Effects of Prebiotic Supplement on Gut Microbiota, Drug Bioavailability and Adverse Effects in Patients with Colorectal Cancer at Different Primary Tumor Locations Receiving Chemotherapy: Study Protocol for a Randomized Clinical Trial

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Abstract

Background: The prevalence of colorectal cancer (CRC) worldwide is a huge challenge to human health. Primary tumor locations found to impact prognosis

and response to therapy. The important role of gut microbiota in the progression and treatment of CRC has led to many attempts of alleviating chemotherapy-induced adverse effects using microecologics. However, the underlying mechanism of the difference in the prognosis of different primary tumor locations and the synergistic effect of prebiotics on chemotherapy need to be further elucidated. This study aims to explore the differences in tumor microbiota and examine the effectiveness of xylooligosaccharides (XOS) on gut microbiota, adverse effects, and bioavailability of chemotherapy drugs in CRC patients at different primary tumor locations.

Methods: This is a double-blinded, randomized, parallel controlled clinical trial. Participants with left-sided CRC (LSCRC, n = 50) and right-sided CC (RSCC, n = 50) will randomly allocated to prebiotic group (n = 25) or control group (n = 25) and will receive either a daily XOS (3 g/d) or placebo, respectively, for 12 weeks. The primary outcomes will be the differences in the mucosa microbiota composition at different tumor locations, and differences in gut microbiota composition, adverse effects, and blood concentration of capecitabine posttreatment. The secondary outcomes will include other blood indicators, short-chain fatty acids (SCFAs) concentration, quality of life, and mental health.

Discussion: This study will reveal the potential benefits of prebiotic for improving the gut microbiota composition, alleviating the adverse effects, and improving the efficacy of chemotherapy in patients with CRC. In addition, this study will provide data on the different distribution of tumor microbiota and the different changes of gut microbiota during treatment in LSCRC and RSCC, which may provide novel insights into personalized cancer treatment strategies based on primary tumor locations and gut microbiota in the future.

Trial registration: Chinese Clinical Trial Registry(www.chictr.org.cn): ChiCTR2100046237. Registered on 12 May 2021.

Introduction

Worldwide, colorectal cancer (CRC) has become the third-ranked cancer in incidence and the second-ranked in mortality. It is estimated that 935,000 people died of CRC in 2020 according to the 2020 Global Cancer Statistics [1]. Lacking of physical activity, obesity, and diet were all independent factors related to the risk of CRC [2]. Some studies suggest that factors causing the imbalance of intestinal flora may also be significant to the carcinogenesis of CRC [3, 4]. Based on location, CRC can be classified into left-sided CRC (LSCRC) and right-sided CC(RSCC). RSCC is defined as any tumor occurring in the cecum, ascending colon, hepatic flexure, or first two-thirds of the transverse colon, while the LSCRC is defined as any tumor occurring in the latter one-third of the transverse colon, splenic flexure, descending colon, sigmoid colon and rectum [5]. Different primary tumor locations differ in incidence, pathogenesis, clinical

characteristics, survival prognosis, molecular biological characteristics, and gut microbiota [6]. Several studies have demonstrated that patients with LSCRC had a significantly better prognosis compared with those with RSCC [7–11].

Increasing evidence supports the character of intestinal flora in the pathogenesis and treatment of CRC. *Fusobacterium nucleatum* adheres, invades, and induces carcinogenic and inflammatory responses via its unique FadA adhesion to activate the growth of CRC cells [12]. Other than the direct effect of specific bacteria on partial tissues, the broader bacterial community may regulate the risk and progression of CRC via competitive rejection and other mechanisms. Gut microbiota-dependent short-chain fatty acids (SCFAs) generation fermented from dietary fiber exert protection against colorectal tumorigenesis in rodents [13].

At present, cytotoxic drugs are generally applied for postoperative adjuvant therapy or advanced CRC patients, usually accompanied by a series of adverse effects (such as constipation, diarrhea, nausea, and bone marrow suppression) and individual differences in drug efficacy [14, 15]. In recent years, with the rise of ‘pharmacomicrobiomics’, the significance of the intestinal flora in regulating the host response to chemotherapeutic drugs is increasingly recognized [16]. Intestinal flora can modulate the anti-tumor activity of chemotherapy drugs [17]. The killing effectiveness of 5-Fu on CRC can be enhanced by bacterial-derived urolithin A [18]. Importantly, proteobacteria can promote the deglycosylation of capecitabine [19]. Moreover, some studies have reported that probiotics/prebiotics can reduce the adverse effects of chemotherapy. *Lactobacillus casei* variety *rharnosus* plays a preventive role in FOLFOX-related intestinal mucositis in CRC-bearing mice [20]. In CRC patients, *L. rharnosus* GG consumption reduced the incidence of 5-FU-induced severe diarrhea and abdominal discomfort compared with guar gum fiber [21]. Lu et al. found that synbiotics (containing a variety of probiotics and prebiotic ingredients) can help relieve postoperative chemotherapy-related adverse effects, further improving the quality of life (QoL) in CRC patients [22]. Hence, targeting the gut microbiota in clinical practice to modulate the efficacy and toxicity of chemotherapeutic drugs has a promising future.

Although there have been some reports on the effects of microecologies on modulating intestinal flora and reducing adverse effects in patients with CRC during chemotherapy, they are still very limited. Through our current knowledge, using microecologies to interfere with the bioavailability of chemotherapeutic agents in a microbiota-dependent manner has not been reported, as well as differences in the intervention effect of prebiotics on the intestinal flora of CRC patients at different primary tumor locations.

Xylooligosaccharide (XOS) is a mixture of oligosaccharides consist of 2–9 xylose units connected by β -1,4-glycosidic bonds [23, 24], which can scape digestion by host enzymes in the small intestine and enter the large intestine directly, and served as major substrates for gut bacterial growth. XOS can be utilized by beneficial bacteria first in the intestine, enriching bifidobacteria and lactobacillus, and inhibiting the proliferation of harmful bacteria such as *E. coli* and *Salmonella enteritidis* to reduce the production of harmful substances [25–27]. The proliferation effect of XOS on bifidobacteria is about 20

times that of other functional oligosaccharides, with higher selectivity [28, 29]. XOS can facilitate the generation of SCFAs such as acetic acid, propionic acid, and butyric acid [30], as well as other organic acids such as lactic acid, succinate, formic acid, isobutyric acid, and isohexanoic acid [31], which play important roles in preventing various intestinal diseases. XOS was found to increase the moisture content of stool and improve constipation [32]. In addition, XOS have a strong ability to adsorb pathogens, thereby preventing diarrhea [33]. Evidence from in vitro and animal research indicates that XOS play roles in inhibiting the secretion of inflammatory cytokines and have an effect on anti-inflammatory and anti-tumor [34, 35]. Chronic toxicology studies on XOS show that it is safe and reliable with no toxicity in humans, dogs, rodents, and other animals [36–38].

This study will explore the difference of tumor microbiota and the different gut microbiota changes during treatment between LSCRC and RSCC. This will give us a better understanding of the tumor microenvironment, a better understanding of the mechanism of cancer occurrence and progression, and provide a basis for new precision treatment and diagnosis methods in the future. Prebiotics are potentially innovative, inexpensive, and easy-to-persist interventions which can be used for the conservative management of CRC patients. This trial aims to explore the effect of XOS on increasing the bioavailability of cytotoxic drug, reducing the adverse effects of chemotherapy, and improving the QoL of CRC patients by modifying gut microbiota.

Methods And Analysis

Study design

This study is a single-center, randomized, parallel controlled clinical trial conducted at the Affiliated Hospital of Jiangnan University in Wuxi, China. Participants with LSCRC and RSCC will receive prebiotic or placebo intervention during postoperative chemotherapy for three months. The study flow is shown in Fig. 1. Study procedures and data collection are shown in Fig. 2 based on SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) [39].

Inclusion criteria

1. Patients diagnosed with primary CRC who will be treated with XELOX chemotherapy;
2. Patients aged 18–80 years;
3. Patients with an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 or 1, absolute number of neutrophils $\geq 1.5 \times 10^9/L$, platelets $\geq 100 \times 10^9/L$, serum creatinine ≤ 1.5 times upper limit of normal (ULN), total bilirubin ≤ 1.5 times ULN, aspartate transaminase/alanine transaminase ≤ 2.5 times ULN and carcinoembryonic antigen within the normal range;
4. Patients have not received preoperative neoadjuvant radiotherapy and chemical treatment;
5. Written informed consent.

Non-inclusion criteria

1. Suffer from other tumors at the same time;
2. Patient has other digestive tract diseases except gastrointestinal tumors, such as inflammatory bowel disease, acute gastroenteritis, etc.;
3. Those who have taken antibiotics, drugs or food containing probiotics within 2 weeks;
4. Those with active infections or those with mental illness, cardiovascular and cerebrovascular diseases, severe cardiopulmonary dysfunction, and other serious diseases that are not suitable for chemotherapy;
5. Those who have undergone multiple courses of chemotherapy, extensive radiotherapy, advanced age, bone marrow metastasis, severe infection, adrenal insufficiency, and severe illness;
6. Women during pregnancy;
7. Patients who cannot receive treatment on time and cannot cooperate fully.

Exclusion criteria

Patients who change medication regimen in the chemotherapy cycle, patients who receive antibiotics or other probiotics/ prebiotics during the intervention, individuals who do not take the prebiotics consistently, and individuals who suffer complications that may be associated with prebiotics will be excluded.

Randomization and blinding

After signing the informed consent form and complete baseline questionnaires, patients with LSCRC (n= 50) and RSCC (n= 50) will be randomly divided into prebiotic group (n=25) and control group (n=25) in a 1:1 allocation ratio, respectively. The random number will be generated by a computer and sealed in envelopes by an investigator who will not be involved in running the study, and placed in a safe place. Once a participant meets the enrollment conditions and signs the informed consent can open one envelope in the order of enrollment time. Prebiotic and placebo will be packaged in opaque bags without any graphics, and their appearance and smell are the same. A third party who has no direct involvement will stick the "A" and "B" codes on the packaging boxes. Before data analysis, participants and investigators will not know the contents of the bags, allocation, and study treatments.

Setting and interventions

Posters and leaflets will be accessible in the outpatient and inpatient departments of the hospital so that interested candidates with CRC can get in touch with investigators to participate in the screening process. The eligible participants will be invited for the first visit, and two trained investigators will explain the

study procedures in detail to them: (1) The purpose of this study, whether there are risks and discomforts, whether it needs to be paid, whether it is completely voluntary, etc.; (2) The types of samples collected in this study and the details of the collection; (3) The personal information of the subjects, research-related measurement indicators, and physical examination information will be kept confidential. Informed consent will be voluntarily signed by individuals who agree to participate in this study. Basic information questionnaires, including body mass index, age, gender, and dietary habit etc., will be conducted through interviews. Blood and stool samples will be obtained as baseline samples from the participants before preoperative bowel preparation. During surgery, three samples from three different sites including tumor tissue, paracancerous tissue, and normal tissue will be collected in sterile tubes and stored in liquid nitrogen, then will be transferred to the -80°C refrigerator until use. The participants will visit the hospital for postoperative chemotherapy about 3 weeks after surgery. Since the chemotherapy regimen will change with the cancer stage, only participants who receive XELOX chemotherapy (oxaliplatin + capecitabine) will continue to participate in this trial.

Participants in the prebiotic group (n = 25 per group) will be asked to consume prebiotic (XOS, 3 g/day) along with routine capecitabine therapy. Participants in the control group (n = 50 per group) will be asked to consume placebo (maltodextrin, 3 g/day). Prebiotics and placebo are white powder, packed in 3g/ bag. Since maltodextrin is colorless and tasteless after dissolution, it can reduce the chance of the placebo effect interfering with results. Each prebiotic bag (produced by Heagreen Company, Henan, China) contains 40.64% xylobiose, 27.75% xylotriose, 14.16% xylotetraose, 7.14% xylopentaoase, 7.8% xylohexaoase. Each box contains 30 bags. Participants will be instructed to mix one bag prebiotic or placebo with water and consume it every day. Participants will be given a box of prebiotics each time they visit the hospital for chemotherapy. The intervention will last for 12 weeks. Participants will be monitored for prebiotic consumption at each delivery of prebiotics and weekly telephone follow-up. Throughout the intervention, volunteers will be required to maintain their lifestyle, diet, as well as medications. They will be asked to collect stool and blood samples before (3 week) and after (15 week) the intervention and complete questionnaires in each chemotherapy cycle. Participants are asked to inform the investigators in time if there is any adverse event that may be associated with prebiotic. These adverse events will be recorded by investigators. Protocol modifications will be communicated to the ethics committee through an official modification request, and the participants will be notified by telephone.

Data collection and sample handling

The overall study design is depicted in Fig. 1. Assessment of participants will be conducted at the perioperative (baseline = week 0), pre-treatment (week 3), post-treatment (week 15), and in each chemotherapy cycle. The primary outcomes will be the differences in the microbiota composition at different CRC tumor sites and differences in gut microbiota composition, adverse reactions, blood concentration in patients with CRC treated for 12 weeks with XOS or placebo. The secondary outcomes will include other blood indicators, SCFAs concentration, QoL, and mental health.

Gut microbial composition

Tissue samples including tumor, para-carcinoma mucosa (2 cm away from the tumor), and normal mucosa (as far as possible from the tumor) will be obtained from LSCRC and RSCC participants during surgery. Each tissue will be cut into small pieces with a volume of 1 cubic centimeter and put into the cryopreserved tubes. After being frozen in liquid nitrogen, the tissue will be stored in the -80 °C refrigerator for later use.

A total of 6 g stool samples from each participant will be collected in the morning for intestinal flora examination at baseline prior to preoperative bowel preparation and at 3 weeks (pre-treatment) and 15 weeks (post-treatment). Briefly, participants will be instructed to: (1) exhaust urine before placing disposable stool tray to prevent contamination of stool; (2) pass stool on the tray; (3) use a sterile spoon to dig up about 2g stool from middle and posterior segment of stool and inserted into a sterilized screw cap containers marked with the participant's code and sampling date. For the purpose of reducing the change of microbiota composition in the stools, samples will be temporarily stored in a foam box with ice packs and transferred to the -80°C refrigerator in the laboratory within two hours.

The total DNA of frozen samples will be extracted via QIAamp (QIAGEN) stool DNA kit according to manufacturer's instructions. DNA integrity and size will be assessed by the NanoDrop ND-1000 spectrophotometer (Thermo, USA) and 1% agarose gel electrophoresis. DNA samples will be kept at -80°C for later use. The bacterial 16S rRNA V3–V4 regions will be amplified by polymerase chain reaction (PCR). High-throughput sequencing will be performed on an Illumina platform.

A sequence similarity threshold of 97% will be used to select operational taxonomic units (OTUs) and then perform taxonomy assignments via the Usearch platform (version 7.1 <http://drive5.com/uparse/>). Principal co-ordinates analysis (PCoA) on weighted UniFrac distances will be measured on all OTUs by QIIME. Alpha diversity will be calculated through the Shannon index, Simpson index, and Chao1 metrics using mothur (version v.1.30.1 http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity). Linear discriminant analysis (LDA) will be performed on the analysis software LEfSe (<http://huttenhower.sph.harvard.edu/galaxy/>). Subsequently, the communities or species that have significant differences in the sample division will be selected.

Stool SCFA profiling

Stool samples aliquots of 50 mg g of dry weight each, will add with 50 µL phosphoric acid, 100 µL isohexanoic acid solution, and 400 µL diethyl ether to homogenize. After centrifugation, the supernatant will be separated for gas chromatography-mass spectrometry (GC-MS) analysis. Stool SCFA profiling will be conducted using an Agilent Technology 6890 GC with autosamplers and 5973 MS Detection and Chemstation Data System (Agilent, Singapore). Chromatographic conditions will be as follows: Agilent HP-INNOWAX capillary column (30 m *0.25 mm ID*0.25 µm); shunt injection, injection volume 1µL, shunt ratio 10:1; injection port temperature of 250°C; ion source temperature of 230°C; initial oven temperature

of 90°C, 10°C/min up to 120°C, 5°C/min up to 150°C, 25°C/min up to 250°C, and keep at 250°C for 2 min. Helium will be used as gas carrier at a constant flow rate of 1 mL/min.

Blood tests

Venous blood samples will be collected before preoperative bowel preparation, at 3 weeks (pre-treatment) and 15 weeks (post-treatment). After fasting for 12 hours, venous blood will be collected into a coagulating tube in the morning and centrifuged at 3000 rpm for 10 minutes for obtaining the upper serum (Thermo, USA). In addition to some of the usual blood biochemical indexes, we will use commercial enzyme-linked immunosorbent assay (ELISA) kits to assess the levels of inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interferon gamma (IFN- γ). Cellular immune indices, including T-lymphocytes (CD3+), helper inducer T cells (CD3+CD4+), suppressor/cytotoxic T cells (CD3+CD8+) will be measured by BD-FACS Canto II flow cytometer (Becton Dickinson, USA).

For the purpose of evaluating the bioavailability of chemotherapy drugs in participants, the blood concentration of capecitabine will be measured at 15 weeks (after intervention). On the third day of the fifth cycle of treatment (taking capecitabine for at least four meals), 5 mL venous blood samples will be collected 2 hours after taking medicine in the morning, put into a tube with ethylene diamine tetraacetic acid (EDTA) anticoagulant, and sent to the pharmacy department of the hospital for testing immediately.

Adverse effects

Adverse effects, including nausea, vomiting, diarrhea, leukopenia, anemia, thrombocytopenia, diarrhea, oral mucosal reaction, peripheral neuropathy, hand-foot syndrome, pigmentation, and abnormal liver function etc., will be assessed in each chemotherapy cycle using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 [40]. All adverse effects will be recorded from grades I to IV according to severity.

Food records

In order to minimize the interference of personal diet on the gut microbiota and record changes in dietary habits in time, volunteers will be asked to complete a 24-hour food record each time a stool sample is collected. They need to describe all foods and beverages consumed in detail including ingredients, weight, and cooking style. Daily nutrient intakes will be calculated based on the China Food Composition 2009 [41].

Health-related QoL

QoL will be assessed in each chemotherapy cycle using Chinese version of QoL questionnaire-cancer 30 (QLQ-C30) formulated by the European Organization for Research and Treatment of Cancer. The questionnaire has 30 items, including 5 functional scales, 3 symptom scales, 6 individual measures items, and a global health [42].

Mental health

The prevalence of anxiety and depression in CRC patients is 13%-57%[43], and one of the important factors is the adverse effects of chemotherapy [44]. Hospital Anxiety and Depression Scale (HADS), an effective questionnaire with 14 items, will be used to assess depression and anxiety in each chemotherapy cycle. Each item is on a 4-point scale and final scores are proportional to the degree of anxiety and depression.

Sample size calculation

Since there is no clinical trial assessing the effect of intestinal flora on the bioavailability of chemotherapy drugs and the intervention endpoints of intestinal flora are currently unable to determine, the sample size is estimated based on the data from a previous clinical trial [22] which assessed the effects of synbiotics intervention on chemotherapy-related adverse effects (including diarrhea, appetite loss, nausea, and vomiting) for CRC patients received post-operative chemotherapy, showing that 20 volunteers per group had 90% power at an alpha level of 0.05 to detect significant differences. Considering the long duration of the study (3 months) and the expected withdrawal of participants during the intervention, we plan to recruit $n = 25$ participants per group ($n = 100$ total).

Data analysis

The visiting time of the participants is in accordance with the treatment course, so as to facilitate the retention of the participants. The measured data for each visit will be collected on paper and then recorded electronically on a secure, locked office computer. The paper version of the data will be locked in a bookcase. Only the investigators running in the study will have access to the final study dataset.

The obtained data will be analyzed by SPSS software V26.0 (IBM, USA). Continuous data will be expressed as mean $\pm$ standard deviation (SD), and categorical data will be expressed as the number of cases (n) and percentage (%). Differences in parameter continuous variables and asymmetric variables between groups will be analyzed through independent samples t test and Mann-Whitney U test, respectively. Using a paired t test to assess the effect of the intervention in each group. The normality of data will be assessed using the Kolmogorov-Smirnov test.

Results will be regarded as statistically significant if p values < 0.05 .

Discussion

CRC is one of the most prevalent cancers world-wide. Postoperative adjuvant chemotherapy for CRC is a helpful attempt in recent years. However, the adverse effects during chemotherapy and the inter-individual variation in drug efficacy are still relatively big problems. Moreover, the different primary tumor locations may result in differences in intestinal flora composition and prognosis. Efforts to find a kind of safe food with therapeutic effect and to evaluate its impact on patients with CRC at different primary tumor locations are critical. Studies have revealed the vital part of intestinal flora in the progression and treatment of CRC [3, 12, 45], and the concept of “pharmacomicrobiomics” is increasingly recognized [46]. Targeting intestinal flora, therefore, may modulate the efficacy and adverse effect of chemotherapy.

Both animal and human studies provide strong evidence that microecologies play essential roles in preventing chemotherapy-induced mucositis [47]. Bowen et.al. [48] have revealed the effects of VSL#3, a mixture of *Lactobacilli*, *bifidobacteria*, and *streptococcus*, on preventing diarrhea following chemotherapy with irinotecan in rats. Synbiotics have significantly reduced the occurrence of severe lymphopenia and diarrhea, increased levels of *Bifidobacterium* and *Lactobacillus* species and concentrations of SCFAs in esophageal cancer patients undergoing neoadjuvant chemotherapy [49]. Dietary supplementation of prebiotics (oligofructose and inulin) can enhance the efficacy of six cytotoxic drugs and prolong the lifespan of rodents with transplantable malignant tumors [51, 52]. XOS is a mixture of oligosaccharides which can be considered as a prebiotic[50]. XOS can modulate the diversity of intestinal flora, effectively multiply favorable bacteria such as bifidobacteria, and produce beneficial metabolites including SCFAs [53]. In addition, it has been reported that XOS are beneficial to type 2 diabetes mellitus [54], diarrhea [33], and constipation [32]. However, so far, there is no study evaluating the role of XOS consumption on the intestinal flora in patients with CRC.

This study will reveal the potential benefits of prebiotic for improving the gut microbiota composition, alleviating the adverse effects, and improving the efficacy of chemotherapy in patients with CRC. In addition, our study will provide data on the different distribution of tumor microbiota and the different changes of gut microbiota during treatment in LSCRC and RSCC, which may provide novel insights into personalized cancer treatment strategies based on primary tumor locations and gut microbiota in the future.

Trial Status

Recruitment began in June 2021 and is expected to be completed by May 2022.

Abbreviations

CRC: Colorectal cancer; SCFAs: Short-chain fatty acids; LSCC: Left-sided colorectal cancer; RSCC: Right-sided colon cancer; QoL: Quality of life; XOS: Xylooligosaccharide; SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials; ULN: Upper limit of normal; PCR: Polymerase chain reaction;

OTUs: Operational taxonomic units; PCoA: Principal co-ordinates analysis; LDA: Linear discriminant analysis; GC-MS: Gas chromatography-mass spectrometry; ELISA: Enzyme-linked immunosorbent assay; TNF- α : Tumor necrosis factor- α ; IL-1 β : Interleukin-1 β ; IFN- γ : Interferon gamma; CD3+: T-lymphocytes; CD3+CD4+: Helper inducer T cells; CD3+CD8+: Suppressor/cytotoxic T cells; EDTA: Ethylene diamine tetraacetic acid; CTCAE: Common Terminology Criteria for Adverse Events; QLQ-C30: QoL questionnaire-cancer 30; HADS: Hospital Anxiety and Depression Scale.

Declarations

Ethics approval and consent to participate

This study protocol (version 1, May 8, 2021) has been approved by an ethical committee of the Affiliated Hospital of Jiangnan University, Wuxi, China (LS2021026) and was registered at the Chinese Clinical Trial Registry (www.chictr.org.cn, ChiCTR2100046237). Informed consent will be voluntarily signed by individuals who agree to participate in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Availability of data and materials

Not applicable.

Authors' contributions

All authors took part in the design of this study. YML was responsible for writing the entire manuscript, and FZ and WZ contributed to manuscript revising. FZ was responsible for obtaining ethical approvals. FZ, WZ, and HC contributed to the acquisition of financial support. BJF, CQB, JMX, HXC, YM, XPC, and XG participated in the recruitment of participants. All authors have read and approved the final manuscript.

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Figures

Figure 1

Study flowchart

	STUDY PERIOD						
	Enrollment	Allocation	Post-allocation				Close-out
TIMEPOINT	0	WK3 Before chemo	1st cycle chemo	2st cycle chemo	3st cycle chemo	4st cycle chemo	WK15 5st cycle chemo
ENROLLMENT:							
Eligibility screen	X						
Informed consent	X						
Allocation		X					
INTERVENTIONS:							
Left-sided prebiotic group			←→				
Left-sided control group			←→				
Right-sided prebiotic group			←→				
Right-sided control group			←→				
ASSESSMENTS:							
Mucosa collection	X						
Stool collection	X	X					X
SCFAs concentration		X					X
Blood concentration							X
Other blood tests	X	X					X
Adverse effects			X	X	X	X	X
Quality of life		X	X	X	X	X	X
Mental health		X	X	X	X	X	X
Food record	X	X					X

Figure 2

SPIRIT diagram: schedule of enrollment, interventions, and assessments

Supplementary Files

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- [SPIRITchecklist.docx](#)