is the objective response rate (ORR) to the RSF regimen in metastatic colorectal cancer (mCRC) patients who have failed standard first- and second-line therapies. ORR will be evaluated based on RECIST v1.1 criteria through imaging assessments conducted at predefined intervals throughout the treatment period. ORR is defined as the proportion of patients who achieve either a partial or complete response.

Secondary outcomes

Secondary endpoints will evaluate additional aspects of efficacy, safety, and quality of life, including:

- Progression-Free Survival (PFS): Defined as the time from treatment initiation to disease progression or death from any cause, whichever occurs first.
- Overall Survival (OS): Measured as the time from treatment initiation to death from any cause.
- Disease Control Rate (DCR): Defined as the proportion of patients achieving CR, PR, or SD lasting at least 6 months.
- Adverse Event Rates: Assessed according to the NCI-CTCAE v4.0 criteria, including the frequency and severity of treatment-related toxicities.
- Quality of Life (QoL): Evaluated using validated tools such as the EQ-5D-5 L questionnaire.

Safety assessments will encompass all reported adverse events, classified and documented in accordance with Good Clinical Practice (GCP) guidelines. Additional exploratory endpoints include potential biomarkers predictive of response or resistance to the RSF regimen.

Statistical analysis

The primary outcome measure is the objective response rate (ORR) of the RSF regimen in refractory mCRC patients, which will be analyzed based on the intention-to-treat (ITT) population. Descriptive statistics will be used to summarize baseline clinicopathological characteristics and safety data. Continuous variables will be reported as means and standard deviations (SDs), while categorical variables will be presented as frequencies and percentages. The Kaplan-Meier method will be applied to estimate progression-free survival (PFS) and overall survival (OS). Survival differences will be assessed using the log-rank test. Cox proportional hazards regression models will be employed for univariate and multivariate analyses, and hazard ratios (HRs) with 95% confidence intervals (CIs) will be calculated. Statistical analyses will be performed using SPSS (version 25.0; IBM Corp., Armonk, NY, USA) and R software (version 4.0.3). A two-sided p-value < 0.05 will be considered statistically significant.

Recruitment and consent

Patients will be recruited and screened at participating institutions, listed in appendix 1. Eligible participants will be identified based on the inclusion and exclusion criteria specified in the protocol. Oncologists will assess potential participants for eligibility, provide comprehensive information about the study, and obtain written informed consent before enrollment. Participation in the study is entirely voluntary, and patients may withdraw at any time without obligation to provide a reason. The informed consent form, which outlines the study's objectives, procedures, potential risks, and benefits, will be provided to all participants prior to enrollment and is included as the appendix 2 to this manuscript (V5/2023-02-06).

Data collection, monitoring, and auditing

The schedule of assessments is detailed in Table 2.

The principal investigator, along with designated study staff, will monitor and evaluate clinical data, safety, and efficacy outcomes recorded in medical records. All data will be systematically documented in case report forms (CRFs) and submitted to the dedicated data management team designated by the principal investigator at West China Hospital, Sichuan University. Data verification

will be conducted by the monitor in accordance with the "Study Monitoring Procedures" established for this trial.

This Phase II trial is exploratory and does not involve major anticipated safety concerns. As such, data monitoring will be conducted internally by the principal investigator and study staff. Regular meetings, either in person or remotely, will ensure adherence to the study protocol and address emerging issues promptly. Given the study's nature, external data monitoring committees are not planned.

Any severe adverse events or critical findings documented in the CRFs will be reported to the Ethics Committee of West China Hospital within 30 days of occurrence. Internal monitoring measures will ensure compliance with Good Clinical Practice (GCP) guidelines and the study protocol.

Protocol amendments

Any modifications to the protocol will be conducted in accordance with Good Clinical Practice (GCP) guidelines and approved by the institutional ethics committees prior to implementation.

Confidentiality

All participant information, including clinical and demographic data, will be treated with the utmost confidentiality. Data access will be strictly limited to authorized personnel involved in the study. Patient names and other personally identifiable information will not be included in electronic case report forms (eCRFs) or shared with any unauthorized third parties. To ensure privacy, each participant will be assigned a unique study identification number, which will be used for all data-related documentation. All data management procedures will adhere to ethical and regulatory standards, including the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. Any disclosure of information outside the study team will require prior written consent from participants. These measures aim to maintain participant privacy and ensure compliance with ethical standards throughout the study.

Patient and public involvement

Patients were not directly involved in the design of this protocol. However, oncologists specializing in colorectal cancer have contributed to patient eligibility criteria and treatment planning. The study outcomes will be shared with patients and the broader community through accessible platforms, including educational seminars and publications.

Trial status

Recruitment for the trial opened in Feb-2023 and is expected to last until Apr-2026.

Discussion

The RS regimen, combining S-1 and raltitrexed, provides a promising strategy for treating metastatic colorectal cancer (mCRC) patients who have failed standard first- and second-line therapies. S-1 enhances 5-FU bioavailability by inhibiting dihydropyrimidine dehydrogenase (DPD) [21], while raltitrexed targets thymidylate synthase (TS) [22], an essential enzyme for DNA synthesis. Unlike 5-FU, which binds to the pyrimidine-binding site of TS, raltitrexed disrupts DNA synthesis through a different mechanism, making it effective in cases resistant to 5-FU. This combination directly addresses resistance mechanisms and amplifies anti-tumor effects, providing a strong foundation for the treatment of refractory mCRC. Prior studies have demonstrated significant improvements in progression-free survival (PFS) and overall survival (OS) with the RS regimen compared to conventional third-line therapies [18–20]. Our Phase II study further validated the clinical benefit, reporting an objective response rate (ORR) of 13.0% [19]. These findings strongly support the RS regimen as an effective treatment option for heavily pretreated mCRC patients.

Adding fruquintinib, a selective VEGFR inhibitor, to the RS regimen could further enhance therapeutic potential by targeting tumor angiogenesis. Fruquintinib has shown efficacy in patients previously treated with anti-VEGF therapies like bevacizumab, which is commonly used in first- and second-line treatments [6, 11]. As a result, fruquintinib offers an effective alternative for patients with limited treatment options.

Compared to standard monotherapies like regorafenib and fruquintinib, which have shown limited ORRs despite improving PFS, the RSF regimen is expected to achieve a higher ORR by combining chemotherapy with targeted therapy. We have prioritized ORR as the primary endpoint, rather than PFS or OS, because it provides a direct, measurable indication of treatment efficacy. ORR will provide valuable insights into the potential benefit of the RSF regimen for refractory mCRC patients. For patients in relatively better physical condition, this combination could enhance sustained disease control or even offer a path toward no evidence of disease (NED).

A key challenge with VEGFR inhibitors, including fruquintinib and regorafenib, is the risk of adverse events, particularly hand-foot syndrome. Notably, S-1 also induces this side effect. Fruquintinib was selected over regorafenib because regorafenib is more likely to cause severe hand-foot syndrome and fatigue [6, 9–11]. To minimize these risks, fruquintinib will be administered at a reduced dose of 5 mg daily for 14 days in a 3-week cycle, which aligns with the dosing schedules of S-1 and raltitrexed. Additionally, the protocol allows for further dose reduction of fruquintinib if necessary. This

adjustment is designed to balance toxicity while maintaining therapeutic efficacy.

In conclusion, the RSF regimen—combining raltitrexed, S-1 and fruquintinib—represents a promising therapeutic approach for mCRC patients who have failed standard treatments. By leveraging complementary mechanisms, this combination may significantly improve treatment outcomes and provide a new option for this challenging patient population. Future clinical studies will be essential to confirm its efficacy, safety, and potential for biomarker-guided patient selection.

Abbreviations

BSA	Body Surface Area
CEA	Carcinoembryonic Antigen
CI	Confidence Interval
CR	Complete Response
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Disease Control Rate
DPD	Dihydropyrimidine Dehydrogenase
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic Data Collection System
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HFSR	Hand-Foot Skin Reaction
ITT	Intention-to-Treat
mCRC	Metastatic Colorectal Cancer
mOS	Median Overall Survival
mPFS	Median Progression-Free Survival
NCI	National Cancer Institute
NED	No Evidence of Disease
ORR	Objective Response Rate
OS	Overall Survival
PFS	Progression-Free Survival
QoL	Quality of Life
RECIST	Response Evaluation Criteria in Solid Tumors
RSA	Raltitrexed, S-1, and Bevacizumab Combination Regimen
RSF	Raltitrexed, S-1, and Fruquintinib Combination Regimen
SD	Stable Disease
TS	Thymidylate Synthase
ULN	Upper Limit of Normal
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
WBC	White Blood Cell

Supplementary Information

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Supplementary Material 1: Appendix 1: List of participating centers

Supplementary Material 2: Appendix 2: The informed consent form of this trial

Supplementary Material 3: Appendix 3: Beijing Xisike Funding Confirmation

Supplementary Material 4: Appendix 4: The ethics approval of this trial

Supplementary Material 5: Appendix 5: The SPIRIT checklist

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Author contributions

Weibing Leng and Deyun Luo contributed to the study design. Weibing Leng, Zhenpeng Wen and Han Wang contributed to the data analysis, and manuscript preparation. Peng Cao, and Jiyan Liu provided expert medical advice and contributed to data interpretation. Deyun Luo and Meng Qiu oversaw the clinical aspects of the study, including patient recruitment and monitoring.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. The protocol has been reviewed and approved by the Ethics Committee of West China Hospital (Approval No. 2023-71) and is supposed to be approved by all the participating institutes. The current protocol version is V5.0/2023-01-10. The study has been registered with the U.S. National Institutes of Health (clinicaltrials.gov) under registration number NCT06427005. Written informed consent will be obtained from all participants prior to enrollment, and their confidentiality will be strictly maintained throughout the study.

Competing interests

The authors declare no competing interests.

Role of funders and sponsor

This is an investigator-initiated and investigator-led trial, sponsored by Beijing Xisike Clinical Oncology Research Foundation. The funders have had no involvement in the design of the trial or the writing of the protocol, and will not participate in data collection, analysis, or interpretation during the study. The corresponding author has full access to all study documentation and bears the final responsibility for the decision to submit the protocol for publication.

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