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Study Protocol

Project Name:

Short-Term Nutritional Enhancement Combined With Health Education in Postoperative Colorectal Cancer Patients: A Randomized Controlled Trial

Study Institution: School of Public Health, Xi'an Jiaotong University

NCT Number: NCT ID not yet assigned

Protocol Version and Date: Version 1.0, October 10, 2024

Brief Title	Short-Term Nutritional Enhancement Combined With Health Education in Postoperative Colorectal Cancer Patients: A Randomized Controlled Trial
Official Title	The Effects and Mechanisms of Short-Term Nutritional Enhancement Combined With Health Education on Clinical Outcomes in Postoperative Patients With Colorectal Cancer: A Multicenter, Open-Label, Randomized Controlled Trial
Investigational Product	N/A
Clinical Phase	Patients with colorectal cancer staged IIa, IIb, or IIIa without distant metastasis
Study Objectives	**Primary Objectives** 1. To evaluate the short-term effects of personalized nutritional enhancement combined with structured health education on the nutritional status of patients with colorectal cancer. This will be assessed through a comprehensive set of indicators, including laboratory tests, anthropometric measurements, psychological evaluations, dietary assessments, physical activity levels, and sleep patterns. Comparisons will be made between intervention and control groups. 2. To determine the long-term impact of nutritional enhancement combined with health education on clinical outcomes in colorectal cancer patients. This will be evaluated using survival analysis and Cox proportional hazards models to examine overall survival, prognosis, and associated prognostic factors. **Secondary Objectives** 1. To improve patients' quality of life: Through nutritional intervention and health education, this study aims to enhance the overall quality of life of patients with colorectal cancer, reduce treatment-related side effects, and improve psychological well-being. 2. To improve the effectiveness of oncology nutrition education: The study will evaluate the positive impact of nutritional education on patients' nutritional status, enhance adherence and engagement with nutrition-related interventions, and ensure effective implementation of the intervention protocols. **Exploratory Objectives** 1. To identify potential metabolism-related biomarkers: Using integrated metabolomics analyses, the study will investigate metabolic

	alterations associated with colorectal cancer and aim to discover novel biomarkers to support individualized therapy. Specific evaluation metrics include changes in metabolite profiles and their correlation with clinical outcomes. 2. To assess the effectiveness of Internet-based health education: This objective explores the efficacy of video-based and online platform-delivered health education in colorectal cancer patients, compared to traditional education formats. Evaluation tools include platform usage analytics and patient feedback questionnaires. 3. To assess the effects of nutritional intervention on inflammation and immune response: By analyzing serum inflammatory markers and immune indicators, the study will evaluate the impact of nutritional intervention on systemic inflammation and immune function. Specific markers include C-reactive protein (CRP), leukocyte count, and cytokine levels.
Study Design	A prospective, multicenter, open-label, randomized controlled clinical trial.
Study Population	Approximately 360 patients with colorectal cancer will be enrolled.

Research Process

Participant Enrollment and Study Workflow

Identify Potential Participants

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Confirm Eligibility Criteria

↓

Obtain Informed Consent

↓

Randomization

→ Intervention Group

→ Control Group (Standard Care)

Study Arms

- **Group A:** Nutrition Education + Nutrition Enhancement
- **Group B:** Nutrition Education Only
- **Group C:** Nutrition Enhancement Only
- **Group D:** Basic Nutrition Management (Control)

Follow-up Timeline

T0, T1, T2, T3, T6, T12 (Baseline and follow-up at months 1, 2, 3, 6, 12), annually up to 5 years

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Sample and Data Collection & Mechanistic Analysis

Metabolomic Analysis of Key Metabolites in CRC Patients

- Blood sample collection and preservation
- Blood serum untargeted metabolomics analysis
    1. Sample pre-processing
    2. Serum separation
    3. Mass spectrometry analysis
- Blood serum targeted metabolomics analysis
    1. Raw data processing
    2. Pathway enrichment and differential metabolite screening
    3. Identification of non-targeted metabolites
    4. Bioinformatics analysis of key metabolites
    - Lipidomic/high-resolution targeted metabolomics

- Validation of differential metabolites

Gut Microbiota and Functional Profiling in CRC Patients

- Fecal sample collection and storage
- Shotgun metagenomic sequencing
  1. DNA extraction from fecal samples
  2. Library construction and sequencing
  3. Taxonomic and functional annotation of metagenomic data
  4. KEGG Orthology (KO) annotation
- Microbiome analysis
  - α-diversity analysis
  - β-diversity analysis
  - Taxonomic differential analysis
  - Functional pathway differential analysis
  - Visualization of microbial alterations

Additional Analyses

- Host-related clinical and laboratory parameter analysis
  - Inflammatory markers
  - Nutritional status
  - Physical activity and sleep assessments
  - Biochemical indicators
  - Immune cytokines
  - Clinical endpoints
  - Other disease-related parameters

Integrated Analysis

- Multi-omics and clinical data integration
- Analysis of differences between intervention groups and the control group

Participant Recruitment and Screening Procedures

Participants will be recruited across multiple hospitals nationwide. Eligible patients will be identified by clinical healthcare providers based on the study's inclusion criteria. Research posters introducing the study will be distributed to patients. Study staff will follow up with potential participants to assess their interest and conduct eligibility screening. Screening may be conducted

	either in person or via online interviews. Patients who are unable to complete the study, such as those planning to become pregnant, will be excluded. Online interviews will not assess adherence at this stage. Prior to any study-related measurements during the screening visit, all potential participants must provide written informed consent. After eligibility is confirmed, participants will undergo baseline assessments and be formally enrolled in the study. Individuals participating in the screening visit will not be enrolled unless explicitly authorized by the study investigators.
Inclusion Criteria	● Signed informed consent ● Age ≥ 18 years ● Pathologically confirmed diagnosis of colon or rectal cancer ● Mentally alert and capable of communication ● Willing to participate in follow-up, with an estimated life expectancy of more than 6 months ● Cancer stage IIa, IIb, or IIIa
Exclusion Criteria	● Nutritional risk screening score of mPG-SGA < 2 or NRS-2002 < 3 ● Diagnosed with AIDS ● History of organ transplantation ● Pregnant or breastfeeding women ● Concurrent participation in another interventional clinical trial ● Inability to care for oneself independently ● Inability to engage in physical activity during the perioperative period ● Severe comorbid conditions (e.g., uncontrolled cardiovascular disease, severe hepatic or renal dysfunction) ● Known allergy or intolerance to components of the nutritional supplements used in the study
Outcome Measures	**Primary Outcomes** 1. **Change in Nutritional Status** The degree of improvement in nutritional status in the intervention groups compared to the control group, assessed by laboratory tests, anthropometric analysis, and body composition measurements. 2. **Improvement in Quality of Life** Quality of life changes will be assessed through validated questionnaires and psychological evaluations, with a focus on physical function, social function, and emotional well-being. **Secondary Outcomes** 1. **Survival and Prognosis** Survival outcomes and prognostic factors will be analyzed using Kaplan-Meier survival

	curves and Cox proportional hazards models to evaluate the impact of nutritional intervention on long-term survival and prognosis. 2. **Complications and Adverse Effects** The incidence of treatment-related complications and adverse effects will be compared between intervention and control groups to determine whether nutritional intervention reduces toxicity or other negative outcomes. 3. **Patient Adherence and Effectiveness of Nutrition Education** Patient adherence to nutritional and educational interventions will be assessed, along with the effectiveness of educational videos and counseling in improving nutrition-related knowledge and behavioral changes. **Exploratory Outcomes** 1. **Gut Microbiota Alterations** Metagenomic analyses will be used to evaluate the impact of nutritional intervention on gut microbial composition and function, and to identify key taxa associated with CRC progression. 2. **Metabolomic Profiling** Untargeted serum metabolomics will be performed to assess the effects of nutritional intervention on metabolic pathways, aiming to identify novel biomarkers and metabolic signatures associated with outcomes. 3. **Mechanistic Insights via Single-Cell Analysis** Single-cell RNA sequencing will be used to investigate how nutritional interventions modulate metabolic and immune pathways that influence clinical outcomes, with the goal of supporting personalized treatment strategies.
Safety and Tolerability Parameters	**Primary Safety Parameters** 1. **Adverse Events (AEs) and Serious Adverse Events (SAEs):** All AEs and SAEs during the intervention and follow-up phases will be recorded and evaluated, including incidence, severity, duration, and potential causal relationship to the intervention. 2. **Treatment-Related Adverse Reactions:** Particular attention will be given to gastrointestinal symptoms, metabolic disturbances, and psychological burdens potentially associated with nutritional supplementation or health education. **Secondary Safety Parameters**

	1. **Changes in Laboratory Parameters:** Regular monitoring of complete blood count, liver and renal function, and electrolyte balance will be conducted to assess physiological impacts of the intervention. 2. **Vital Signs Monitoring:** Blood pressure, heart rate, body temperature, and other vital signs will be recorded periodically to evaluate the clinical safety of the intervention. **Tolerability Parameters** 1. **Patient Adherence and Acceptance:** Adherence and acceptance will be assessed through questionnaires and interviews, capturing any discomfort, resistance, or compliance issues experienced during the intervention. 2. **Overall Tolerability of the Interventions:** Clinical observation and patient feedback will be used to evaluate the tolerability of both the nutritional supplements and the health education content.
Other Parameters	**Disease Characteristics:** • Type and stage of diagnosed tumor (e.g., stage IIa, IIb, or IIIa colorectal cancer) • Histopathological diagnosis • Prior cancer treatments (e.g., surgery, radiotherapy, chemotherapy) • Karnofsky Performance Status (KPS) score **Laboratory and Anthropometric Data:** • Laboratory parameters: complete blood count, liver function, renal function, electrolytes • Anthropometrics: height, weight, body mass index (BMI), waist circumference, hip circumference **Nutritional Status Assessment:** • Nutritional Risk Screening (NRS-2002) • Patient-Generated Subjective Global Assessment (mPG-SGA) • Dietary status using the Simplified Dietary Self-Assessment Tool (SDSAT) • Physical activity and sleep status (PA tool) **Quality of Life and Psychological Status:** • Quality of life assessed using the EORTC QLQ-C30 questionnaire • Anxiety and depression status assessed using the Hospital Anxiety and Depression Scale (HAD)

	• Nutrition education compliance and dietary knowledge-attitude-practice (KAP) questionnaire -
Study Arms and Randomization	Participants will be coded sequentially according to enrollment order (e.g., 01, 02, 03…). After coding, participants will be stratified based on baseline nutritional status and further grouped by cancer stage using block randomization. A computer-generated random seed will be used to ensure allocation concealment. Participants will then be randomly assigned to one of four arms: **Group A: Nutritional Enhancement + Health Education** Participants will receive standard inpatient treatment plus personalized nutritional support based on calculated energy and protein requirements, designed by a multidisciplinary nutrition support team. Additionally, participants will receive structured health education delivered through short videos covering nutrition, physical activity, and psychological support. A dedicated team of experts will provide ongoing consultation on nutrition-related issues. **Group B: Health Education Only** Participants will receive standard inpatient treatment plus health education. The education component consists of short videos addressing nutrition, exercise, and psychological well-being. Content is delivered via an online platform, and expert consultation will be available for nutrition-related questions. **Group C: Nutritional Enhancement Only** Participants will receive standard inpatient care along with personalized nutritional support designed by the nutrition support team based on individual energy and protein requirements. **Group D: Basic Nutritional Management (Control Group)** Participants in this group will receive standard inpatient care without additional nutritional support or health education. They will eat according to personal capacity and appetite. No individualized nutrition counseling or recommended supplementation will be provided.
Study Interventions and Products	**Personalized Nutritional Support Program** The individualized nutritional support plan for colorectal cancer patients will be tailored to each participant's specific nutritional status and clinical needs. The plan will aim for ≥80% of estimated energy requirements, ≥80% of protein targets, and 100% of micronutrient requirements. Nutritional prescriptions will be developed by a multidisciplinary nutrition support team and adjusted regularly to maintain adherence to these targets. **Health Education Program**

	A digital “Internet Plus” health education platform will be developed and applied to deliver structured health education to patients and their families. The platform will include weekly online videos, science-based educational articles, and interactive Q&A sessions. Content will cover nutrition, physical activity, and psychological support. The intervention will last for 12 weeks, structured as follows: • **Weeks 1–4:** Intensive education phase, with 6 nutrition videos, 6 exercise videos, and 3 psychological support videos released each week • **Weeks 5–12:** Maintenance phase, with one routine follow-up per week (online or in person) • **Week 12:** Comprehensive evaluation at the conclusion of the intervention The video content is based on the team’s published book titled *"12-Week Cancer Rehabilitation: Nutrition, Exercise, and Psychology"* (People's Medical Publishing House, ISBN 978-7-117-35805-7). Selected content from the book has been adapted into the educational materials used in the study. **Nutrition Monitoring and Evaluation Tools** Standardized tools will be used to assess and monitor participants’ nutritional status and quality of life throughout the intervention. These include: • Patient-Generated Subjective Global Assessment (mPG-SGA) • Simplified Dietary Self-Assessment Tool (SDSAT) • Physical Activity Assessment Tool (PA) **Biobank and Data Analysis Platform** Blood serum and tumor tissue samples will be collected and stored to establish a biobank. These samples will be analyzed using metagenomic and metabolomic technologies to explore potential biological mechanisms and identify novel biomarkers associated with nutritional intervention. **Clinical Trial Management System** A dedicated clinical trial management system (CTMS) will be developed and implemented to ensure standardized and systematic handling of participant recruitment, randomization, data collection, follow-up, and data management. This system is intended to enhance operational efficiency and ensure the integrity and quality of research data.

Study Phases	• **Intervention Phase:**
	  ○ **Nutritional Enhancement Intervention:** Conducted for 14 days post-enrollment
	  ○ **Health Education Intervention:** Implemented over a 12-week period post-enrollment
	• **Follow-Up Phase:**

	○ Follow-up assessments will be conducted at 1, 2, 3, 6, and 12 months, annually up to 5 years after the nutritional enhancement intervention concludes.
Study Description	This clinical study aims to evaluate whether short-term personalized nutritional support, when combined with structured health education, can improve nutritional status, quality of life, and clinical outcomes in patients who have undergone surgery for colorectal cancer (CRC). Colorectal cancer is one of the most common cancers worldwide, and many patients experience malnutrition and poor physical condition during treatment, which can negatively affect recovery and long-term survival. In this multicenter, randomized, controlled clinical trial, approximately 360 postoperative CRC patients will be enrolled and randomly assigned to one of four groups: (A) nutritional enhancement combined with health education, (B) health education alone, (C) nutritional enhancement alone, or (D) standard care (control group). Nutritional support will include individualized diet counseling and oral nutritional supplements tailored to each patient's needs. Health education will be delivered using an “Internet Plus” approach, including weekly educational videos and expert consultations focusing on nutrition, physical activity, and mental health. The primary objectives are to determine whether these interventions can improve patients' short-term nutritional status and quality of life. Secondary outcomes include the impact of interventions on long-term survival, treatment-related side effects, patient adherence to nutrition recommendations, and psychological well-being. This study will also investigate the biological mechanisms underlying the clinical effects by analyzing changes in the gut microbiome, blood-based metabolic profiles, and immune responses. Blood, stool, and tumor tissue samples will be collected and analyzed using advanced techniques, including untargeted metabolomics, metagenomics, and single-cell sequencing. This trial is designed to provide evidence for the integration of nutritional strategies into routine cancer care, and to guide the development of more personalized, effective nutrition-based therapies for colorectal cancer patients. Participants will be followed for up to annually up to 5 years to evaluate both clinical outcomes and biological markers of response.
Statistical Analysis	**1. Sample Size** This study plans to recruit a total of 360 patients with colorectal cancer, who will be randomly assigned to four groups, with 90 participants in each group. The sample size was determined based on both statistical power considerations and practical feasibility. 1.1 Sample Size Calculation The sample size calculation was based on the following assumptions and parameters:

- Primary endpoint: improvement in nutritional status (e.g., body weight, body fat, muscle mass, and serum protein levels)
- Effect size (δ): 0.3
- Standard deviation between groups (σ): 0.6
- Significance level (α): 0.05 (two-sided)
- Statistical power (1–β): 80%

Based on the calculation, at least 75 participants per group are required. To account for an estimated 20% dropout rate, 90 participants per group will be recruited, totaling 360 participants across the four study arms.

$$n = \frac{2(\sigma^2 + \sigma_b^2)(z_{\alpha/2} + z_\beta)^2}{\delta^2}$$

2. Interim Analysis

To ensure ethical and scientific integrity, an **interim analysis** will be conducted at the midpoint of the study.

- **Timing:** Once all participants have completed the 6-month follow-up
- **Scope:** Assessment of the primary endpoint (nutritional status improvement), secondary endpoints (quality of life, complications, adverse effects), and safety outcomes (adverse events and serious adverse events)
- **Decision Criteria:** Based on the interim results, the study team will determine whether the observed effect size or safety profile warrants adjustments in sample size or early termination of the trial. If significant treatment effects or safety concerns are identified, protocol modifications or study cessation will be considered.

3. Analysis Populations

Three analytical datasets will be established for data evaluation:

- **Full Analysis Set (FAS):** Includes all randomized participants who received at least one intervention. Analysis will follow the **Intention-to-Treat (ITT)** principle.
- **Per Protocol Set (PPS):** Includes participants who strictly adhered to the study protocol and completed all scheduled visits and interventions. Used to evaluate the true efficacy of the interventions.
- **Safety Analysis Set (SAS):** Includes all participants who received at least one intervention and have available safety data. Used to assess the safety and tolerability of the interventions.

	4. Descriptive Statistics Descriptive statistics will summarize and present: • **Demographic characteristics:** age, sex, marital status, education, occupation • **Baseline characteristics:** body weight, mPG-SGA score, quality of life scores • **Intervention data:** weight changes at each visit, laboratory results, questionnaire responses Statistical methods will include means, standard deviations, medians, interquartile ranges, frequencies, and percentages as appropriate.
	5. Statistical Methods for Data Analysis Statistical methods will be selected based on the objectives and types of data collected: • **Primary Endpoint Analysis:** Repeated Measures ANOVA will be used to assess changes in nutritional status across time points and between groups. Kaplan-Meier survival analysis and Cox proportional hazards models will be applied to evaluate overall survival and progression-free survival. • **Secondary Endpoint Analysis:** Multivariate regression analysis will be used to explore factors influencing quality of life, complications, and adverse effects. Categorical variables between groups will be compared using chi-square tests or Fisher's exact tests. • **Exploratory Endpoint Analysis:** Bioinformatic tools will be used to analyze gut microbiota and metabolomics data. Principal component analysis (PCA) and clustering methods will be used to identify metabolic features and potential biomarkers. • **Safety Analysis:** Descriptive statistics and proportion comparisons will be applied to analyze the incidence of adverse events and serious adverse events. Logistic regression will be used to assess predictors of safety outcomes. • **Data Handling and Missing Data:** Multiple imputation will be employed to address missing data and ensure robustness of results.
Principal Investigator Team	The study will be conducted by the team of Professor Xiaoqin Luo at the School of Public Health, Xi'an Jiaotong University.

Appendix 1: Roles and Responsibilities

PRIMARY PROTOCOL DEVELOPMENT TEAM

Name	Title	Role in the Study
Xiaoqin Luo	Professor	Study design, protocol drafting and revision
Hexiang Yang		Study design, protocol drafting and revision
Haige Cao		Statistical analysis planning, protocol revision
Yaxin Yu		Protocol review and revision

Study Oversight Committee

The research team led by Professor Xiaoqin Luo at Xi'an Jiaotong University will establish the following committee to monitor the progress of the study and oversee data quality and integrity.

Data Review and Monitoring Committee

Position	Role
CS Researcher / Chief Scientist	Chair / Lead Presenter
CS Manager	Committee Member
Data Manager	Committee Member
Biostatistician	Committee Member
Statistical Programmer	Committee Member
Medical Monitor	Committee Member
Senior Methodology Lead	Committee Member
Scientist	Committee Member

Appendix 2: Study Schedule of Events

Variable	SV	Folllow-up					
		1th month	2th month	3th month	6th month	1year	Every year until the tenth year
Demographic information	√						
Disease status	√						
KPS Scoring	√	√	√	√	√	√	√
Laboratory examination	√						
Anthropometry	√						
Nutrition survey	√	√	√	√	√	√	√
EORTC QLQ C30	√	√	√	√	√	√	√
Hospital anxiety and expression scale	√	√	√	√	√	√	√
Nutrition-Supply	√	√	√	√	√	√	√
SDSAT	√	√	√	√	√	√	√
PA	√	√	√	√	√	√	√
KAP	√	√	√	√	√	√	√
Adverse Event Recording	√	√	√	√	√	√	√
Short-Term Clinical Outcome		√	√	√	√	√	√