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Combination of Compound Kushen injection with first-line treatment versus first-line treatment alone for advanced colorectal cancer: a study protocol for a multicenter, openlabel, randomized controlled trial

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Abstract

Background The treatment of advanced colorectal cancer (CRC) has progressed slowly, with chemotherapy combined with targeted therapy being the first-line treatment for the disease, but the improvement in efficacy is not satisfactory. Compound Kushen injection (CKI) is one of the representative drugs of anti-cancer Chinese herbal injection drugs, which has been widely used in the adjunct treatment of cancer in China. The aim of this trial is to evaluate the efficacy and safety of CKI combined with first-line treatment of advanced CRC.

Methods This is a multicenter, randomized, open-label controlled clinical trial in which 320 patients with advanced CRC will be randomly assigned to the treatment group or the control group in a 1:1 ratio. Both groups will receive at least 4 cycles of first-line therapy (FOLFOX/FOLFIRI/CAPEOX ± cetuximab/bevacizumab) in 14–21 day cycles, and the experimental group will receive additional CKI with a cumulative dose of 200 ml per cycle. Patients who achieve a complete response, partial response, or stable disease after 4–6 months will receive maintenance therapy until disease progression or another endpoint event, such as toxicity or death, occurs.. Follow-up will occur every 3 months until death or loss to follow-up. The primary outcome of this study will be progression-free survival (PFS). Secondary outcomes will be overall survival (OS), 1-year OS rate, 1-year PFS rate, objective response rate,disease control rate, symptoms and quality of life evaluation. Safety outcomes will be incidence of adverse events.

Discussion This study will be the first randomized controlled trial to investigate the efficacy and safety of CKI when combined with first-line treatment in the treatment of advanced CRC, with PFS as the primary outcome. It aims to clarify the clinical advantages and therapeutic effect of CKI in the treatment of advanced CRC. To identify

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the benefit population of CKI in the treatment of patients with advanced CRC, an enrichment design based on biomarkers will be utilized. Metabolomics and gut microbiota analysis will be conducted on biological samples to explore the metabolic and gut microbiota differences associated with the efficacy of CKI, guiding further research into its mechanism of action.

Trial registration ClinicalTrials.govNCT05894694. Registered on 4 August 2023.

Keywords Compound Kushen injection, Advanced colorectal cancer, Chinese herbal medicine, Randomized controlled trial, Progression-free survival

Introduction

Background and rationale {6a}

According to International Agency for Research on Cancer (IARC), the new cases of colorectal cancer (CRC) in 2020 was about 1.88 million, and the deaths reached about 0.92 million [1]. CRC is the third malignant tumor in the world, ranking the second cause of death of malignant tumors [2]. About 60% of newly diagnosed CRC patients have advanced disease, with 22% of patients developing distant metastases [3]. According to the American Cancer Society (https://www.cancer.org/) [4], the overall 5-year overall survival (OS) rate for patients with CRC is 63%—67%, and for patients with advanced CRC is about 13–18%. Over the past decade, the aging population, coupled with industrialization and shifts in dietary patterns in China, has led to a notable upward trend in both the incidence and mortality rates of CRC [5]. Additionally, the 5-year OS rate for CRC in China ranges from 56.9%—57.6%, placing the country among those with a significant burden of CRC globally [6]. Therefore, the development and implementation of an effective prevention and treatment strategy for CRC tailored to the unique characteristics of China holds significant practical importance. FOLFOX/FOLFIRI/CAPEOX ± cetuximab/bevacizumab is recommended as the first-line treatment for unresectable MSS/MSI-L/pMMR patients with advanced CRC, according to National Comprehensive Cancer Network (NCCN) and Chinese Society of Clinical Oncology (CSCO) guidelines [7–9].

Traditional Chinese medicine (TCM) plays a pivotal role in the holistic management of cancer, with a survey indicating that approximately 80% of Chinese cancer patients received TCM treatment in China [10]. Anticancer proprietary Chinese medicine is an important part of comprehensive treatment of cancer. One article showed that the number of randomised controlled trials (RCTs) related to Compound Kushen injection (CKI) was the largest among TCM anti-cancer injections [11]. The addition of CKI on the basis of first-line treatment may have clinical significance in the treatment of advanced CRC. CKI is extracted and processed from Sophora flavescens and white Smilax glabra Roxb. Its main active ingredients alkaloids, such as matrine and oxymatrine can activate pro-apoptotic genes, regulate cell cycle, block DNA synthesis and replication of tumor cells, and thus directly inhibit the proliferation of tumor cells [12].

Matrine, one of the active components of CKI, can enhance the expression of miR-22, thereby obstructing Wnt/β-catenin and MEK/ERK pathways, then evoked colon cancer (CC) cell apoptosis and G0/G1 cell cycle arrest [13]. Matrine might inhibit the epithelial-mesenchymal transition (EMT) process and MAPK signaling pathway through downregulation Claudin-9, thereby inhibiting vasculogenic mimicry formation, proliferation, and invasion of CC cells [14]. An RCT of 78 patients with advanced CRC showed that CKI combined with FOLFOX4 could increase overall response rate (ORR) by 12.8% and prolong the 1-year progression-free survival (PFS) rate by 15.39% [15]. A network meta-analysis indicated CKI combined with chemotherapy for CRC had significantly improved the clinical efficacy, reduced the incidence of adverse reactions, and been safe [16]. Despite the potential advantages of incorporating CKI into the first-line treatment regimen for patients with advanced CRC, the impact on long-term survival remains uncertain. The existing evidence base is limited in quality, and there is a pressing need for well-conducted, large-scale RCTs to provide definitive insights into its efficacy and safety.

Objectives {7}

The objectives of this trial are to assess the long-term efficacy and safety of combination of CKI with first-line chemotherapy ± cetuximab/bevacizumab for patients with advanced CRC.

Trial design {8}

This is a prospective multicentre, openlabel, randomized controlled trial. A total of 320 patients with advanced CRC patients receiving the first line treatment, based on the inclusion and exclusion criteria, will be recruited from November 2022 through December 2025. The patients will be randomized at a 1:1 ratio to the experimental group and the control group and treated with combination of CKI with first-line chemotherapy ± cetuximab/bevacizumab and first-line chemotherapy ± cetuximab/

bevacizumab, respectively. The investigator will observe and record subjects' disease progression or remission and adverse drug reactions and analyse the data. Open label will be used in the trial. This protocol was designed according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 Statement [17] and registered at ClinicalTrials.gov NCT05894694 (4 August 2023).

Methods

Participants, interventions, and outcomes

Study setting {9}

Guang'anmen Hospital (GAMH), China Academy of Chinese Medical Sciences is the initiator and responsible for this programme. Up to now, 13 upper first-class hospitals across 11 provinces in China have participated as subcentres for patient recruitment, diagnosis and treatment, and data collection (Table 1).

Eligibility criteria {10}

(1) Inclusion criteria

① Patients with advanced CRC confirmed by pathology or cytology and receiving first-line therapy;
② Aged ≥ 18 years old, male or female;
③ ECOG score 0-2 points;
④ Expected survival ≥ 3 months;
⑤ According to RECIST1.1 criteria, at least one detectable lesion;
⑥ Voluntarily join the study, sign informed consent, compliance with good cooperation with follow-up.

(2) Exclusion criteria

① Combined with other malignant primary tumors;
② Immunohistochemistry/polymerase chain reaction/second-generation sequencing results suggest MSI-H/dMMR patients;
③ Patients with recurrence and metastasis within 6 months after radical tumor surgery;
④ Patients who have previously or are undergoing cancer immunotherapy; Patients undergoing radiation therapy;
⑤ Pregnant or lactating women; women of childbearing age and their spouses can not take effective contraceptive measures during and within 6 months after the end of clinical study;
⑥ Psychiatric patients;
⑦ Patients with severe, uncontrolled organic disease or infection, such as decompensated heart, lung, kidney failure caused by intolerance to chemotherapy;
⑧ Patients who have received clinical trials of small molecule drugs within 28 days or received clinical trials of large molecule drugs within 3 months;
⑨ Patients who are known to be allergic to or intolerant of study drugs.

Who will take informed consent {26a}

Patients with advanced CRC who meet all inclusion criteria and do not meet any exclusion criteria will obtain informed consent. Before each patient is enrolled in the study, a trained investigator will fully and comprehensively introduce the purpose, procedures, and possible risks of the study to the patient or his/her representative

Table 1 List of the participating hospitals

Number	Hospital name	Region (in China)
01	Guang'anmen Hospital, China Academy of Chinese Medical Science	Beijing
02	The First Affiliated Hospital of Guangzhou University of Chinese Medicine	Guangzhou
03	Hunan Cancer Hospital	Changsha
04	Anhui Chest Hospital	Hefei
05	Jiangsu Province Hospital of Chinese Medicine	Nanjing
06	Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine	Shanghai
07	The Affiliated Hospital of Shandong University of Traditional Chinese Medicine	Jinan
08	Chongqing Traditional Chinese Medicine Hospital	Chongqing
09	Shanxi Provincial People's Hospital	Taiyuan
10	Integrated Hospital of Traditional Chinese Medieine, Southern Medical UniversityInner Mongolia Hospital of Traditional Chinese Medicine	Huhehaote
11	Peking University Cancer Hospital Inner Mongolia Hospital	Huhehaote
12	Chongqing Cancer Hospital	Chongqing
13	Anyang Cancer Hospital	Anyang

Table 2 Chemotherapy regimen ± cetuximab/bevacizumab for advanced CRC

Chemotherapy regimens	Specific drug	Dosage	Time and usage of drug	Time and period
mFOLFOX6	oxaliplatin	85mg/m^2	d1 ivgtt	14 days is a period
	LV	400mg/m^2	d1 ivgtt	
	5-FU	400mg/m^2 → 1200mg/m^2	d1 iv → d2-3 ivgtt	
CAPEOX	oxaliplatin	130mg/m^2	d1 ivgtt	21 days is a period
	capecitabine	1000mg/m^2	d1-14, bid, po	
FOLFIRI	irinotecan	180mg/m^2	d1 ivgtt	14 days is a period
	LV	400mg/m^2	d1 ivgtt	
	5-FU	400mg/m^2 → 1200mg/m^2	d1 iv → d2-3 ivgtt	

When RAS and BRAF are wild-type, the above chemotherapy regimen is combined with intravenous infusion of bevacizumab 5 mg/kg on day 1; or cetuximab 400 mg/m^2 first intravenous infusion over 2 h and then 250 mg/m^2 intravenous infusion over 60 min once a week or cetuximab 500 mg/m^2 intravenous infusion over 2 h once a week

When RAS or BRAF is mutant, bevacizumab 5 mg/kg ivgtt is added to the above chemotherapy regimen

and sign a written informed consent form. Patients or their representatives should be informed that their participation is entirely voluntary and they have the right to withdraw from the study at any time. Additionally, it should be clarified to them that their decision to participate or not will have no impact on their regular treatment. During the research process, the personal privacy and data confidentiality of all participants will be protected, and their personal information will not be disclosed.

Interventions

Explanation for the choice of comparators {6b} According to NCCN guidelines (Version V1, 2022), the control group receive first-line regimen (FOLFOX/FOLFIRI/CAPEOX ± cetuximab/bevacizumab) in 14–21 day cycles. Specific first-line regimens are shown in Table 2.

Intervention description {11a} The experimental group receive the CKI in addition to the control group regimen. CKI (specifications: 5 ml/tube): ivgtt, 20ml/time, diluted with 200ml sodium chloride injection, qd. The cumulative dose of 200ml should be reached for each cycle of CKI in combination with chemotherapy. CKI is produced and provided by Shanxi Zhendong Pharmaceutical Co., Ltd. (Changzhi, China).

Treatment period After 4–6 months of treatment, patients with complete response (CR), partial response (PR), or stable disease (SD) entered the maintenance phase. The control group receive the maintenance treatment (5-FU/LV/capecitabine ± bevacizumab) in 14–21 day cycles, while the experimental group receive the CKI in addition to the control group regimen. Patients will continue to receive treatment until progressive disease (PD) or an endpoint event. The flow chart of the study design is shown in Fig. 1. If a patient experiences disease recurrence, discontinues treatment due to intolerance, or for other reasons, they will enter a follow-up observation phase, with follow-ups conducted every 3 months until the endpoint event occurs (death or loss of follow-up).

Restrictions on concomitant medications and concomitant therapies {11d}

(1) During the trial treatment, drugs for bone metastases, marrow suppression, nausea and vomiting, diarrhea, abnormal liver and kidney function, infection and other symptomatic treatment may be used in combination, but they should be truthfully recorded.
(2) Immune checkpoint inhibitors are prohibited.
(3) It is prohibited to use traditional Chinese medicines, Chinese patent medicines with anti-tumor effects and other anti-tumor drugs or treatment means other than those specified in the protocol.

Criteria for withdrawals or dropouts from the trial {11b}

(1) Unexpected events occurred during the treatment and could not adhere to the completion of at least 4 cycles of treatment;
(2) The patient voluntarily withdrew the informed consent form and voluntarily requested withdrawal;
(3) The patient was pregnant or lost to follow-up;
(4) The patient developed poor compliance and was unable to continue the clinical study;

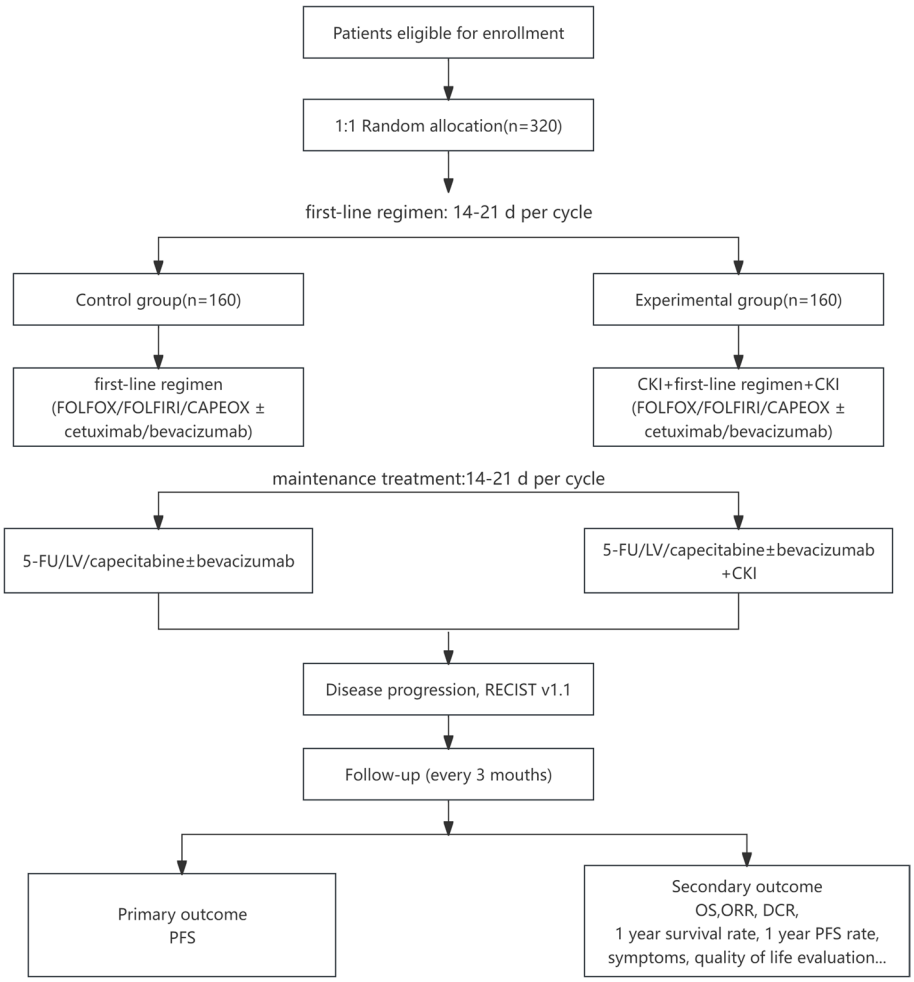

Fig. 1 Flow chart of the study. CKI, Compound Kuhsen injection; PFS, progression-free survival; ORR, objective response rate

(5) The sponsor terminated the study or administrative authorities withdrew the trial;
(6) Other conditions that the investigators considered necessary to withdraw from the study.

Handling of withdrawals or dropouts from the trial {11c}

The investigator is dedicated to ensuring the continuation of proper treatment for each patient unless it is in the best interest of the patient to discontinue participation in the study. Should a patient's participation in the study treatment be terminated, the investigator is obliged to diligently assess the patient's outcomes to the fullest extent possible, ensuring that valuable data is captured and analyzed for the benefit of the research. After discontinuing treatment or withdrawing from the study, patients should enter a follow-up period during which they will undergo periodic follow-ups (once every three months, telephone follow-up is acceptable) to ascertain their survival status, with observations continuing until the occurrence of a terminal event in the patient.

Outcomes {12}

(1) Primary outcome

PFS: the time from randomization to tumor progression or death. Patients who have not experienced progression by the cutoff date will have their data censored as of the date of their last follow-up contact.

(2) Secondary outcomes

OS: the time from randomization to death due to any cause.

1-year survival rate: the proportion of patients who survive for more than 1 year from enrollment in the treatment in total patients.

1 year PFS rate: the proportion of the total patients who does not with tumor progression or death within 1 year from enrollment.

ORR: Based on imaging studies, the proportion of patients whose tumor volume reduction has reached the predetermined value and can be maintained for the minimum required duration. Response generally refers to the period from the start of response until tumor progression is confirmed, including CR + PR cases. CR is defined as disappearance of all target lesions and maintained for at least 4 weeks; PR is defined as ≥ 30% reduction in the sum of the long diameters of target lesions and maintained for at least 4 weeks.

Disease Control Rate (DCR): The percentage of patients with advanced cancer who achieve CR, PR, or SD as a result of therapeutic intervention, as determined by imaging studies, including the proportion of CR + PR + SD. SD is defined as the sum of the largest diameters of target lesions does not decrease to the extent of PR, or the sum of the largest diameters of target lesions increases by less than 20%.

Symptoms and quality of life evaluation: Quality of life is evaluated using the Functional Assessment of Cancer Therapy-Colorectal (FACT-C), ECOG scores, and KPS scores. Symptoms of CRC were evaluated using the M.D. Anderson Symptom Inventory.

Safety outcomes

According to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE v.5.0), adverse events (AEs) are monitored in patients every two months from baseline to disease progression or death. The incidence rate of drug-related AEs (referring to the proportion of patients who experience adverse events caused by the drugs) will be calculated.

Plans for biological specimens {33}

After signing the informed consent form, blood samples (8ml), midstream morning urine (15ml) and morning stool (5g) were collected and stored before enrollment, after every 2 cycles of treatment and at the time of disease progression. Blood samples need to be separated into plasma and blood cells. The aliquoted blood, urine, and fecal specimens must be stored in a freezer at −80 degrees Celsius and then centrally transported to Guang'anmen Hospital using a dry ice cold chain. The retention of biological samples will be obtained with the informed consent of each participant. In the current trial, blood samples will be tested for metabolomics and stool samples for gut microbiota and will be used to support research in the future.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Beyond the scope of this study, patient medical records, as well as blood, urine, and stool samples, may be utilized in subsequent research endeavors. This potential use is clearly outlined in the informed consent form, which is informed to patients by their physicians. Patient is fully voluntary to sign this statement or refuse.

Participant timeline {13}

During the screening and enrollment phase, patients are required to complete the collection of demographic information, vital signs, physical examination, performance status assessment, clinical symptoms, and related examinations and imaging tests. Referring to the guidelines, the chemotherapy regimen is set to have a cycle of either 14 or 21 days. All patients in this study will be followed up every 3 months from the end of treatment until the endpoint event (death or loss to follow-up). Patients who discontinue treatment or withdraw from the study should also enter the follow-up period. See Table 3 for the participant timeline.

Sample size {14}

The Log-rank test for two groups of survival data is designed with a 1:1 experimental group to control group ratio. According to literature reports, the median PFS for advanced CRC treated with chemotherapy (FOLFOX6 or FOLFIRI) combined with targeted therapy (bevacizumab or cetuximab) is about 8.2 months. Assuming the experimental group has a 4-month longer PFS than the control group, with an alpha level of 0.05, a power of 1-beta (80%), and an expected enrollment time of 24 months for all participants, the expected duration of the clinical trial is 36 months. Using the software PASS 15, it is calculated that 128 cases are needed for both the experimental and control groups. Considering a 20% dropout rate, the final determination is 160 cases for each group, totaling 320 cases.

Recruitment {15}

In order to complete such large amount patients' selection as soon as possible, we selected 13 hospitals localized in 11 different provinces in China. In addition, recruitment will be accomplished through posters and website advertisements at outpatient clinics, hospitals. Thus, we will obtain timely enrolment to the greatest extent possible.

Table 3 Schedule of screening, intervention and follow-up of the trial

Items	Screening	Treatment periods				Follow-up periods
Time line	d −7–0	d 14 ± 7/21 ± 7	d 28 ± 7/42 ± 7	d 42 ± 7/63 ± 7	…	Once every 3 months
Check with inclusion and exclusion criteria	X					
Consent form	X					
Demographic data	X					
Diagnosis data	X					
vital signs	X	X	X	X	X	
physical examination	X	X	X	X	X	X
Body condition score	X	X	X	X	X	X
clinical sign	X	X	X	X	X	
DXR or CT	X		X		X	X
MRI、B-mode ultrasound	*		*		*	*
bone scanning	*		*		*	*
colonoscopy	X		*		*	*
tumor markers	X	X	X	X	X	X
MDASI-TCM	X	X	X	X	X	
FACT-C	X	X	X	X	X	
Blood routine	X	X	X	X	X	X
hepatic and renal function	X	X	X	X	X	X
Urine and stool routine	X	*	X	*	X	*
lipid profile	X	*	X	*	X	X
Electrocardiograph	X	X	X	X	X	X
Adverse events		X	X	X	X	
drug combination		X	X	X	X	
therapeutic evaluation		X		X		X
treatment condition	X	X	X	X	X	X

Note: [1] For imaging examinations, the same examination method should be used for each follow-up after the initial screening and enrollment for the same body part, with a review every 2-3 treatment cycles or every 2 months during the treatment phase, and every 3 months during the follow-up phase. [2] Selectively perform head, chest, abdominal, and bone scans based on the situation. [3] If CT/MRI examinations have already been conducted for the same body part, then abdominal films and ultrasound examinations are not necessary. In the table, an asterisk (*) indicates that the examination is optional

Assignment of interventions

Allocation

Sequence generation {16a} Eligible patients will be randomized in a 1:1 ratio to receive first-line chemotherapy ± cetuximab/bevacizumab or CKI combined with first-line chemotherapy ± cetuximab/bevacizumab immediately after signing the informed consent form. The randomization sequence is generated by computer, and randomization will be performed by one physician in GAMH.

Concealment mechanism {16b} Central randomization will be performed using the IWRS system, allocation scheme concealment was implemented, and randomization will be performed using a minimized dynamic randomization method with random factors taking into account: gender (male, female), age (< 60 years, ≥ 60 years), and location (left colorectal, right colon).

Implementation {16c} Steps of central randomization:

(1) Cases will be screened for trial entry based on eligibility criteria by physicians;
(2) The physician will log in the website of central randomization system, enter relevant information (such as patient name abbreviation, age, gender, telephone number, etc.) and click to determine, and the system will generate the grouping results of the case and the corresponding randomization number;
(3) According to the group assignment results, if a participant has been administered the wrong group of medication, no correction will be made and the original drug treatment will continue. Detailed information about the drug treatment will be recorded in the medical records.

Blinding {17a}

The design of this trial is open-label, so unblinding will not occur in any circumstances.

Data collection, management, and analysis

Data collection

Plans for data collection {18a} Case report forms (CRFs) will be used to collect data. Within 3 days after the end of the observation course of each patient, the researcher should submit the study medical records, informed consent and other materials to the subject leader of the center for review and archiving. At the same time, the data in the CRFs will be uploaded to the online web-based electronic data capture system for data collection. This data platform features verification and proofreading capabilities, with modified data clearly highlighted in red. Patients' information on the electronic case report form (e-CRF) will be anonymized using initials and a unique identifier code.

Plans to promote participant retention and complete follow-up {18b} Data from all participants, including those who discontinue treatment or deviate from the intervention protocol, will be collected faithfully according to the study protocol. Providing blood draw subsidies, transportation allowances, and frequent follow-up phone calls will be conducted to improve adherence to interventions. For patients lost to follow-up, attempts to contact the patient must be made and documented.

Data management {19}

To ensure that all data recorded in the CRFs are consistent with the source materials, a clinical research coordinator will be assigned to assist the physician in recording and managing all visits and examinations. Each center will be equipped with dedicated file cabinets, with a designated person responsible for the storage of trial documents. At the end of the study, all original data will be kept and reviewed by the study group and archived after the trial is concluded.

Confidentiality {27}

Patient medical records, blood, urine, and stool samples will be anonymized through encoding and encryption before being securely stored at the GAMH. Access to these data and samples will be strictly limited to researchers. We will make every effort to protect the privacy of our patients' personal medical information to the extent permitted by law.

Statistical methods

Statistical methods for primary and secondary outcomes {20a} SAS 9.4 statistical software will be used by an independent, professional statistician. Full analysis set and per protocol set analysis will be performed simultaneously for efficacy indicators. Safety set analysis will be performed for AEs. All statistical tests will be performed using two-sided tests, and a P value of less than or equal to 0.05 was considered statistically significant. Measurement data shall describe mean, standard deviation, median, minimum and maximum, and enumeration data shall describe frequency and percentage. The use of t-tests and rank sum tests for the analysis of quantitative data, and the use of chi-square tests and Ridit analysis for the analysis of categorical data. The use of Kaplan–Meier method, Wilcoxon rank sum test, or log-rank test for the analysis of survival data.

Methods for additional analyses {20b} The use of the Cox proportional hazards regression model for multivariable survival analysis, with subgroup analysis based on stratification factors (gender, age, and tumor location). At the same time, using PFS as the boundary, while clarifying the survival benefits of CKI in the treatment of advanced CRC, an enrichment design approach is adopted. Through correlation analysis, the advantageous targets of CKI are summarized.

Methods in analysis to handle protocol nonadherence and any statistical methods to handle missing data {20c} Analyze data for outliers and perform professional analysis of outliers to decide whether to choose them. Data will be analyzed for missing values, and professional analysis will be performed for missing values to decide whether to include dropout or data transfer. The proportion of drop-out cases shall not exceed 20%, otherwise it shall be analyzed and explained.

Plans to give access to the full protocol, participant-level data, and statistical code {31c} The full protocol, participant-level data, and statistical code during the current study will be available from the corresponding author upon reasonable request.

Oversight and monitoring

Composition of the the coordinating centre and trial steering committee {5d}

The trial coordinating centre of the study is established by GAMH and consists of the undertaker and core research members. Investigators from GAMH act as coordinating investigators, tasked with the crucial role of facilitating

communication and collaboration between participating hospitals in the trial. They are dedicated to advancing and overseeing the trial's progress, supported by the Contract Research Organization. The trial steering committee consists of the GAMH clinical research team. The committee is responsible for the investigator training, coordination and communication with cooperative units. Periodically hold subject meetings every month to coordinate the progress of the project and solve practical problems in the process of project implementation.

Composition of the data monitoring committee, its role, and reporting structure {21a}

In this study, a Data Monitoring Committee (DMC) will be established and an independent expert group with relevant expertise and experience is planned. This committee is responsible for regularly reviewing cumulative data from ongoing clinical statistics in this trial to protect the safety of patients, ensure the reliability of the trial, and the validity of the trial results. If necessary, the DMC may adjust and modify the elements of the ongoing trial based on the collected data, such as intervention dose, study population, or effect size and error for sample size estimation.

Interim analyses {21b}

The DMC shall conduct interim analyses based on the progress of the clinical trial to assess efficacy and futility, and continues the trial with modifications to the protocol after the interim analysis, such as adjusting the sample size.

Adverse event reporting and harms {22}

If any AEs, including serious adverse events (SAEs), occur during the clinical trial, regardless of causal relationship with the CKI, the attending physician should take necessary measures to give treatment and rescue. AEs occurring during the trial should be recorded in the CRF, including the type, severity, time of occurrence, duration, management measures, and the course of management. A comprehensive analysis should be conducted to determine if there is any association with the experimental drug and the control drug being used. After an AE occurs, the investigator may decide whether the patient should discontinue the clinical trial based on the condition. If a SAE occurs during the trial, the investigator should report it to the principal investigator at their respective sub-center within 24 h of becoming aware of the event. The principal investigator of the research unit should then immediately report to GAMH, which will subsequently report to the hospital's ethics committee. Patients discontinued from the trial due to SAE should be followed up until symptoms disappear or treatment is discontinued.

Frequency and plans for auditing trial conduct {23}

After each center completes the enrollment of the first patient, the monitor will conduct one inspection. Following enrollment, each sub-center will be inspected once a month including checking the original data of participants and counting the biological samples. Meet the basic monitoring needs, according to the specific implementation process of the project will be adjusted accordingly.

Plans for communicating important protocol amendments to relevant parties {25}

Should there be any suggested modifications to the protocol, such changes will be thoroughly documented in protocol amendments. These amendments must undergo a rigorous approval process by the ethics committee and regulatory agencies before implementation.

Provisions for post-trial care {30}

Doctors and research institutions will do their best to prevent and treat any harm that may result from this research. If a SAE occurs in the clinical study, the medical expert committee will determine whether it is related to the study, and if it is confirmed to be relevant, the research unit will bear the reasonable cost of treatment and the corresponding financial compensation in accordance with Chinese law.

Dissemination plans {31a}

This study will be published no matter whether positive or negative results are obtained, and the personal privacy of patients will not be disclosed in the published articles.

Discussion

CKI has been marketed in China in 1995, with a history of about 30 years so far. CKI, often used in combination with radiotherapy and chemotherapy, is commonly applied in clinical practice to enhance the therapeutic effect and reduce side effects, alleviate cancer pain, and treat ascites caused by cancer [18–20]. Our team found through a search on the China National Knowledge Infrastructure that among all the articles on the treatment of malignant tumors with TCM injections, the number of clinical studies on CKI is the highest. However, there is still a relative scarcity of high-quality, large-sample clinical studies at present.

In previous studies on the treatment of advanced CRC with CKI, the primary endpoints were always ORR and DCR, but they could not fully reflect the long-term efficacy of the drug and the clinical benefits such as prolongation of patient survival [21]. OS is the gold standard

endpoint to assess treatment efficacy in cancer clinical trials. However, detecting a significant improvement in OS often necessitates a large patient cohort and prolong follow-up periods, which can restrict the feasibility of conducting clinical trials with OS as the primary endpoint [22]. In this context, identifying alternative endpoints capable of accurately capturing treatment benefits and providing measurable results earlier is crucial for advancing clinical research in oncology. Both the European Medicines Agency and the United States Food and Drug Administration have recognized PFS as an appropriate endpoint that may be used to demonstrate clinical benefit in oncology [23, 24]. Compared with OS, PFS requires a smaller sample size and shorter follow-up time. The analysis of PFS is not affected by subsequent therapies or crossover and is more proximal to the therapy being evaluated in the trial [25]. Accordingly, PFS is the primary endpoint in this study and patients will be followed for OS. This study will be the first randomized controlled trial to investigate the efficacy and safety of CKI combined with first-line chemotherapy ± targeted therapy in the treatment of advanced CRC with PFS as the primary outcome.

In addition, the strength of this study lies in our approach to adopt an enrichment design, where we will select the beneficial patient population according to biomarkers. This will guide the precise clinical targeting and clarify the therapeutic benefits and safety of CKI in treating patients with advanced CRC.

Blood samples and stool samples will be collected at baseline, after every 2 cycles of treatment, and at the time of disease progression. We will conduct metabolomics analysis on blood samples to explore the metabolic products that contribute to the efficacy differences of CKI, guiding further research into its mechanism of action. Studies have reported that CKI can affect metabolism. For example, after intervening with the active component of CKI, oxo-matrine, in CC cells, it was observed that the consumption of glucose in the cells decreased, and the production of ATP, pyruvate, and lactate was significantly inhibited. The expression of PKM2 and GLUT1 was markedly reduced, and this effect is closely related to the weakening of PKM2-mediated aerobic glycolysis [26]. The gut microbiota is involved in CRC formation, progression and its response to treatment. In patients with CRC, the composition of the gut microbiota tends to differ significantly from that of healthy people, and this dysregulation may be associated with the development of cancer. For instance, certain pathogenic bacteria such as Fusobacterium nucleatum have increased abundance in the gut of patients with CRC, while the abundance of beneficial bacteria like Bifidobacterium and Lactobacillus decreases [27]. The gut microbiota may promote the development of CRC through various mechanisms. These mechanisms include the production of carcinogenic metabolites (such as secondary bile acids), disruption of the intestinal barrier, induction of chronic inflammatory responses, impact on the immune system, and direct interactions with tumor cells [28]. At the same time, the status of intestinal flora may affect the response of patients to chemotherapy, radiotherapy and immunotherapy. For example, the abundance of Fusobacterium nucleatum is associated with a higher recurrence rate and poor prognosis of the disease after chemotherapy. Additionally, the relative abundance of this bacterium has been linked to diminished responses to chemotherapy drugs such as 5-fluorouracil, capecitabine, and oxaliplatin. This phenomenon is associated with the bacterium's role in modulating the activation of the TLR-4 signaling pathway and MyD88-induced autophagy, which in turn affects the tumor microenvironment and the efficacy of immune cells in combating the tumor [29]. Therefore, in this clinical study, fecal samples from patients will be collected to investigate the gut microbiota, exploring whether CKI can exert its therapeutic effects in patients with advanced CRC by influencing the gut microbiota.

Placebos for CKI are difficult to obtain in China, so it is difficult to blind this study. To minimize this bias, all participating hospitals are top-tier tertiary hospitals in China, with comparable levels of diagnostic and treatment capabilities. In addition, the attending physician and a chief physician will evaluate each patient's condition as the disease progresses. Finally, to ensure the reliability of the study results, we will implement quality control measures from four different aspects: (i) training researchers before the project starts, (ii) establishing the DMC and the trial steering committee to manage data, (iii) dual verification of both CRF and e-CRF, and (iv) conducting regular monitoring.

We hope that this trial will provide high-level evidence for the efficacy and safety of CKI combined with first-line chemotherapy ± targeted therapy in advanced CRC, and will explore the clinical characteristics of CKI, aiming to refine its clinical advantages and therapeutic effects in treating advanced CRC.

Trial status

Protocol version number and date:version 3.0 of 25/04/2024.

Recruitment start date: 08/09/2023.

Estimated recruitment end date: 31/12/2025.

Abbreviations

AEs	adverse events
ATP	adenosine triphosphate
CC	colon cancer
CKI	Compound Kushen injection
CR	complete response

CRC	colorectal cancer
CRFs	case report forms
CSCO	Chinese Society of Clinical Oncology
DCR	disease control rate
e-CRF	electronic case report form
EMT	epithelial-mesenchymal transition
GLUT1	glucose transporter 1
IARC	International Agency for Research on Cancer
NCCN	National Comprehensive Cancer Network
ORR	overall response rate
OS	overall survival
PD	progressive disease
PFS	progression-free survival
PKM2	pyruvate kinase isozyme type M2
PR	partial response
RCTs	randomised controlled trials
SAEs	serious adverse events
SD	stable disease
TCM	traditional Chinese medicine

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-024-04725-6.

Additional file 1. Ethical approval document.

Additional file 2. Consent form.

Additional file 3. SPIRIT checklist.

Additional file 4. Funding documentation.

Additional file 5. Product inspection report.

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Authors' contributions {31b}
JW, YG and GZ have contributed equally to this work and share the first authorship. JW, YG, GZ, YG and JL participated in the design the study. RG and XZ were in charge of study coordination. YZ helped to develop the study measures and analyses. JW and YG drafted the manuscript. GZ helped to revise the manuscript. All authors have read and approved the final manuscript. JW, YG and GZ have contributed equally to this work and share the first authorship.

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Data availability {29}
The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate {24}
Ethical approval for the study has been obtained from the institutional review boards of the Guang'anmen Hospital, China Academy of Chinese Medical Sciences (Number: 2023-063-KY-01, 2023-063-KY-02 and 2023-063-KY-03). An informed consent will be obtained from all subjects and/or their legal guardian(s).

Consent for publication {32}
Not applicable

Competing interests {28}
The authors declare no competing interests.

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