BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-067990
Article Type:	Protocol
Date Submitted by the Author:	02-Sep-2022

Complete List of Authors:	Zhou, Da; Medical School of Nanjing University, Research Institute of General Surgery, Jinling Hospital Zhang, Li; Research Institute of General Surgery, Jinling Hospital, Medical School of Nanjing University Liu, Yuxiu; Nanjing Jinling Hospital, Department of Medical Statistics Lu, Mengjie; Shanghai Jiao Tong University School of Medicine Gao, Xuejin; Research Institute of General Surgery, Jinling Hospital, Medical School of Nanjing University Li, Guoli; Medical School of Nanjing University, Department of General Surgery Xiang, Xiaosong; Medical School of Nanjing University, Department of General Surgery Xu, Hao; Nanjing Medical University, Department of General Surgery Li, Gang; Nanjing Medical University, Jiangsu Cancer Hospital, Department of General Surgery Sun, Zhiping; Nanjing Medical University, Affiliated Second Hospital, Department of Clinical Nutrition wei, Wei; Nanjing Medical University, Affiliated Second Hospital, Department of General Surgery Yi, Kui; The Affiliated Jiangning Hospital of Nanjing Medical University, Department of Gastroenteropancreatic Surgery Zuo, Junbo; Zhenjiang First People's Hospital, Department of General Surgery Wu, Yugang; Soochow University, The First People's Hospital of Changzhou, Department of General Surgery Qian, Jun; Changzhou No. 2 People's Hospital, Department of General Surgery Zhou, Jian; First Affiliated Hospital of Soochow University, Department of General Surgery Duan, Kaipeng; First Affiliated Hospital of Soochow University, Department of General Surgery Wu, Yong; Second Affiliated Hospital of Soochow University, Department of General Surgery Tian, Zhiqiang; Nanjing Medical University Affiliated Wuxi People's Hospital, Department of General Surgery Jiang, Chao; Yixing People's Hospital, Department of General Surgery Wang, Xinying; Medical School of Nanjing University, Department of General Surgery

Keywords:	Gastrointestinal tumours < GASTROENTEROLOGY, Nutritional support < GASTROENTEROLOGY, Adult gastroenterology < GASTROENTEROLOGY

Effects of oral immunonutritional supplement on 3-year disease-free survival in gastric cancer patients with pathologic stage III after total gastrectomy (CRUCIAL): study protocol of a multicenter, randomized clinical trial

Da Zhou,[1] Li Zhang,[1] Yuxiu Liu,[2] Mengjie Lu,[3] Xuejin Gao,[1] Guoli Li,[1] Xiaosong Xiang,[1] Hao Xu,[4] Gang Li,[5] Zhiping Sun,[6] Wei Wei,[7] Kui Yi,[8] Junbo Zuo,[9] Yugang Wu,[10] Jun Qian,[11] Jian Zhou,[12] Kaipeng Duan,[12] Yong Wu,[13] Zhiqiang Tian,[14] Chao Jiang,[15] Xinying Wang[1]

[1]Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu, China

[2]Department of Medical Statistics, Jinling Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China

[3]School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[4]Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[5]Department of General Surgery, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[6]Department of Clinical Nutrition, Nanjing Medical University, Affiliated Second Hospital, Nanjing, Jiangsu, China

[7]Department of General Surgery, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[8]Department of Gastroenteropancreatic Surgery, The Affiliated Jiangning Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[9]Department of General Surgery, Zhenjiang First People's Hospital, Zhengjiang, Jiangsu, China

[10]Department of General Surgery, The Third Affiliated Hospital of Soochow University and The First People's Hospital of Changzhou, Changzhou, Jiangsu, China

[11]Department of General Surgery, ChangZhou Second Hospital, the afflicated Hospital of Nanjing Medical University, Changzhou, Jiangsu, China

[12]Department of General Surgery, the First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[13]Department of General Surgery, the Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[14]Department of General Surgery, Wuxi People's Hospital Affiliated to Nanjing Medical University, Wuxi, Jiangsu, China

[15]Department of General Surgery, Yixing People's Hospital, Yixing, Jiangsu, China

Corresponding author: Xinying Wang

Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, 305 Zhongshan East Road, Nanjing, Jiangsu, China

wangxinying@nju.edu.cn

ABSTRACT

Introduction The nutritional status of patients with gastric cancer (GC) after total gastrectomy continues to deteriorate and lasts a long time after discharge, which is an independent risk factor for mortality. Recent guidelines have recommended appropriate nutritional support after discharge for cancer surgery patients with malnutrition or nutritional risk. The evidence on the efficacy of oral immunonutritional supplement and its effect on long-term disease-free survival (DFS) in patients with GC is limited. This study was designed to test the hypothesis that oral immunonutritional supplement compared to diet alone may improve 3-year DFS of GC patients with pathologic stage III after total gastrectomy (nutrition risk screening 2002 score ≥ 3 at discharge).

Methods and analysis This is a pragmatic, open-label, multicenter, randomized controlled study. 696 eligible GC patients with pathologic stage III after total gastrectomy will be randomized in a 1:1 ratio to oral immunonutritional supplement group or normal diet group for six months. The primary endpoint is 3-year DFS after discharge. The following secondary endpoints will be evaluated: 3-year overall survival; unplanned readmission rate at 3 and 6 months after discharge; quality of life, body mass index, and hematological index at 3, 6, and 12 months after discharge; incidence of sarcopenia at 6 and 12 months after discharge; and the tolerance to chemotherapy. The adverse events of oral immunonutritional supplement will also be evaluated during the intervention.

Ethics and dissemination This study was approved by the ethics committee of Jinling Hospital, Nanjing University (Number 2021NZKY-069-01). The present study may validate the effectiveness of oral immunonutritional therapy in improving 3-year DFS for gastric cancer patients with pathologic stage III after total gastrectomy for the first time. The results of this trial will be disseminated in peer-reviewed journals and at scientific conferences.

Trial registration number ClinicalTrials.gov Registry (NCT05253716).

Strengths and limitations of this study

This is a pragmatic, open-label, multicenter, randomized controlled trial providing top-class evidence concerning the efficacy and safety of oral immunonutrition supplement for gastric cancer patients with pathologic stage III after total gastrectomy.

The data will be handled by an Independent Data Monitoring Committee to ensure the safety of the participants.

Oral immunonutrition supplement is a well-studied method with a favorable safety profile in previous trials.

A sample size of 696 is required to detect the efficacy of oral immunonutrition supplement in improving 3-year DFS for gastric cancer patients with pathologic stage III after total gastrectomy, which will take years before the conclusion could be drawn. Longer survival or recurrence status is not assessed in this study.

INTRODUCTION

Malnutrition is common in patients with gastric cancer. It increases postoperative complications, length of stay, and cost of treatment and weakens the effect of chemotherapy and quality of life (QoL), as well as shortens long-term survival.[1-4] In addition to preoperative malnutrition, which most studies are concerned about, the nutritional status after radical gastrectomy will also continue to deteriorate for nearly 1 year; the rate of malnutrition was approximately 30%–50%.[1, 5, 6] Due to postoperative chemotherapy and several other risk factors, including reduced nutritional intake and malabsorption, continuous weight loss, and sarcopenia (an important phenotype of malnutrition) caused by progressive loss of skeletal muscle mass with decreased physical activity persists long after radical gastrectomy.[7, 8] These conditions are more common and severe in patients with pathological stage III gastric cancer or after total gastrectomy: the proportion of skeletal muscle loss of ≥ 5% six months after surgery has been reported to be up to 51% and 55.4%, respectively.[9, 10] Gastric cancer patients who exhibited ≥ 5% skeletal muscle loss have shown a lower 5-year disease-free survival (DFS) rates (33.8% vs 46.2%; P = 0.020).[10] Moreover, postoperative sarcopenia may persist for approximately 1 year and is significantly related to worse 5-year overall survival (OS) in gastric cancer.[11] Therefore, reinforced nutritional support should be offered to this patient population, and more attention should be provided.

The most recent guideline recommends appropriate postdischarge nutritional support for surgical cancer patients with nutritional risks or those who are already malnourished, and several guidelines have recommended oral nutritional supplement (ONS) as the preferred approach.[12, 13] The limited available evidence suggests that consecutive ONS for 3 months postdischarge has a positive effect on maintaining weight, reduces the risk of sarcopenia, and improves chemotherapy tolerance for gastric cancer after surgery.[14, 15] Although ONS can effectively prolong median OS for metastatic gastric cancers,[16] its effect on survival outcomes of gastric cancer patients with pathologic stage III after total gastrectomy remains unclear. Omega-3

polyunsaturated fatty acids (ω-3 PUFAs, including EPA and DHA) are commonly used as nutrients for immunity. Aoife et al. found that enteral nutrition enriched with 2.2 g of EPA/d can maintain skeletal muscle mass after esophagectomy.[17] Furthermore, recent preclinical studies have shown that ω-3 PUFAs can also inhibit gastric cancer-related cell growth and metastasis.[18-20] However, to our knowledge, studies on disease-free survival (DFS) after oral immunonutritional supplement after surgery for advanced gastric cancer are lacking.

The objective of this pragmatic, multicenter, randomized clinical trial (Clinicaltrials. gov identifier: NCT05253716) is to evaluate the efficacy of the postoperative oral immunonutritional supplement for 6 months and its effect on 3-year DFS, 3-year OS, weight maintenance, sarcopenia, QoL, chemotherapy tolerance, and unplanned readmission of gastric cancer patients with pathological stage III after total gastrectomy. We hypothesize that oral immunonutritional supplement will have significantly more benefits than diet alone for the above patients with nutritional risk at discharge.

METHODS AND ANALYSIS

Standard protocol approval, registration, and patient consent

This study will be conducted in accordance with good clinical practice and ethical standards set out in the Declaration of Helsinki of 1964 and its subsequent amendments. The study protocol was approved by the Ethics Committee of Jinling Hospital (Nanjing, China; 17/12/2021; approval No. 2021NZKY-069-01) and was registered on ClinicalTrials.gov (NCT05253716). The medical personnel of the participating institutions will obtain written informed consent from all the patients enrolled in the study, and clarify that they can withdraw at any time without providing a reason and without any effect on their current or future care.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Design and randomization

This study is initiated by the investigator and will be designed as a pragmatic, multicenter, open-label, randomized, and controlled clinical trial. Eligible patients will be randomly assigned to the immunonutrition supplement (INS) or control group (C) using a 1:1 ratio. Centralized permuted block randomization will be implemented using the mobile client-based *Randomization Allocation Tool* (RAT),[21] with stratification by

trial center (13 tertiary general hospitals in China), pathological stage of TNM (IIIA or IIIB or IIIC), and Laurén classification (intestinal-type or not). If a participant is qualified for the trial and has signed the written informed consent, the investigator authorized by each center can input the relevant information into the RAT. The assigned group will be immediately fed back to the interface on the mobile client of the investigator. Participants will accept the specified treatment.

Subjects

Patients will be enrolled in the study when they volunteer and meet the following criteria: consecutive adults (18 years of age or more) gastric cancer patients who have undergone radical total gastrectomy with pathological TNM stage III and nutrition risk screening 2002 (NRS2002) score of ≥3 and the Eastern Cooperative Oncology Group (ECOG) performance status of zero, one, or two at discharge. Patients will be excluded for the following: inability to oral or consume ONS; previous receipt of neoadjuvant chemotherapy; pregnancy; palliative surgery or gastric stump cancer or Borrmann type IV; inability to discontinue oral anticoagulants; acquired immune deficiency syndrome (HIV positive or CD4 < 200/mm^3); severe cardiovascular disease that includes chronic heart failure, angina pectoris, myocardial infarction, arrhythmias (such as atrial fibrillation), or uncontrolled hypertension; severe liver and kidney diseases including active hepatitis, cirrhosis, and uremia; diabetes with complications or uncontrolled by medications; previous use of fish oil capsule >2 times/week; contraindications for fish oil capsule; incomplete grip strength measurement and 5-time chair stand test; and previous enrollment in other studies within the same hospital admission.

Discharge criteria

The discharge criteria are similar for the two groups. They include the ability to mobilize and self-care; oral tolerance of semiliquid food; and no complications requiring hospital treatment. The patient can be discharged when they meet these criteria, and the time of discharge will be recorded.[22]

Assessments

General demographic [gender, age, and body mass index (BMI)] and clinical data, including the American Society of Anesthesiologists score, the Charlson Comorbidity Index, surgical methods (laparoscopy and laparotomy), pathological stage of TNM (IIIA, IIIB, and IIIC), Laurén classification (intestinal and non-intestinal type), and tumor size and location will be collected before discharge after radical gastrectomy. The pathological terms and classification used in this study are compiled using the

eighth edition of the American Joint Committee on Cancer (AJCC) staging system.[23] In addition to the NRS2002 (score range from 0 to 7, 3 or higher are considered nutritional risk) and ECOG (the performance status was classified into 0-5 grades: 0 = asymptomatic, 1 = symptomatic but completely ambulatory, 2 = ambulatory and capable of all self-care, 3 or 4 = generally considered unsuitable for chemotherapy) scores acquired at discharge, the following indicators will be evaluated.

Sarcopenia: Sarcopenia is diagnosed as low skeletal muscle mass plus low skeletal muscle strength or low physical performance, according to the criteria of the Asian Working Group for Sarcopenia 2019 criteria.[24] Abdominal CT images will be selected to calculate the skeletal muscle index of the third lumbar spine (L3MI). Low skeletal muscle mass is defined as L3MI < 40.8 cm^2/m^2 for men and L3MI < 34.9 cm^2/m^2 for women, according to our previously published study.[25] Patients will hold the dynamometer (EH101, China) with maximum force in the dominant hand, and the experiment will be carried out three consecutive times. The maximum value measured after each interval of 1 minute is grip strength;[26] low skeletal muscle strength is defined as grip strength <28.0 kg for men and grip strength < 18.0 kg for women. Physical performance will be assessed using a 5-time chair stand test; low physical performance will be defined as a 5-time chair stand test result of ≥ 12 s. Sarcopenia will be assessed preoperatively and 6 and 12 months after discharge.

Anthropometric indicators: body weight and BMI, calculated as weight (kg)/height (m^2), will be collected at discharge and 3, 6, and 12 months after discharge.

Hematological indicators: hematological indicators including albumin, prealbumin, and hemoglobin will be assessed at discharge and 3, 6, and 12 months after discharge.

QoL: QoL will be assessed at discharge and 3, 6, and 12 months after discharge using the European Organization for Research and Treatment of Cancer (EORTC) core QoL questionnaire (QLQ-C30). The EORTC QLQ-C30 questionnaire consists of 30 items, which can evaluate QoL from a multidimensional perspective and better reflect the connotation of QoL for cancer patients.[27]

ω-3 PUFA intake for the diet: The Food Frequency Questionnaire (FFQ) will be developed to assess the dietary intake of ω-3 PUFAs to rule out its influence on the trial results. FFQ will be completed at baseline, the third month of the intervention, and the end of the intervention.

Toxicity and tolerability of chemotherapy: Chemotherapy toxicity will be monitored at the end of each cycle during chemotherapy by the investigators and graded

according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE, version 5.0). In addition, chemotherapy intolerance (defined as the presence of reduction, delay, or termination) will be assessed and documented.[28]

Tumor recurrence assessments: A minimum follow-up for tumor recurrence assessments of 36 months will be required for each patient. They will include (1) CT or MRI of the chest and abdomen repeated every 6 months for 3 years; (2) tumor marker assessments, including carcinoembryonic antigen and carbohydrate antigen 19-9, assessed at the same frequency as CT or MRI; and (3) mandatory annual endoscopy during follow-up.

Adverse complications and events: All adverse complications and events attributed to interventions (gastrointestinal side effects, such as nausea, vomiting, diarrhea, abdominal pain, abdominal distention, and constipation), including unplanned hospitalizations, will be recorded.

ω-3 PUFA measurement: The red blood cells (RBCs) slurry will be obtained after centrifugation at 700g/min and 4 °C for 10 minutes within 3 hours of extraction of 10 ml of venous blood with an EDTA anticoagulant tube during fasting at 7 a.m[29, 30] The isolated RBCs will be stored at -80 °C to determine the content of fatty acids (FA). We will measure FAs by liquid chromatography tandem mass spectrometry (LCMS/MS),[31] and the data will be presented by measuring EPA and DHA as a percentage of the total FA content. Measurements will be taken before the intervention and at the end of the intervention.

A trained team that includes a gastrointestinal surgeon, an oncologist, a dietitian, and an oncology specialty nurse at each research center will evaluate all of these indicators.

Treatment

A team of experienced surgeons who have performed at least 50 gastric cancer surgeries in the last calendar year will perform gastric cancer surgery in each participating center. Total gastrectomy and standard D2 lymph node dissection will be performed, and more than 15 lymph nodes will be dissected. All patients will receive nutritional counseling at discharge and at the beginning of each cycle of adjuvant chemotherapy.

All enrolled patients will be randomized into two groups: (i) INS group and (ii) C group. In the INS group, patients will consume two bottles per day of a high-calorie, high-protein ONS [iVital Energy™ (vanilla, 200 mL per bottle, 1.5 kcal per mL),

FRESENIUS KABI, Germany] and three capsules of marine fish oil [webber naturals (1.425 g of fish oil per capsule, containing 0.6 g of EPA and 0.3 g of DHA), Canada] per day after discharge for 6 months, in addition to diet. In the C group, patients will receive nutritional counseling and dietary modifications; the intake of protein-rich foods will increase. ONS will also be considered when a dietitian evaluates the medical needs of a patient.

The detailed composition of iVital Energy™ is provided in Table 1. The number of supplements consumed will be counted daily. The researchers will monitor compliance by telephone every week and check the records at each follow-up. Any gastrointestinal side effects will also be recorded to determine the safety of ONS and fish oil consumption.

Table 1 Nutrient contents of the iVital Energy

Items	100ml	NRV%
Energy, kcal	150	7
% from proteins	27	-
% from carbohydrates	34	-
% from fats	39	-
Proteins, g	10	17
Fats, g	6.7	11
Saturated fatty acid, g	0.6	-
Monounsaturated fatty acid, g	4.9	-
Polyunsaturated fatty acids, g	1.2	-
Carbohydrates, g	12.4	4
Vit A, μg	81	10
Vit D, μg	2.5	50
Vit E, mg	3.75	27
Vit B_1, mg	0.14	10
Vit B_6, mg	0.13	9
Vit C, mg	18.8	19
Sodium, mg	55	3
Phosphorus, mg	120	17
Potassium, mg	130	7
Magnesium, mg	14	5
Calcium, mg	205	26
Iron, mg	1.8	12
Zinc, mg	2	13
Selenium, μg	13.5	27

NRV Nutrient Reference Values

Anticancer treatments

Patients will receive 6–8 cycles of fluorouracil-based adjuvant chemotherapy from 3-4 weeks after discharge. Oncologists will decide on a specific treatment regimen (fluorouracil combined with platinum, fluorouracil combined with paclitaxel, or

fluorouracil combined with platinum and paclitaxel) and duration of treatment.

Endpoints

The primary endpoint will be a 3-year DFS post-discharge after radical gastrectomy. DFS is defined as the time from randomization to tumor recurrence of primary cancer, new gastric cancer, distant metastases, or death from any cause, whichever comes first.[32]

The following secondary endpoints will also be evaluated: 3-year OS; unplanned readmission rate (one or more) at 3 and 6 months after discharge; QoL (EORTC QLQ-C30 score), weight, BMI, and hematological index at 3, 6, and 12 months after discharge; incidence of sarcopenia at 6 and 12 months after discharge; and the tolerance to chemotherapy.

The safety of ONS and the fish oil capsule will be evaluated during the intervention by monitoring vital signs (heart rate, pulse, blood pressure, respiration rate) and the incidence of gastrointestinal side effects, as mentioned above.

Table 2 provides a summary of the assessments and related endpoints that will be investigated during the study. The flowchart of the CRUCIAL trial is shown in Figure 1.

Table 2 Summary of scheduled assessment and follow-up during the study

Evaluations	Visit 0	Visit 1-8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13
	discharge	Month: 0-6 after discharge	Month: 12 after discharge	Month: 18 after discharge	Month: 24 after discharge	Month: 30 after discharge	Month: 36 after discharge
Informed consent	X						
Inclusion/ exclusion criteria	X						
Randomization	X						
Demographic data	X						
Clinical data	X						

Blood sample	X	X					
BMI	X	X	X				
Sarcopenia	X	X	X				
Hematological indicators	X	X	X				
QoL (EORTC QLQ-C30)	X	X	X				
ω-3 PUFAs intakes in diet	X	X					
Chemotherapy tolerance		X					
Chemotherapy toxicity		X					
Adherence to interventions		X					
Adverse events		X					
Unplanned readmission		X					
CT or MRI		X	X	X	X	X	X
Tumor markers		X	X	X	X	X	X
Endoscopy			X		X		X
DFS							X
OS							X

BMI body mass index, QoL quality of life, EORTC QLQ-C30 European Organization for Research and Treatment of Cancer core quality of life questionnaire, DFS disease-

free survival, OS overall survival

Benefit for participants

All patients will receive an early nutritional assessment and counseling. The nutritional status of the patients will be regularly monitored. Medical interventions will be provided for any adverse effects during nutritional support.

Potential risks and burdens for research participants

ONS and fish oil capsules may cause discomfort or expose patients to an increased risk of gastrointestinal intolerance, which will be recorded and promptly provided medical treatment. The ONS product in this study is vanilla, which is internationally well accepted and tolerated, as reported in previous studies.[33] As indicated in the ESPEN guidelines in 2016, fish oil and ω-3 fatty acids were mainly well tolerated.[34] Furthermore, the combined dose of the EPA and DHA supplement was up to 5 g/day, which did not increase the risk of spontaneous bleeding episodes or bleeding complications. [35] Therefore, there are no safety concerns for adults regarding the dose of fish oil in this study.

Statistical methods

Sample size

The sample size was calculated according to the primary endpoint using PASS 15.0 software (NCSS, Kaysville, Utah, USA). Based on relevant data available in the previous literature, the 3-year DFS after total gastrectomy for stage III gastric cancer was 36.2%.[36] Assuming this type of patient received only nutritional counseling in the control group, the 3-year DFS was similar, that is, 36%. Taking into account a study power of 80%, an alpha error level at two tails of 5%, and an expected 3-year DFS of 46% in the INS group, 696 patients (348 in each group) will have to be enrolled to allow for a dropout rate of 5% or withdrawal.

Statistical analyses

Analyses of primary and secondary endpoints will be based on the intention-to-treat principle. Survival curves will be estimated using the Kaplan–Meier method and compared with the results of log-rank tests in time-to-event analyzes. The hazard ratios (HR) and 95% confidence intervals (CI) will be derived using Cox proportional hazards models. For the primary endpoint of the 3-year DFS, an additional prespecified analysis of the multivariate Cox proportional hazards model will be used to evaluate the consistency of the group effect. This model will account for clinically important baseline characteristics, including trial center, age, sex, Laurén type, sarcopenia, N

stage, T stage, and TNM stage. The proportional hazards assumption will be evaluated using scaled Schoenfeld residuals. Prespecified subgroup analyzes will include age (≥ 65 vs <65 years old), sex (men vs women), Laurén type (intestinal-type vs not intestinal-type), sarcopenia (yes vs no), N stage (N0 vs N1 vs N2 vs N3), T stage (T1 vs T2 vs T3 vs T4), and TNM stage (IIIA vs IIIB vs IIIC). Cox proportional hazards models with additional interaction variables of the subgroup and group factors will be constructed to estimate the *P* values of interactions in these subgroup analyses.

The normality of the continuous variable will be assessed using the Shapiro-Wilk test. Continuous variables will be presented as means with standard deviations or medians with interquartile ranges, and categorical variables will be shown as frequencies and percentages. The student's t-test will be used for the analysis of continuous variables of normal distribution, and Wilcoxon rank sum test will be used for the analyses of continuous nonnormal distribution or ranking data. The chi-squared test or Fisher's exact test will be used for the analyses of categorical variables.

For the superiority assessment of the primary endpoint of the 3-year DFS, a two-sided *P* value of less than 0.05 will be considered statistical significance. Due to the potential for type I error due to multiplicity, any other inferences drawn from *P* values, or 95% CIs will not be reproducible and should be interpreted as exploratory.

All statistical analyzes will be performed using SAS software, version 9.4 (SAS Institute, Cary, NC) by independent statisticians masked in the allocation of the treatment group.

Study administration

The Clinical Endpoint Committee (CEC) is an independent group of five experts that includes one pathologist, two radiologists, and two clinical specialists. Recurrence of the primary endpoint will be assessed by the CEC, who are masked from the treatment assignment, based on medical history and physical examination combined with imaging evaluation, cytology, or tissue biopsy findings.

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Recruiting process

The trial was registered on 24 February 2022. The first patient was randomised on 1 August 2022. So far, 76 patients had been randomised, and the enrolment keeps to the flowchart.

Data management

The investigators are responsible for the accuracy and timely entry of the data into the electronic case report form (eCRF) according to the study protocol. The study monitor will review the eCRFs and other study documents, and verify the primary data. The final confirmed data set will be locked and analyzed by the trial statistician. In principle, the data set locking cannot be modified. The investigators must keep research documents for a specified period by the regulatory requirements.

ETHICAL AND DISSEMINATION

This study was approved by the ethics committee of Jinling Hospital. We will not begin recruiting at other participating centers of the trial until the local ethics committee approves the study. Site ethical approvals were obtained from ethics committees of the First Afflated Hospital of Nanjing Medical University, the Second Afflated Hospital of Nanjing Medical University, the Affiliated Cancer Hospital of Nanjing Medical University, Nanjing Jiangning Hospital, Zhenjiang First People's Hospital, The Third Affiliated Hospital of Soochow University, Changzhou Second Hospital, The First Affiliated Hospital of Soochow University, The Second Affiliated Hospital of Soochow University, The Affiliated Wuxi People's Hospital of Nanjing Medical University, and Yixing People's Hospital. The results of the study will be presented at national and international medical meetings. Meanwhile, the results will be published in prestigious peer-reviewed medical journals.

DISCUSSION

Malnutrition can occur at any stage during the development, progression, and treatment of gastric cancer.[37] After surgical resection of the tumor, the incidence of malnutrition is 30 to 50% after discharge, which is still common.[6] Malnutrition is more pronounced in patients with pathological stage III gastric cancer or after total gastrectomy,[1, 38, 39] which will significantly shorten long-term survival.[40, 41] A previous randomized controlled trial has demonstrated nutritional support was a feasible approach to increase the median OS of patients with stage IV gastric cancer from 11.9 to 14.8 months.[16] However, oral immunonutritional supplement after discharge is mostly concentrated in nutritional status or inflammatory response in patients with advanced gastric cancer,[42] and lacks high-quality evidence on long-term DFS and OS,

which still requires confirmation.

The ONS is the preferred form of nutritional support for energy and nutrients for specific medical purposes.[43] A previous multicenter randomized controlled trial had demonstrated high tolerability and compliance with long-term oral ONS after gastric cancer surgery.[44] Several studies have found that ONS can reduce the incidence of sarcopenia, improve some parameters of QoL, improve chemotherapy tolerance[15], and delay weight loss after total gastrectomy.[45, 46] However, the duration of ONS is usually shorter (6-12 weeks), resulting in a difference in weight maintenance and gradual decline after 6 months that is insignificant 1 year after surgery compared with the results of the standard diet.[14] This study will ensure that long-term nutritional support (6 months) is provided to patients with a nutritional risk assigned to the INS group after discharge, and improve nutritional status and QoL for 12 months after surgery.

Omega-3 PUFAs, as immunological nutrients, do not only prevent cardiovascular events;[47] they fight sarcopenia by reducing insulin resistance, improving mitochondrial function, inhibiting the inflammatory response and activating the mTOR pathway.[18] Smith et al. confirmed that oral administration of fish oil (1.86g/d EPA+1.5g/d DHA) for 6 months delayed the loss of muscle mass and function, and prevented sarcopenia in healthy older adults without severe adverse events related to ω–3 PUFA.[48] Preclinical studies have confirmed that ω-3 PUFAs can inhibit gastric cancer progress by inducing apoptosis of gastric cancer cells in various ways.[19, 20, 49, 50] In addition, a randomized controlled trial has shown that 2g/d ω-3 PUFAs during neoadjuvant chemotherapy can improve the pathological response rate and the subsequent R0 resection rate.[51] Moreover, ω-3 PUFAs also play a synergistic role with cisplatin to enhance its inhibitory effect on gastric cancer.[52] In this study, the nutrition and anti-tumor recurrence effects of ω-3 PUFAs can be fully played under the premise of ensuring safety.

Several studies have attempted to determine whether nutritional support can improve long-term survival for patients with gastric cancer after discharge. A nationwide cohort study (n=1771, including 218 gastric patients) in France did not show significant improvement in OS (mean follow-up of 33 ± 20 months, P=0.19) after 45 days of oral immunonutrition before digestive oncologic surgery relative to a normal diet.[53] During the postoperative period, a small prospective controlled randomized study (n=98) found that enteral immunonutrition (including arginine, glutamines, and ω-3 PUFAs) continued for 6 days in gastric cancer patients did not significantly prolong

6 months (P = 0.24) OS and 1-year OS (P = 0.83) compared to conventional enteral nutrition.[54] The beneficial effects of immune modulated enteral nutrition were too weak to be significant in these patients because malnourished patients (population who needed nutritional support) were excluded. However, in a randomized controlled trial involving 99 gastric cancer patients treated with enteral nutrition, postoperative enteral immunonutrition lasting 7 days had a positive effect on 6 months OS (HR=0.25, P=0.049) only in malnourished stage IV gastric cancer patients.[55] The three studies did not demonstrate that nutritional support had a positive effect on long-term survival of advanced gastric cancer, due to the exclusion of appropriate patients and the short duration of intervention. More importantly, the primary outcome did not involve DFS. In the present study, we expect that ONS combined with ω-3 PUFAs will reduce postoperative recurrence, improve long-term DFS and OS by reducing sarcopenia, and improve tolerance and efficacy of chemotherapy.

To our knowledge, this is the first study to investigate the efficacy of oral immunonutritional supplement, based on 3-year DFS, 3-year OS, 1-year nutritional status and quality of life, in a population of specific gastric cancers, with the expectation of developing new therapeutic strategies to improve the efficacy of anticancer therapy. The positive results of this multicenter clinical trial will further stimulate larger international randomized trials, which can improve the quality of supportive care for cancer patients and increase access to patients who may benefit from nutritional support in the nonsurgical oncological setting.

Authors' contributions During the study, DZ, LZ, YL, XG and XY developed the study concept and drafted the manuscript. DZ, ML, and YL are responsible for the randomization of patients. DZ, SX, HX, GL, KY, JZ, YW, JQ, JZ, KD, YW, ZT, CJ, WW, ZS, and GL are responsible for recruiting, managing the treatment of the patients and collecting data. All authors have approved the submission of this manuscript for publication.

Funding This study is funded by the National Natural Science Foundation of China (81770531) and the Jiangsu Province Key Research and Development Project (BE2022822).

Competing interests There are no conflicts of interest to declare.

Patient consent for publication Patients or their next of kin consent obtained.

Provenance and peer review Not commissioned; externally peer reviewed

ORCID iDs

Da Zhou http://orcid.org/0000-0003-3062-5952

Mengjie Lu http://orcid.org/0000-0003-4788-7668

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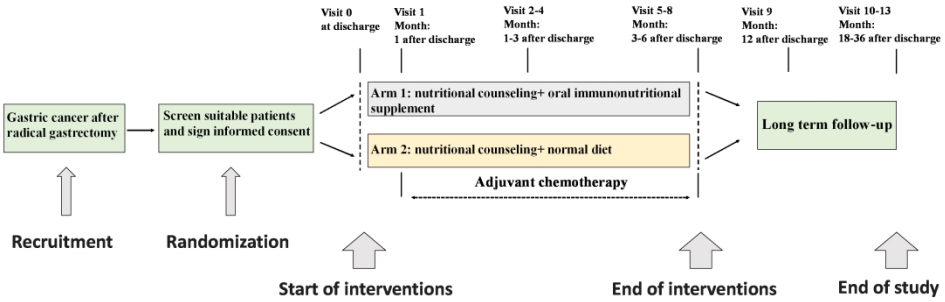
Figure legend:

Figure 1 Flowchart of the CRUCIAL trial



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SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size
Methods: Assignment of interventions (for controlled trials)		
Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effects of oral immunonutritional supplement on 3-year disease-free survival in gastric cancer patients with pathologic stage III after total gastrectomy (CRUCIAL): study protocol of a multicenter, randomized clinical trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-067990.R1
Article Type:	Protocol
Date Submitted by the Author:	27-Dec-2022

Complete List of Authors:	Zhou, Da; Medical School of Nanjing University, Research Institute of General Surgery, Jinling Hospital Liu, Yuxiu; Nanjing Jinling Hospital, Data and Statistics Division of Department of Critical Care Medicine Zhang, Li; Research Institute of General Surgery, Jinling Hospital, Medical School of Nanjing University Lu, Mengjie; Shanghai Jiao Tong University School of Medicine Gao, Xuejin; Research Institute of General Surgery, Jinling Hospital, Medical School of Nanjing University Li, Guoli; Medical School of Nanjing University, Department of General Surgery Xiang, Xiaosong; Medical School of Nanjing University, Department of General Surgery Xu, Hao; Nanjing Medical University, Department of General Surgery Li, Gang; Nanjing Medical University, Jiangsu Cancer Hospital, Department of General Surgery Sun, Zhiping; Nanjing Medical University, Affiliated Second Hospital, Department of Clinical Nutrition wei, Wei; Nanjing Medical University, Affiliated Second Hospital, Department of General Surgery Yi, Kui; The Affiliated Jiangning Hospital of Nanjing Medical University, Department of Gastroenteropancreatic Surgery Zuo, Junbo; Zhenjiang First People's Hospital, Department of General Surgery Wu, Yugang; Soochow University, The First People’s Hospital of Changzhou, Department of General Surgery Qian, Jun; Changzhou No. 2 People's Hospital, Department of General Surgery Zhou, Jian; First Affiliated Hospital of Soochow University, Department of General Surgery Duan, Kaipeng; First Affiliated Hospital of Soochow University, Department of General Surgery Wu, Yong; Second Affiliated Hospital of Soochow University, Department of General Surgery Tian, Zhiqiang; Nanjing Medical University Affiliated Wuxi People's Hospital, Department of General Surgery Jiang, Chao; Yixing People's Hospital, Department of General Surgery Wang, Xinying; Medical School of Nanjing University, Department of

	General Surgery
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Nutrition and metabolism, Surgery
Keywords:	Gastrointestinal tumours < GASTROENTEROLOGY, Nutritional support < GASTROENTEROLOGY, Adult gastroenterology < GASTROENTEROLOGY

Effects of oral immunonutritional supplement on 3-year disease-free survival in gastric cancer patients with pathologic stage III after total gastrectomy (CRUCIAL): study protocol of a multicenter, randomized clinical trial

Da Zhou,[1] Yuxiu Liu,[2] Li Zhang,[1] Mengjie Lu,[3] Xuejin Gao,[1] Guoli Li,[1] Xiaosong Xiang,[1] Hao Xu,[4] Gang Li,[5] Zhiping Sun,[6] Wei Wei,[7] Kui Yi,[8] Junbo Zuo,[9] Yugang Wu,[10] Jun Qian,[11] Jian Zhou,[12] Kaipeng Duan,[12] Yong Wu,[13] Zhiqiang Tian,[14] Chao Jiang,[15] Xinying Wang[1]

[1]Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu, China

[2]Data and Statistics Division of Department of Critical Care Medicine, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu, China

[3]School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[4]Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[5]Department of General Surgery, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[6]Department of Clinical Nutrition, Nanjing Medical University, Affiliated Second Hospital, Nanjing, Jiangsu, China

[7]Department of General Surgery, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[8]Department of Gastroenteropancreatic Surgery, The Affiliated Jiangning Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[9]Department of General Surgery, Zhenjiang First People's Hospital, Zhengjiang, Jiangsu, China

[10]Department of General Surgery, The Third Affiliated Hospital of Soochow University and The First People's Hospital of Changzhou, Changzhou, Jiangsu, China

[11]Department of General Surgery, ChangZhou Second Hospital, the afflicated Hospital of Nanjing Medical University, Changzhou, Jiangsu, China

[12]Department of General Surgery, the First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[13]Department of General Surgery, the Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[14]Department of General Surgery, Wuxi People's Hospital Affiliated to Nanjing Medical University, Wuxi, Jiangsu, China

[15]Department of General Surgery, Yixing People's Hospital, Yixing, Jiangsu, China

Corresponding author: Xinying Wang

Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, 305 Zhongshan East Road, Nanjing, Jiangsu, China

wangxinying@nju.edu.cn

ABSTRACT

Introduction The nutritional status of patients with gastric cancer (GC) after total gastrectomy continues to deteriorate and lasts a long time after discharge, which is an independent risk factor for mortality. Recent guidelines have recommended appropriate nutritional support after discharge for cancer surgery patients with malnutrition or nutritional risk. The evidence on the efficacy of oral immunonutritional supplement and its effect on long-term disease-free survival (DFS) in patients with GC is limited. This study was designed to test the hypothesis that oral immunonutritional supplement compared to diet alone may improve 3-year DFS of GC patients with pathologic stage III after total gastrectomy (nutrition risk screening 2002 score ≥ 3 at discharge).

Methods and analysis This is a pragmatic, open-label, multicenter, randomized controlled study. 696 eligible GC patients with pathologic stage III after total gastrectomy will be randomized in a 1:1 ratio to oral immunonutritional supplement group or normal diet group for six months. The primary endpoint is 3-year DFS after discharge. The following secondary endpoints will be evaluated: 3-year overall survival; unplanned readmission rate at 3 and 6 months after discharge; quality of life, body mass index, and hematological index at 3, 6, and 12 months after discharge; incidence of sarcopenia at 6 and 12 months after discharge; and the tolerance to chemotherapy. The adverse events of oral immunonutritional supplement will also be evaluated during the intervention.

Ethics and dissemination This study was approved by the ethics committee of Jinling Hospital, Nanjing University (Number 2021NZKY-069-01). The present study may validate the effectiveness of oral immunonutritional therapy in improving 3-year DFS for gastric cancer patients with pathologic stage III after total gastrectomy for the first time. The results of this trial will be disseminated in peer-reviewed journals and at scientific conferences.

Trial registration number ClinicalTrials.gov Registry (NCT05253716).

Strengths and limitations of this study

⇒ This is a pragmatic, open-label, multicenter, randomized controlled trial providing high quality clinical evidence concerning the efficacy and safety of oral immunonutrition supplement for gastric cancer patients with pathologic stage III after total gastrectomy.

⇒ The data will be handled by an Independent Data Monitoring Committee to ensure

the safety of the participants. Oral immunonutrition supplement is a well-studied method with a favorable safety profile in previous trials.

⇒ This is the first study to evaluate the effect of oral immunonutrition supplementation on long-term disease-free survival in patients with gastric cancer after discharge.

⇒ Longer survival or recurrence status is not assessed in this study.

INTRODUCTION

Malnutrition is common in patients with gastric cancer. It increases postoperative complications, length of stay, and cost of treatment and weakens the effect of chemotherapy and quality of life (QoL), as well as shortens long-term survival.[1-4] In addition to preoperative malnutrition, which most studies are concerned about, the nutritional status after radical gastrectomy will also continue to deteriorate for nearly 1 year; the rate of malnutrition was approximately 30%–50%.[1, 5, 6] Due to postoperative chemotherapy and several other risk factors, including reduced nutritional intake and malabsorption, continuous weight loss, and sarcopenia (an important phenotype of malnutrition) caused by progressive loss of skeletal muscle mass with decreased physical activity persists long after radical gastrectomy.[7, 8] These conditions are more common and severe in patients with pathological stage III gastric cancer or after total gastrectomy: the proportion of skeletal muscle loss of ≥ 5% six months after surgery has been reported to be up to 51% and 55.4%, respectively.[9, 10] Gastric cancer patients who exhibited ≥ 5% skeletal muscle loss have shown a lower 5-year disease-free survival (DFS) rates (33.8% vs 46.2%; P = 0.020).[10] Moreover, postoperative sarcopenia may persist for approximately 1 year and is significantly related to worse 5-year overall survival (OS) in gastric cancer.[11] Therefore, reinforced nutritional support should be offered to this patient population, and more attention should be provided.

The most recent guideline recommends appropriate postdischarge nutritional support for surgical cancer patients with nutritional risks or those who are already malnourished, and several guidelines have recommended oral nutritional supplement (ONS) as the preferred approach.[12, 13] The limited available evidence suggests that consecutive ONS for 3 months postdischarge has a positive effect on maintaining weight, reduces the risk of sarcopenia, and improves chemotherapy tolerance for gastric cancer after surgery.[14, 15] Although ONS can effectively prolong median OS for metastatic gastric cancers,[16] its effect on survival outcomes of gastric cancer patients with pathologic stage III after total gastrectomy remains unclear. Omega-3

polyunsaturated fatty acids (ω-3 PUFAs, including EPA and DHA) are commonly used as nutrients for immunity. Aoife et al. found that enteral nutrition enriched with 2.2 g of EPA/d can maintain skeletal muscle mass after esophagectomy.[17] Furthermore, recent preclinical studies have shown that ω-3 PUFAs can also inhibit gastric cancer-related cell growth and metastasis.[18-20] However, to our knowledge, studies on disease-free survival (DFS) after oral immunonutritional supplement after surgery for advanced gastric cancer are lacking.

The objective of this pragmatic, multicenter, randomized clinical trial (Clinicaltrials. gov identifier: NCT05253716) is to evaluate the efficacy of the postoperative oral immunonutritional supplement for 6 months and its effect on 3-year DFS, 3-year OS, weight maintenance, sarcopenia, QoL, chemotherapy tolerance, and unplanned readmission of gastric cancer patients with pathological stage III after total gastrectomy. We hypothesize that oral immunonutritional supplement will have significantly more benefits than diet alone for the above patients with nutritional risk at discharge.

METHODS AND ANALYSIS

Standard protocol approval, registration, and patient consent

This study will be conducted in accordance with good clinical practice and ethical standards set out in the Declaration of Helsinki of 1964 and its subsequent amendments. The study protocol was approved by the Ethics Committee of Jinling Hospital (Nanjing, China; 17/12/2021; approval No. 2021NZKY-069-01) and was registered on ClinicalTrials.gov (NCT05253716). The medical personnel of the participating institutions will obtain written informed consent from all the patients enrolled in the study, and clarify that they can withdraw at any time without providing a reason and without any effect on their current or future care.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Design and randomization

This study is initiated by the investigator and will be designed as a pragmatic, multicenter, open-label, randomized, and controlled clinical trial. Eligible patients will be randomly assigned to the immunonutrition supplement (INS) or control group (C) using a 1:1 ratio. Centralized permuted block randomization will be implemented using the mobile client-based *Randomization Allocation Tool* (RAT),[21] with stratification

by trial center (13 tertiary general hospitals in China), pathological stage of TNM (IIIA or IIIB or IIIC), and Laurén classification (intestinal-type or not). If a participant is qualified for the trial and has signed the written informed consent, the investigator authorized by each center can input the relevant information into the RAT. The assigned group will be immediately fed back to the interface on the mobile client of the investigator. Participants will accept the specified treatment.

Subjects

Prior to surgery, the principal investigator at each study center will be responsible for recruiting subjects. Patients will be enrolled in the study when they volunteer and meet the following criteria: consecutive adults (18 years of age or more) gastric cancer patients who have undergone radical total gastrectomy with pathological TNM stage III and nutrition risk screening 2002 (NRS2002) score of ≥3 and the Eastern Cooperative Oncology Group (ECOG) performance status of zero, one, or two at discharge. Patients will be excluded for the following: inability to oral or consume ONS; previous receipt of neoadjuvant chemotherapy; pregnancy; palliative surgery or gastric stump cancer or Borrmann type IV; inability to discontinue oral anticoagulants; acquired immune deficiency syndrome (HIV positive or CD4 < 200/mm^3); severe cardiovascular disease that includes chronic heart failure, angina pectoris, myocardial infarction, arrhythmias (such as atrial fibrillation), or uncontrolled hypertension; severe liver and kidney diseases including active hepatitis, cirrhosis, and uremia; diabetes with complications or uncontrolled by medications; previous use of fish oil capsule >2 times/week; contraindications for fish oil capsule; incomplete grip strength measurement and 5-time chair stand test; and previous enrollment in other studies within the same hospital admission.

Discharge criteria

The discharge criteria are similar for the two groups. They include the ability to mobilize and self-care; oral tolerance of semiliquid food; tube feeding is not required; and no complications requiring hospital treatment. The patient can be discharged when they meet these criteria, and the time of discharge will be recorded.[22]

Assessments

General demographic [gender, age, and body mass index (BMI)] and clinical data, including the American Society of Anesthesiologists score, the Charlson Comorbidity Index, surgical methods (laparoscopy and laparotomy), pathological stage of TNM (IIIA, IIIB, and IIIC), Laurén classification (intestinal and non-intestinal type), and

tumor size and location will be collected before discharge after radical gastrectomy. The pathological terms and classification used in this study are compiled using the eighth edition of the American Joint Committee on Cancer (AJCC) staging system.[23] In addition to the NRS2002 (score range from 0 to 7, 3 or higher are considered nutritional risk) and ECOG (the performance status was classified into 0-5 grades: 0 = asymptomatic, 1 = symptomatic but completely ambulatory, 2 = ambulatory and capable of all self-care, 3 or 4 = generally considered unsuitable for chemotherapy) scores acquired at discharge, the following indicators will be evaluated.

Sarcopenia: Sarcopenia is diagnosed as low skeletal muscle mass plus low skeletal muscle strength or low physical performance, according to the criteria of the Asian Working Group for Sarcopenia 2019 criteria.[24] Abdominal CT images will be selected to calculate the skeletal muscle index of the third lumbar spine (L3MI). Low skeletal muscle mass is defined as L3MI < 40.8 cm^2/m^2 for men and L3MI < 34.9 cm^2/m^2 for women, according to our previously published study.[25] Patients will hold the dynamometer (EH101, China) with maximum force in the dominant hand, and the experiment will be carried out three consecutive times. The maximum value measured after each interval of 1 minute is grip strength;[26] low skeletal muscle strength is defined as grip strength <28.0 kg for men and grip strength < 18.0 kg for women. Physical performance will be assessed using a 5-time chair stand test; low physical performance will be defined as a 5-time chair stand test result of ≥ 12 s. Sarcopenia will be assessed preoperatively and 6 and 12 months after discharge.

Anthropometric indicators: body weight and BMI, calculated as weight (kg)/height (m^2), will be collected at discharge and 3, 6, and 12 months after discharge.

Hematological indicators: hematological indicators including albumin, prealbumin, and hemoglobin will be assessed at discharge and 3, 6, and 12 months after discharge.

QoL: QoL will be assessed at discharge and 3, 6, and 12 months after discharge using the European Organization for Research and Treatment of Cancer (EORTC) core QoL questionnaire (QLQ-C30). The EORTC QLQ-C30 questionnaire consists of 30 items, which can evaluate QoL from a multidimensional perspective and better reflect the connotation of QoL for cancer patients.[27]

ω-3 PUFA intake for the diet: The Food Frequency Questionnaire (FFQ) will be developed to assess the dietary intake of ω-3 PUFAs to rule out its influence on the trial results. FFQ will be completed at baseline, the third month of the intervention, and the end of the intervention.

Toxicity and tolerability of chemotherapy: Chemotherapy toxicity will be monitored at the end of each cycle during chemotherapy by the investigators and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE, version 5.0). In addition, chemotherapy intolerance (defined as the presence of reduction, delay, or termination) will be assessed and documented.[28]

Tumor recurrence assessments: A minimum follow-up for tumor recurrence assessments of 36 months will be required for each patient. They will include (1) CT or MRI of the chest and abdomen repeated every 6 months for 3 years; (2) tumor marker assessments, including carcinoembryonic antigen and carbohydrate antigen 19-9, assessed at the same frequency as CT or MRI; and (3) mandatory annual endoscopy during follow-up.

Adverse complications and events: All adverse complications and events attributed to interventions (gastrointestinal side effects, such as nausea, vomiting, diarrhea, abdominal pain, abdominal distention, and constipation), including unplanned hospitalizations, will be recorded.

ω-3 PUFA measurement: The red blood cells (RBCs) slurry will be obtained after centrifugation at 700g/min and 4 °C for 10 minutes within 3 hours of extraction of 10 ml of venous blood with an EDTA anticoagulant tube during fasting at 7 a.m[29, 30] The isolated RBCs will be stored at -80 °C to determine the content of fatty acids (FA). We will measure FAs by liquid chromatography tandem mass spectrometry (LCMS/MS),[31] and the data will be presented by measuring EPA and DHA as a percentage of the total FA content. Measurements will be taken before the intervention and at the end of the intervention.

A trained team that includes a gastrointestinal surgeon, an oncologist, a dietitian, and an oncology specialty nurse at each research center will evaluate all of these indicators.

Treatment

A team of experienced surgeons who have performed at least 50 gastric cancer surgeries in the last calendar year will perform gastric cancer surgery in each participating center. Total gastrectomy and standard D2 lymph node dissection will be performed, and more than 15 lymph nodes will be dissected. All patients will receive nutritional counseling at discharge and at the beginning of each cycle of adjuvant chemotherapy.

All enrolled patients will be randomized into two groups within 24 hours before

discharge: (i) INS group and (ii) C group. In the INS group, patients will consume two bottles per day of a high-calorie, high-protein ONS [iVital Energy™ (vanilla, 200 mL per bottle, 1.5 kcal per mL), FRESENIUS KABI, Germany] and three capsules of marine fish oil [webber naturals (1.425 g of fish oil per capsule, containing 0.6 g of EPA and 0.3 g of DHA), Canada] per day after discharge for 6 months, in addition to diet. In the C group, patients will receive nutritional counseling and dietary modifications; the intake of protein-rich foods will increase. ONS will also be considered when a dietitian evaluates the medical needs of a patient.

The detailed composition of iVital Energy™ is provided in Table 1. The number of supplements consumed will be counted daily. The researchers will monitor compliance by telephone every week and check the records at each follow-up. In addition, participants will be asked to bring back the remaining iVital Energy™ and fish oil capsules at each follow-up visit for counting to measure compliance. Any gastrointestinal side effects will also be recorded to determine the safety of ONS and fish oil consumption.

Table 1 Nutrient contents of the iVital Energy

Items	100ml	NRV%
Energy, kcal	150	7
% from proteins	27	-
% from carbohydrates	34	-
% from fats	39	-
Proteins, g	10	17
Fats, g	6.7	11
Saturated fatty acid, g	0.6	-
Monounsaturated fatty acid, g	4.9	-
Polyunsaturated fatty acids, g	1.2	-
Carbohydrates, g	12.4	4
Vit A, μg	81	10
Vit D, μg	2.5	50
Vit E, mg	3.75	27
Vit B_1, mg	0.14	10
Vit B_6, mg	0.13	9
Vit C, mg	18.8	19
Sodium, mg	55	3
Phosphorus, mg	120	17
Potassium, mg	130	7
Magnesium, mg	14	5
Calcium, mg	205	26
Iron, mg	1.8	12
Zinc, mg	2	13
Selenium, μg	13.5	27

NRV Nutrient Reference Values

Anticancer treatments

Patients will receive 6–8 cycles of fluorouracil-based adjuvant chemotherapy from 3-4 weeks after discharge. Oncologists will decide on a specific treatment regimen (fluorouracil combined with platinum, fluorouracil combined with paclitaxel, or fluorouracil combined with platinum and paclitaxel) and duration of treatment.

Endpoints

The primary endpoint will be a 3-year DFS post-discharge after radical gastrectomy. DFS is defined as the time from randomization to tumor recurrence of primary cancer, new gastric cancer, distant metastases, or death from any cause, whichever comes first.[32]

The following secondary endpoints will also be evaluated: 3-year OS; unplanned readmission rate (one or more) at 3 and 6 months after discharge; QoL (EORTC QLQ-C30 score), weight, BMI, and hematological index at 3, 6, and 12 months after discharge; incidence of sarcopenia at 6 and 12 months after discharge; and the tolerance to chemotherapy.

The safety of ONS and the fish oil capsule will be evaluated during the intervention by monitoring vital signs (heart rate, pulse, blood pressure, respiration rate) and the incidence of gastrointestinal side effects, as mentioned above.

Table 2 provides a summary of the assessments and related endpoints that will be investigated during the study. The flowchart of the CRUCIAL trial is shown in Figure 1.

Table 2 Summary of scheduled assessment and follow-up during the study

Evaluations	Visit 0	Visit 1-8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13
	discharge	Month: 0-6 after discharge	Month: 12 after discharge	Month: 18 after discharge	Month: 24 after discharge	Month: 30 after discharge	Month: 36 after discharge
Informed consent	X						
Inclusion/ exclusion criteria	X						
Randomization	X						

Demographic data	X						
Clinical data	X						
Blood sample	X	X					
BMI	X	X	X				
Sarcopenia	X	X	X				
Hematological indicators	X	X	X				
QoL (EORTC QLQ-C30)	X	X	X				
ω-3 PUFAs intakes in diet	X	X					
Chemotherapy tolerance		X					
Chemotherapy toxicity		X					
Adherence to interventions		X					
Adverse events		X					
Unplanned readmission		X					
CT or MRI		X	X	X	X	X	X
Tumor markers		X	X	X	X	X	X
Endoscopy			X		X		X

DFS							X
OS							X

BMI body mass index, QoL quality of life, EORTC QLQ-C30 European Organization for Research and Treatment of Cancer core quality of life questionnaire, DFS disease-free survival, OS overall survival

Benefit for participants

All patients will receive an early nutritional assessment and counseling. The nutritional status of the patients will be regularly monitored. Medical interventions will be provided for any adverse effects during nutritional support.

Potential risks and burdens for research participants

ONS and fish oil capsules may cause discomfort or expose patients to an increased risk of gastrointestinal intolerance, which will be recorded and promptly provided medical treatment. The ONS product in this study is vanilla, which is internationally well accepted and tolerated, as reported in previous studies.[33] As indicated in the ESPEN guidelines in 2016, fish oil and ω-3 fatty acids were mainly well tolerated.[34] Furthermore, the combined dose of the EPA and DHA supplement was up to 5 g/day, which did not increase the risk of spontaneous bleeding episodes or bleeding complications. [35] Therefore, there are no safety concerns for adults regarding the dose of fish oil in this study.

Statistical methods

Sample size

The sample size was calculated according to the primary endpoint using PASS 15.0 software (NCSS, Kaysville, Utah, USA). Based on relevant data available in the previous literature, the 3-year DFS after total gastrectomy for stage III gastric cancer was 36.2%.[36] Assuming this type of patient received only nutritional counseling in the control group, the 3-year DFS was similar, that is, 36%. Taking into account a study power of 80%, an alpha error level at two tails of 5%, and an expected 3-year DFS of 46% in the INS group, 696 patients (348 in each group) will have to be enrolled to allow for a dropout rate of 5% or withdrawal.

Statistical analyses

Analyses of primary and secondary endpoints will be based on the intention-to-treat principle. Survival curves will be estimated using the Kaplan–Meier method and

compared with the results of log-rank tests in time-to-event analyzes. The hazard ratios (HR) and 95% confidence intervals (CI) will be derived using Cox proportional hazards models. For the primary endpoint of the 3-year DFS, an additional prespecified analysis of the multivariate Cox proportional hazards model will be used to evaluate the consistency of the group effect. This model will account for clinically important baseline characteristics, including trial center, age, sex, Laurén type, sarcopenia, N stage, T stage, and TNM stage. The proportional hazards assumption will be evaluated using scaled Schoenfeld residuals. Prespecified subgroup analyzes will include age (≥ 65 vs <65 years old), sex (men vs women), Laurén type (intestinal-type vs not intestinal-type), sarcopenia (yes vs no), N stage (N0 vs N1 vs N2 vs N3), T stage (T1 vs T2 vs T3 vs T4), and TNM stage (IIIA vs IIIB vs IIIC). Cox proportional hazards models with additional interaction variables of the subgroup and group factors will be constructed to estimate the *P* values of interactions in these subgroup analyses.

The normality of the continuous variable will be assessed using the Shapiro-Wilk test. Continuous variables will be presented as means with standard deviations or medians with interquartile ranges, and categorical variables will be shown as frequencies and percentages. The student's t-test will be used for the analysis of continuous variables of normal distribution, and Wilcoxon rank sum test will be used for the analyses of continuous nonnormal distribution or ranking data. The chi-squared test or Fisher's exact test will be used for the analyses of categorical variables.

For the superiority assessment of the primary endpoint of the 3-year DFS, a two-sided *P* value of less than 0.05 will be considered statistical significance. Due to the potential for type I error due to multiplicity, any other inferences drawn from *P* values, or 95% CIs will not be reproducible and should be interpreted as exploratory.

All statistical analyzes will be performed using SAS software, version 9.4 (SAS Institute, Cary, NC) by independent statisticians masked in the allocation of the treatment group.

Study administration

The Clinical Endpoint Committee (CEC) is an independent group of five experts that includes one pathologist, two radiologists, and two clinical specialists. Recurrence of the primary endpoint will be assessed by the CEC, who are masked from the treatment assignment, based on medical history and physical examination combined with imaging evaluation, cytology, or tissue biopsy findings.

The Clinical Endpoint Committee (CEC) is an independent group of five experts including one pathologist, two radiologists, and two clinical specialists. Recurrence of the primary endpoint will be assessed by the CEC, which is masked to treatment assignment, based on medical history and physical examination combined with imaging evaluation, cytology, or tissue biopsy findings.

Recruiting process

The trial was registered on 24 February 2022. The first patient was randomised on 1 August 2022. So far, 76 patients had been randomised, and the enrolment keeps to the flowchart.

Data management

The investigators are responsible for the accuracy and timely entry of the data into the electronic case report form (eCRF) according to the study protocol. The study monitor will review the eCRFs and other study documents, and verify the primary data. The final confirmed data set will be locked and analyzed by the trial statistician. In principle, the data set locking cannot be modified. The investigators must keep research documents for a specified period by the regulatory requirements.

ETHICAL AND DISSEMINATION

This study was approved by the ethics committee of Jinling Hospital. We will not begin recruiting at other participating centers of the trial until the local ethics committee approves the study. Site ethical approvals were obtained from ethics committees of the First Afflated Hospital of Nanjing Medical University, the Second Afflated Hospital of Nanjing Medical University, the Affiliated Cancer Hospital of Nanjing Medical University, Nanjing Jiangning Hospital, Zhenjiang First People's Hospital, The Third Affiliated Hospital of Soochow University, Changzhou Second Hospital, The First Affiliated Hospital of Soochow University, The Second Affiliated Hospital of Soochow University, The Affiliated Wuxi People's Hospital of Nanjing Medical University, and Yixing People's Hospital. The results of the study will be presented at national and international medical meetings. Meanwhile, the results will be published in prestigious peer-reviewed medical journals.

DISCUSSION

Malnutrition can occur at any stage during the development, progression, and treatment of gastric cancer.[37] After surgical resection of the tumor, the incidence of malnutrition is 30 to 50% after discharge, which is still common.[6] Malnutrition is more pronounced in patients with pathological stage III gastric cancer or after total

gastrectomy,[1, 38, 39] which will significantly shorten long-term survival.[40, 41] A previous randomized controlled trial has demonstrated nutritional support was a feasible approach to increase the median OS of patients with stage IV gastric cancer from 11.9 to 14.8 months.[16] However, oral immunonutritional supplement after discharge is mostly concentrated in nutritional status or inflammatory response in patients with advanced gastric cancer,[42] and lacks high-quality evidence on long-term DFS and OS, which still requires confirmation.

The ONS is the preferred form of nutritional support for energy and nutrients for specific medical purposes.[43] A previous multicenter randomized controlled trial had demonstrated high tolerability and compliance with long-term oral ONS after gastric cancer surgery.[44] Several studies have found that ONS can reduce the incidence of sarcopenia, improve some parameters of QoL, improve chemotherapy tolerance[15], and delay weight loss after total gastrectomy.[45, 46] However, the duration of ONS is usually shorter (6-12 weeks), resulting in a difference in weight maintenance and gradual decline after 6 months that is insignificant 1 year after surgery compared with the results of the standard diet.[14] This study will ensure that long-term nutritional support (6 months) is provided to patients with a nutritional risk assigned to the INS group after discharge, and improve nutritional status and QoL for 12 months after surgery.

Omega-3 PUFAs, as immunological nutrients, do not only prevent cardiovascular events;[47] they fight sarcopenia by reducing insulin resistance, improving mitochondrial function, inhibiting the inflammatory response and activating the mTOR pathway.[18] Smith et al. confirmed that oral administration of fish oil (1.86g/d EPA+1.5g/d DHA) for 6 months delayed the loss of muscle mass and function, and prevented sarcopenia in healthy older adults without severe adverse events related to ω–3 PUFA.[48] Preclinical studies have confirmed that ω-3 PUFAs can inhibit gastric cancer progress by inducing apoptosis of gastric cancer cells in various ways.[19, 20, 49, 50] In addition, a randomized controlled trial has shown that 2g/d ω-3 PUFAs during neoadjuvant chemotherapy can improve the pathological response rate and the subsequent R0 resection rate.[51] Moreover, ω-3 PUFAs also play a synergistic role with cisplatin to enhance its inhibitory effect on gastric cancer.[52] In this study, the nutrition and anti-tumor recurrence effects of ω-3 PUFAs can be fully played under the premise of ensuring safety.

Several studies have attempted to determine whether nutritional support can

improve long-term survival for patients with gastric cancer after discharge. A nationwide cohort study (n=1771, including 218 gastric patients) in France did not show significant improvement in OS (mean follow-up of 33 ± 20 months, P=0.19) after 45 days of oral immunonutrition before digestive oncologic surgery relative to a normal diet.[53] During the postoperative period, a small prospective controlled randomized study (n=98) found that enteral immunonutrition (including arginine, glutamines, and ω-3 PUFAs) continued for 6 days in gastric cancer patients did not significantly prolong 6 months (P = 0.24) OS and 1-year OS (P = 0.83) compared to conventional enteral nutrition.[54] The beneficial effects of immune modulated enteral nutrition were too weak to be significant in these patients because malnourished patients (population who needed nutritional support) were excluded. However, in a randomized controlled trial involving 99 gastric cancer patients treated with enteral nutrition, postoperative enteral immunonutrition lasting 7 days had a positive effect on 6 months OS (HR=0.25, P=0.049) only in malnourished stage IV gastric cancer patients.[55] The three studies did not demonstrate that nutritional support had a positive effect on long-term survival of advanced gastric cancer, due to the exclusion of appropriate patients and the short duration of intervention. More importantly, the primary outcome did not involve DFS. In the present study, we expect that ONS combined with ω-3 PUFAs will reduce postoperative recurrence, improve long-term DFS and OS by reducing sarcopenia, and improve tolerance and efficacy of chemotherapy.

To our knowledge, this is the first study to investigate the efficacy of oral immunonutritional supplement, based on 3-year DFS, 3-year OS, 1-year nutritional status and quality of life, in a population of specific gastric cancers, with the expectation of developing new therapeutic strategies to improve the efficacy of anticancer therapy. The positive results of this multicenter clinical trial will further stimulate larger international randomized trials, which can improve the quality of supportive care for cancer patients and increase access to patients who may benefit from nutritional support in the nonsurgical oncological setting.

Authors' contributions During the study, DZ and YL contributed equally as first authors. DZ, YL, LZ, XG and XY developed the study concept and drafted the manuscript. DZ, ML, and YL are responsible for the randomization of patients. DZ, SX, HX, GL, KY, JB-Z, YW, JQ, JZ, KD, YW, ZT, CJ, WW, ZS, and GL-L are responsible for recruiting, managing the treatment of the patients and collecting data. All authors have read and approved of the final manuscript.

Funding This study is funded by the National Natural Science Foundation of China (81770531) and the Jiangsu Province Key Research and Development Project (BE2022822).

Competing interests There are no conflicts of interest to declare. There is no competing interest in the iVital EnergyTM. FRESENIUS KABI does not participate in data collection, analysis and interpretation of results.

Patient consent for publication Patients or their next of kin consent obtained.

Provenance and peer review Not commissioned; externally peer reviewed

ORCID iDs

Da Zhou http://orcid.org/0000-0003-3062-5952

Mengjie Lu http://orcid.org/0000-0003-4788-7668

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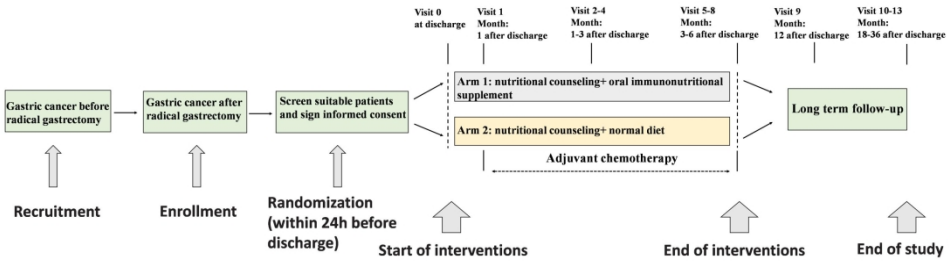
Figure legend:

Figure 1 Flowchart of the CRUCIAL trial



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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title Page 2	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration Page 4	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version n/a	3	Date and version identifier
Funding Page 17	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Page 15 and 17
	5b	Name and contact information for the trial sponsor Page 3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities Page 15
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) Page 14 and 15
Introduction		
Background and rationale Page 5 and 6	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses Page 6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Page 6

Methods: Participants, interventions, and outcomes

Study setting Page 6 and 15	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria Page 7	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered Page 9 and 10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) Page 10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) Page 10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Page 10 and 11
Outcomes Page 11	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline Page 27	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size Page 13	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment n/a	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation Page 6	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
Allocation concealment mechanism Page 6	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation Page 17	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking) n/a	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Page 7-9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols n/a
Data management Page 15	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Page 13-14
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) Page 14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) n/a

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Page 14-15
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Page 14
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Page 10, 11, 13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor n/a

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Page 6
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) n/a
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) n/a
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable n/a
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial n/a
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Page 18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators n/a
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation n/a

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions n/a
	31b	Authorship eligibility guidelines and any intended use of professional writers n/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code n/a
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates n/a
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Page 11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Effects of oral immunonutritional supplement on 3-year disease-free survival in gastric cancer patients with pathologic stage III after total gastrectomy (CRUCIAL): study protocol of a multicenter, randomized clinical trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-067990.R2
Article Type:	Protocol
Date Submitted by the Author:	07-Mar-2023

Complete List of Authors:	Zhou, Da; Medical School of Nanjing University, Research Institute of General Surgery, Jinling Hospital Liu, Yuxiu; Nanjing Jinling Hospital, Data and Statistics Division of Department of Critical Care Medicine Zhang, Li; Research Institute of General Surgery, Jinling Hospital, Medical School of Nanjing University Lu, Mengjie; Shanghai Jiao Tong University School of Medicine Gao, Xuejin; Research Institute of General Surgery, Jinling Hospital, Medical School of Nanjing University Li, Guoli; Medical School of Nanjing University, Department of General Surgery Xiang, Xiaosong; Medical School of Nanjing University, Department of General Surgery Xu, Hao; Nanjing Medical University, Department of General Surgery Li, Gang; Nanjing Medical University, Jiangsu Cancer Hospital, Department of General Surgery Sun, Zhiping; Nanjing Medical University, Affiliated Second Hospital, Department of Clinical Nutrition wei, Wei; Nanjing Medical University, Affiliated Second Hospital, Department of General Surgery Yi, Kui; The Affiliated Jiangning Hospital of Nanjing Medical University, Department of Gastroenteropancreatic Surgery Zuo, Junbo; Zhenjiang First People's Hospital, Department of General Surgery Wu, Yugang; Soochow University, The First People’s Hospital of Changzhou, Department of General Surgery Qian, Jun; Changzhou No. 2 People's Hospital, Department of General Surgery Zhou, Jian; First Affiliated Hospital of Soochow University, Department of General Surgery Duan, Kaipeng; First Affiliated Hospital of Soochow University, Department of General Surgery Wu, Yong; Second Affiliated Hospital of Soochow University, Department of General Surgery Tian, Zhiqiang; Nanjing Medical University Affiliated Wuxi People's Hospital, Department of General Surgery Jiang, Chao; Yixing People's Hospital, Department of General Surgery Wang, Xinying; Medical School of Nanjing University, Department of

	General Surgery
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Nutrition and metabolism, Surgery
Keywords:	Gastrointestinal tumours < GASTROENTEROLOGY, Nutritional support < GASTROENTEROLOGY, Adult gastroenterology < GASTROENTEROLOGY

Effects of oral immunonutritional supplement on 3-year disease-free survival in gastric cancer patients with pathologic stage III after total gastrectomy (CRUCIAL): study protocol of a multicenter, randomized clinical trial

Da Zhou,[1] Yuxiu Liu,[2] Li Zhang,[1] Mengjie Lu,[3] Xuejin Gao,[1] Guoli Li,[1] Xiaosong Xiang,[1] Hao Xu,[4] Gang Li,[5] Zhiping Sun,[6] Wei Wei,[7] Kui Yi,[8] Junbo Zuo,[9] Yugang Wu,[10] Jun Qian,[11] Jian Zhou,[12] Kaipeng Duan,[12] Yong Wu,[13] Zhiqiang Tian,[14] Chao Jiang,[15] Xinying Wang[1]

[1]Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu, China

[2]Data and Statistics Division of Department of Critical Care Medicine, Jinling Hospital, Medical School of Nanjing University, Nanjing, Jiangsu, China

[3]School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[4]Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[5]Department of General Surgery, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[6]Department of Clinical Nutrition, Nanjing Medical University, Affiliated Second Hospital, Nanjing, Jiangsu, China

[7]Department of General Surgery, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[8]Department of Gastroenteropancreatic Surgery, The Affiliated Jiangning Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

[9]Department of General Surgery, Zhenjiang First People's Hospital, Zhengjiang, Jiangsu, China

[10]Department of General Surgery, The Third Affiliated Hospital of Soochow University and The First People's Hospital of Changzhou, Changzhou, Jiangsu, China

[11]Department of General Surgery, ChangZhou Second Hospital, the afflicated Hospital of Nanjing Medical University, Changzhou, Jiangsu, China

[12]Department of General Surgery, the First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[13]Department of General Surgery, the Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[14]Department of General Surgery, Wuxi People's Hospital Affiliated to Nanjing Medical University, Wuxi, Jiangsu, China

[15]Department of General Surgery, Yixing People's Hospital, Yixing, Jiangsu, China

Corresponding author: Xinying Wang

Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, 305 Zhongshan East Road, Nanjing, Jiangsu, China

wangxinying@nju.edu.cn

ABSTRACT

Introduction The nutritional status of patients with gastric cancer (GC) after total gastrectomy continues to deteriorate and lasts a long time after discharge, which is an independent risk factor for mortality. Recent guidelines have recommended appropriate nutritional support after discharge for cancer surgery patients with malnutrition or nutritional risk. The evidence on the efficacy of oral immunonutritional supplement and its effect on long-term disease-free survival (DFS) in patients with GC is limited. This study was designed to test the hypothesis that oral immunonutritional supplement compared to diet alone may improve 3-year DFS of GC patients with pathologic stage III after total gastrectomy (nutrition risk screening 2002 score ≥ 3 at discharge).

Methods and analysis This is a pragmatic, open-label, multicenter, randomized controlled study. 696 eligible GC patients with pathologic stage III after total gastrectomy will be randomized in a 1:1 ratio to oral immunonutritional supplement group or normal diet group for six months. The primary endpoint is 3-year DFS after discharge. The following secondary endpoints will be evaluated: 3-year overall survival; unplanned readmission rate at 3 and 6 months after discharge; quality of life, body mass index, and hematological index at 3, 6, and 12 months after discharge; incidence of sarcopenia at 6 and 12 months after discharge; and the tolerance to chemotherapy. The adverse events of oral immunonutritional supplement will also be evaluated during the intervention.

Ethics and dissemination This study was approved by the ethics committee of Jinling Hospital, Nanjing University (Number 2021NZKY-069-01). The present study may validate the effectiveness of oral immunonutritional therapy in improving 3-year DFS for gastric cancer patients with pathologic stage III after total gastrectomy for the first time. The results of this trial will be disseminated in peer-reviewed journals and at scientific conferences.

Trial registration number ClinicalTrials.gov Registry (NCT05253716).

Strengths and limitations of this study

⇒ This is a pragmatic, open-label, multicenter, randomized controlled trial providing high quality clinical evidence concerning the efficacy and safety of oral immunonutrition supplement for gastric cancer patients with pathologic stage III after total gastrectomy.

⇒ The data will be handled by an Independent Data Monitoring Committee to ensure

the safety of the participants. Oral immunonutrition supplement is a well-studied method with a favorable safety profile in previous trials.

⇒ This is the first study to evaluate the effect of oral immunonutrition supplementation on long-term disease-free survival in patients with gastric cancer after discharge.

⇒ Longer survival or recurrence status is not assessed in this study.

INTRODUCTION

Malnutrition is common in patients with gastric cancer. It increases postoperative complications, length of stay, and cost of treatment and weakens the effect of chemotherapy and quality of life (QoL), as well as shortens long-term survival.[1-4] In addition to preoperative malnutrition, which most studies are concerned about, the nutritional status after radical gastrectomy will also continue to deteriorate for nearly 1 year; the rate of malnutrition was approximately 30%–50%.[1, 5, 6] Due to postoperative chemotherapy and several other risk factors, including reduced nutritional intake and malabsorption, continuous weight loss, and sarcopenia (an important phenotype of malnutrition) caused by progressive loss of skeletal muscle mass with decreased physical activity persists long after radical gastrectomy.[7, 8] These conditions are more common and severe in patients with pathological stage III gastric cancer or after total gastrectomy: the proportion of skeletal muscle loss of ≥ 5% six months after surgery has been reported to be up to 51% and 55.4%, respectively.[9, 10] Gastric cancer patients who exhibited ≥ 5% skeletal muscle loss have shown a lower 5-year disease-free survival (DFS) rates (33.8% vs 46.2%; P = 0.020).[10] Moreover, postoperative sarcopenia may persist for approximately 1 year and is significantly related to worse 5-year overall survival (OS) in gastric cancer.[11] Therefore, reinforced nutritional support should be offered to this patient population, and more attention should be provided.

The most recent guideline recommends appropriate postdischarge nutritional support for surgical cancer patients with nutritional risks or those who are already malnourished, and several guidelines have recommended oral nutritional supplement (ONS) as the preferred approach.[12, 13] The limited available evidence suggests that consecutive ONS for 3 months postdischarge has a positive effect on maintaining weight, reduces the risk of sarcopenia, and improves chemotherapy tolerance for gastric cancer after surgery.[14, 15] Although ONS can effectively prolong median OS for metastatic gastric cancers,[16] its effect on survival outcomes of gastric cancer patients with pathologic stage III after total gastrectomy remains unclear. Omega-3

polyunsaturated fatty acids (ω-3 PUFAs, including EPA and DHA) are commonly used as nutrients for immunity. Aoife et al. found that enteral nutrition enriched with 2.2 g of EPA/d can maintain skeletal muscle mass after esophagectomy.[17] Furthermore, recent preclinical studies have shown that ω-3 PUFAs can also inhibit gastric cancer-related cell growth and metastasis.[18-20] However, to our knowledge, studies on disease-free survival (DFS) after oral immunonutritional supplement after surgery for advanced gastric cancer are lacking.

The objective of this pragmatic, multicenter, randomized clinical trial (Clinicaltrials. gov identifier: NCT05253716) is to evaluate the efficacy of the postoperative oral immunonutritional supplement for 6 months and its effect on 3-year DFS, 3-year OS, weight maintenance, sarcopenia, QoL, chemotherapy tolerance, and unplanned readmission of gastric cancer patients with pathological stage III after total gastrectomy. We hypothesize that oral immunonutritional supplement will have significantly more benefits than diet alone for the above patients with nutritional risk at discharge.

METHODS AND ANALYSIS

Standard protocol approval, registration, and patient consent

This study will be conducted in accordance with good clinical practice and ethical standards set out in the Declaration of Helsinki of 1964 and its subsequent amendments. The study protocol (version 2.0) was approved by the Ethics Committee of Jinling Hospital (Nanjing, China; 17/12/2021; approval No. 2021NZKY-069-01) and was registered on ClinicalTrials.gov (NCT05253716). The medical personnel of the participating institutions will obtain written informed consent from all the patients enrolled in the study, and clarify that they can withdraw at any time without providing a reason and without any effect on their current or future care. The translated patient consent form is attached as an online supplemental file.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Design and randomization

This study is initiated by the investigator and will be designed as a pragmatic, multicenter, open-label, randomized, and controlled clinical trial. Eligible patients will be randomly assigned to the immunonutrition supplement (INS) or control group (C) using a 1:1 ratio. Centralized permuted block randomization will be implemented using

the mobile client-based *Randomization Allocation Tool* (RAT),[21] with stratification by trial center (13 tertiary general hospitals in China), pathological stage of TNM (IIIA or IIIB or IIIC), and Laurén classification (intestinal-type or not). If a participant is qualified for the trial and has signed the written informed consent, the investigator authorized by each center can input the relevant information into the RAT. The assigned group will be immediately fed back to the interface on the mobile client of the investigator. Participants will accept the specified treatment.

Subjects

Prior to surgery, the principal investigator at each study center will be responsible for recruiting subjects. Patients will be enrolled in the study when they volunteer and meet the following criteria: consecutive adults (18 years of age or more) gastric cancer patients who have undergone radical total gastrectomy with pathological TNM stage III and nutrition risk screening 2002 (NRS2002) score of ≥3 and the Eastern Cooperative Oncology Group (ECOG) performance status of zero, one, or two at discharge. Patients will be excluded for the following: inability to oral or consume ONS; previous receipt of neoadjuvant chemotherapy; pregnancy; palliative surgery or gastric stump cancer or Borrmann type IV; inability to discontinue oral anticoagulants; acquired immune deficiency syndrome (HIV positive or CD4 < 200/mm^3); severe cardiovascular disease that includes chronic heart failure, angina pectoris, myocardial infarction, arrhythmias (such as atrial fibrillation), or uncontrolled hypertension; severe liver and kidney diseases including active hepatitis, cirrhosis, and uremia; diabetes with complications or uncontrolled by medications; previous use of fish oil capsule >2 times/week; contraindications for fish oil capsule; incomplete grip strength measurement and 5-time chair stand test; and previous enrollment in other studies within the same hospital admission.

Discharge criteria

The discharge criteria are similar for the two groups. They include the ability to mobilize and self-care; oral tolerance of semiliquid food; tube feeding is not required; and no complications requiring hospital treatment. The patient can be discharged when they meet these criteria, and the time of discharge will be recorded.[22]

Assessments

General demographic [gender, age, and body mass index (BMI)] and clinical data, including the American Society of Anesthesiologists score, the Charlson Comorbidity Index, surgical methods (laparoscopy and laparotomy), pathological stage of TNM

(IIIA, IIIB, and IIIC), Laurén classification (intestinal and non-intestinal type), and tumor size and location will be collected before discharge after radical gastrectomy. The pathological terms and classification used in this study are compiled using the eighth edition of the American Joint Committee on Cancer (AJCC) staging system.[23] In addition to the NRS2002 (score range from 0 to 7, 3 or higher are considered nutritional risk) and ECOG (the performance status was classified into 0-5 grades: 0 = asymptomatic, 1 = symptomatic but completely ambulatory, 2 = ambulatory and capable of all self-care, 3 or 4 = generally considered unsuitable for chemotherapy) scores acquired at discharge, the following indicators will be evaluated.

Sarcopenia: Sarcopenia is diagnosed as low skeletal muscle mass plus low skeletal muscle strength or low physical performance, according to the criteria of the Asian Working Group for Sarcopenia 2019 criteria.[24] Abdominal CT images will be selected to calculate the skeletal muscle index of the third lumbar spine (L3MI). Low skeletal muscle mass is defined as L3MI < 40.8 cm^2/m^2 for men and L3MI < 34.9 cm^2/m^2 for women, according to our previously published study.[25] Patients will hold the dynamometer (EH101, China) with maximum force in the dominant hand, and the experiment will be carried out three consecutive times. The maximum value measured after each interval of 1 minute is grip strength;[26] low skeletal muscle strength is defined as grip strength <28.0 kg for men and grip strength < 18.0 kg for women. Physical performance will be assessed using a 5-time chair stand test; low physical performance will be defined as a 5-time chair stand test result of ≥ 12 s. Sarcopenia will be assessed preoperatively and 6 and 12 months after discharge.

Anthropometric indicators: body weight and BMI, calculated as weight (kg)/height (m^2), will be collected at discharge and 3, 6, and 12 months after discharge.

Hematological indicators: hematological indicators including albumin, prealbumin, and hemoglobin will be assessed at discharge and 3, 6, and 12 months after discharge.

QoL: QoL will be assessed at discharge and 3, 6, and 12 months after discharge using the European Organization for Research and Treatment of Cancer (EORTC) core QoL questionnaire (QLQ-C30). The EORTC QLQ-C30 questionnaire consists of 30 items, which can evaluate QoL from a multidimensional perspective and better reflect the connotation of QoL for cancer patients.[27]

ω-3 PUFA intake for the diet: The Food Frequency Questionnaire (FFQ) will be developed to assess the dietary intake of ω-3 PUFAs to rule out its influence on the trial results. FFQ will be completed at baseline, the third month of the intervention, and the

end of the intervention.

Toxicity and tolerability of chemotherapy: Chemotherapy toxicity will be monitored at the end of each cycle during chemotherapy by the investigators and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE, version 5.0). In addition, chemotherapy intolerance (defined as the presence of reduction, delay, or termination) will be assessed and documented.[28]

Tumor recurrence assessments: A minimum follow-up for tumor recurrence assessments of 36 months will be required for each patient. They will include (1) CT or MRI of the chest and abdomen repeated every 6 months for 3 years; (2) tumor marker assessments, including carcinoembryonic antigen and carbohydrate antigen 19-9, assessed at the same frequency as CT or MRI; and (3) mandatory annual endoscopy during follow-up.

Adverse complications and events: All adverse complications and events attributed to interventions (gastrointestinal side effects, such as nausea, vomiting, diarrhea, abdominal pain, abdominal distention, and constipation), including unplanned hospitalizations, will be recorded.

ω-3 PUFA measurement: The red blood cells (RBCs) slurry will be obtained after centrifugation at 700g/min and 4 °C for 10 minutes within 3 hours of extraction of 10 ml of venous blood with an EDTA anticoagulant tube during fasting at 7 a.m.[29, 30] The isolated RBCs will be stored at -80 °C to determine the content of fatty acids (FA). We will measure FAs by liquid chromatography tandem mass spectrometry (LCMS/MS),[31] and the data will be presented by measuring EPA and DHA as a percentage of the total FA content. Measurements will be taken before the intervention and at the end of the intervention.

A trained team that includes a gastrointestinal surgeon, an oncologist, a dietitian, and an oncology specialty nurse at each research center will evaluate all of these indicators.

Treatment

A team of experienced surgeons who have performed at least 50 gastric cancer surgeries in the last calendar year will perform gastric cancer surgery in each participating center. Total gastrectomy and standard D2 lymph node dissection will be performed, and more than 15 lymph nodes will be dissected. All patients will receive nutritional counseling at discharge and at the beginning of each cycle of adjuvant chemotherapy.

All enrolled patients will be randomized into two groups within 24 hours before discharge: (i) INS group and (ii) C group. In the INS group, patients will consume two bottles per day of a high-calorie, high-protein ONS [iVital Energy™ (vanilla, 200 mL per bottle, 1.5 kcal per mL), FRESENIUS KABI, Germany] and three capsules of marine fish oil [webber naturals (1.425 g of fish oil per capsule, containing 0.6 g of EPA and 0.3 g of DHA), Canada] per day after discharge for 6 months, in addition to diet. In the C group, patients will receive nutritional counseling and dietary modifications; the intake of protein-rich foods will increase. ONS will also be considered when a dietitian evaluates the medical needs of a patient.

The detailed composition of iVital Energy™ is provided in Table 1. The number of supplements consumed will be counted daily. The researchers will monitor compliance by telephone every week and check the records at each follow-up. In addition, participants will be asked to bring back the remaining iVital Energy™ and fish oil capsules at each follow-up visit for counting to measure compliance. Any gastrointestinal side effects will also be recorded to determine the safety of ONS and fish oil consumption.

Table 1 Nutrient contents of the iVital Energy

Items	100ml	NRV%
Energy, kcal	150	7
% from proteins	27	-
% from carbohydrates	34	-
% from fats	39	-
Proteins, g	10	17
Fats, g	6.7	11
Saturated fatty acid, g	0.6	-
Monounsaturated fatty acid, g	4.9	-
Polyunsaturated fatty acids, g	1.2	-
Carbohydrates, g	12.4	4
Vit A, μg	81	10
Vit D, μg	2.5	50
Vit E, mg	3.75	27
Vit B_1, mg	0.14	10
Vit B_6, mg	0.13	9
Vit C, mg	18.8	19
Sodium, mg	55	3
Phosphorus, mg	120	17
Potassium, mg	130	7
Magnesium, mg	14	5
Calcium, mg	205	26
Iron, mg	1.8	12
Zinc, mg	2	13

Selenium, μg	13.5	27

NRV Nutrient Reference Values

Anticancer treatments

Patients will receive 6–8 cycles of fluorouracil-based adjuvant chemotherapy from 3-4 weeks after discharge. Oncologists will decide on a specific treatment regimen (fluorouracil combined with platinum, fluorouracil combined with paclitaxel, or fluorouracil combined with platinum and paclitaxel) and duration of treatment.

Endpoints

The primary endpoint will be a 3-year DFS post-discharge after radical gastrectomy. DFS is defined as the time from randomization to tumor recurrence of primary cancer, new gastric cancer, distant metastases, or death from any cause, whichever comes first.[32]

The following secondary endpoints will also be evaluated: 3-year OS; unplanned readmission rate (one or more) at 3 and 6 months after discharge; QoL (EORTC QLQ-C30 score), weight, BMI, and hematological index at 3, 6, and 12 months after discharge; incidence of sarcopenia at 6 and 12 months after discharge; and the tolerance to chemotherapy.

The safety of ONS and the fish oil capsule will be evaluated during the intervention by monitoring vital signs (heart rate, pulse, blood pressure, respiration rate) and the incidence of gastrointestinal side effects, as mentioned above.

Table 2 provides a summary of the assessments and related endpoints that will be investigated during the study. The flowchart of the CRUCIAL trial is shown in Figure 1.

Table 2 Summary of scheduled assessment and follow-up during the study

Evaluations	Visit 0	Visit 1-8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13
	discharge	Month: 0-6 after discharge	Month: 12 after discharge	Month: 18 after discharge	Month: 24 after discharge	Month: 30 after discharge	Month: 36 after discharge
Informed consent	X						
Inclusion/ exclusion criteria	X						

Randomization	X						
Demographic data	X						
Clinical data	X						
Blood sample	X	X					
BMI	X	X	X				
Sarcopenia	X	X	X				
Hematological indicators	X	X	X				
QoL (EORTC QLQ-C30)	X	X	X				
ω-3 PUFAs intakes in diet	X	X					
Chemotherapy tolerance		X					
Chemotherapy toxicity		X					
Adherence to interventions		X					
Adverse events		X					
Unplanned readmission		X					
CT or MRI		X	X	X	X	X	X
Tumor markers		X	X	X	X	X	X

Endoscopy			X		X		X
DFS							X
OS							X

BMI body mass index, QoL quality of life, EORTC QLQ-C30 European Organization for Research and Treatment of Cancer core quality of life questionnaire, DFS disease-free survival, OS overall survival

Benefit for participants

All patients will receive an early nutritional assessment and counseling. The nutritional status of the patients will be regularly monitored. Medical interventions will be provided for any adverse effects during nutritional support.

Potential risks and burdens for research participants

ONS and fish oil capsules may cause discomfort or expose patients to an increased risk of gastrointestinal intolerance, which will be recorded and promptly provided medical treatment. The ONS product in this study is vanilla, which is internationally well accepted and tolerated, as reported in previous studies.[33] As indicated in the ESPEN guidelines in 2016, fish oil and ω-3 fatty acids were mainly well tolerated.[34] Furthermore, the combined dose of the EPA and DHA supplement was up to 5 g/day, which did not increase the risk of spontaneous bleeding episodes or bleeding complications. [35] Therefore, there are no safety concerns for adults regarding the dose of fish oil in this study.

Statistical methods

Sample size

The sample size was calculated according to the primary endpoint using PASS 15.0 software (NCSS, Kaysville, Utah, USA). Based on relevant data available in the previous literature, the 3-year DFS after total gastrectomy for stage III gastric cancer was 36.2%.[36] Assuming this type of patient received only nutritional counseling in the control group, the 3-year DFS was similar, that is, 36%. Taking into account a study power of 80%, an alpha error level at two tails of 5%, and an expected 3-year DFS of 46% in the INS group, 696 patients (348 in each group) will have to be enrolled to allow for a dropout rate of 5% or withdrawal.

Statistical analyses

Analyses of primary and secondary endpoints will be based on the intention-to-treat principle. Survival curves will be estimated using the Kaplan–Meier method and compared with the results of log-rank tests in time-to-event analyzes. The hazard ratios (HR) and 95% confidence intervals (CI) will be derived using Cox proportional hazards models. For the primary endpoint of the 3-year DFS, an additional prespecified analysis of the multivariate Cox proportional hazards model will be used to evaluate the consistency of the group effect. This model will account for clinically important baseline characteristics, including trial center, age, sex, Laurén type, sarcopenia, N stage, T stage, and TNM stage. The proportional hazards assumption will be evaluated using scaled Schoenfeld residuals. Prespecified subgroup analyzes will include age (≥ 65 vs <65 years old), sex (men vs women), Laurén type (intestinal-type vs not intestinal-type), sarcopenia (yes vs no), N stage (N0 vs N1 vs N2 vs N3), T stage (T1 vs T2 vs T3 vs T4), and TNM stage (IIIA vs IIIB vs IIIC). Cox proportional hazards models with additional interaction variables of the subgroup and group factors will be constructed to estimate the *P* values of interactions in these subgroup analyses.

The normality of the continuous variable will be assessed using the Shapiro-Wilk test. Continuous variables will be presented as means with standard deviations or medians with interquartile ranges, and categorical variables will be shown as frequencies and percentages. The student's t-test will be used for the analysis of continuous variables of normal distribution, and Wilcoxon rank sum test will be used for the analyses of continuous nonnormal distribution or ranking data. The chi-squared test or Fisher's exact test will be used for the analyses of categorical variables.

For the superiority assessment of the primary endpoint of the 3-year DFS, a two-sided *P* value of less than 0.05 will be considered statistical significance. Due to the potential for type I error due to multiplicity, any other inferences drawn from *P* values, or 95% CIs will not be reproducible and should be interpreted as exploratory.

All statistical analyzes will be performed using SAS software, version 9.4 (SAS Institute, Cary, NC) by independent statisticians masked in the allocation of the treatment group.

Study administration

The Clinical Endpoint Committee (CEC) is an independent group of five experts that includes one pathologist, two radiologists, and two clinical specialists. Recurrence of the primary endpoint will be assessed by the CEC, who are masked from the

treatment assignment, based on medical history and physical examination combined with imaging evaluation, cytology, or tissue biopsy findings.

The Clinical Endpoint Committee (CEC) is an independent group of five experts including one pathologist, two radiologists, and two clinical specialists. Recurrence of the primary endpoint will be assessed by the CEC, which is masked to treatment assignment, based on medical history and physical examination combined with imaging evaluation, cytology, or tissue biopsy findings.

Recruiting process

The trial was registered on 24 February 2022. The first patient was randomised on 1 August 2022. So far, 76 patients had been randomised, and the enrolment keeps to the flowchart.

Data management

The investigators are responsible for the accuracy and timely entry of the data into the electronic case report form (eCRF) according to the study protocol. The study monitor will review the eCRFs and other study documents, and verify the primary data. The final confirmed data set will be locked and analyzed by the trial statistician. In principle, the data set locking cannot be modified. The investigators must keep research documents for a specified period by the regulatory requirements.

ETHICAL AND DISSEMINATION

This study was approved by the ethics committee of Jinling Hospital. We will not begin recruiting at other participating centers of the trial until the local ethics committee approves the study. Site ethical approvals were obtained from ethics committees of the First Afflated Hospital of Nanjing Medical University, the Second Afflated Hospital of Nanjing Medical University, the Affiliated Cancer Hospital of Nanjing Medical University, Nanjing Jiangning Hospital, Zhenjiang First People's Hospital, The Third Affiliated Hospital of Soochow University, Changzhou Second Hospital, The First Affiliated Hospital of Soochow University, The Second Affiliated Hospital of Soochow University, The Affiliated Wuxi People's Hospital of Nanjing Medical University, and Yixing People's Hospital. The results of the study will be presented at national and international medical meetings. Meanwhile, the results will be published in prestigious peer-reviewed medical journals.

DISCUSSION

Malnutrition can occur at any stage during the development, progression, and treatment of gastric cancer.[37] After surgical resection of the tumor, the incidence of

malnutrition is 30 to 50% after discharge, which is still common.[6] Malnutrition is more pronounced in patients with pathological stage III gastric cancer or after total gastrectomy,[1, 38, 39] which will significantly shorten long-term survival.[40, 41] A previous randomized controlled trial has demonstrated nutritional support was a feasible approach to increase the median OS of patients with stage IV gastric cancer from 11.9 to 14.8 months.[16] However, oral immunonutritional supplement after discharge is mostly concentrated in nutritional status or inflammatory response in patients with advanced gastric cancer,[42] and lacks high-quality evidence on long-term DFS and OS, which still requires confirmation.

The ONS is the preferred form of nutritional support for energy and nutrients for specific medical purposes.[43] A previous multicenter randomized controlled trial had demonstrated high tolerability and compliance with long-term oral ONS after gastric cancer surgery.[44] Several studies have found that ONS can reduce the incidence of sarcopenia, improve some parameters of QoL, improve chemotherapy tolerance[15], and delay weight loss after total gastrectomy.[45, 46] However, the duration of ONS is usually shorter (6-12 weeks), resulting in a difference in weight maintenance and gradual decline after 6 months that is insignificant 1 year after surgery compared with the results of the standard diet.[14] This study will ensure that long-term nutritional support (6 months) is provided to patients with a nutritional risk assigned to the INS group after discharge, and improve nutritional status and QoL for 12 months after surgery.

Omega-3 PUFAs, as immunological nutrients, do not only prevent cardiovascular events;[47] they fight sarcopenia by reducing insulin resistance, improving mitochondrial function, inhibiting the inflammatory response and activating the mTOR pathway.[18] Smith et al. confirmed that oral administration of fish oil (1.86g/d EPA+1.5g/d DHA) for 6 months delayed the loss of muscle mass and function, and prevented sarcopenia in healthy older adults without severe adverse events related to ω–3 PUFA.[48] Preclinical studies have confirmed that ω-3 PUFAs can inhibit gastric cancer progress by inducing apoptosis of gastric cancer cells in various ways.[19, 20, 49, 50] In addition, a randomized controlled trial has shown that 2g/d ω-3 PUFAs during neoadjuvant chemotherapy can improve the pathological response rate and the subsequent R0 resection rate.[51] Moreover, ω-3 PUFAs also play a synergistic role with cisplatin to enhance its inhibitory effect on gastric cancer.[52] In this study, the nutrition and anti-tumor recurrence effects of ω-3 PUFAs can be fully played under the

premise of ensuring safety.

Several studies have attempted to determine whether nutritional support can improve long-term survival for patients with gastric cancer after discharge. A nationwide cohort study (n=1771, including 218 gastric patients) in France did not show significant improvement in OS (mean follow-up of 33 ± 20 months, P=0.19) after 45 days of oral immunonutrition before digestive oncologic surgery relative to a normal diet.[53] During the postoperative period, a small prospective controlled randomized study (n=98) found that enteral immunonutrition (including arginine, glutamines, and ω-3 PUFAs) continued for 6 days in gastric cancer patients did not significantly prolong 6 months (P = 0.24) OS and 1-year OS (P = 0.83) compared to conventional enteral nutrition.[54] The beneficial effects of immune modulated enteral nutrition were too weak to be significant in these patients because malnourished patients (population who needed nutritional support) were excluded. However, in a randomized controlled trial involving 99 gastric cancer patients treated with enteral nutrition, postoperative enteral immunonutrition lasting 7 days had a positive effect on 6 months OS (HR=0.25, P=0.049) only in malnourished stage IV gastric cancer patients.[55] The three studies did not demonstrate that nutritional support had a positive effect on long-term survival of advanced gastric cancer, due to the exclusion of appropriate patients and the short duration of intervention. More importantly, the primary outcome did not involve DFS. In the present study, we expect that ONS combined with ω-3 PUFAs will reduce postoperative recurrence, improve long-term DFS and OS by reducing sarcopenia, and improve tolerance and efficacy of chemotherapy.

To our knowledge, this is the first study to investigate the efficacy of oral immunonutritional supplement, based on 3-year DFS, 3-year OS, 1-year nutritional status and quality of life, in a population of specific gastric cancers, with the expectation of developing new therapeutic strategies to improve the efficacy of anticancer therapy. At the same time, compared to previous studies, patients will be able to receive oral immunonutritional supplement /nutritional counseling longer after discharge to cope with the postoperative chemotherapy in this study. Furthermore, although iVital Energy™ and fish oil capsules are taken separately, they provide more ω-3 PUFAs than other immunonutritional preparations, potentially providing more benefit to patients. However, there are some limitations to this study. Firstly, patients receiving preoperative chemotherapy before gastrectomy will be excluded from this study, excluding these patients will reduce the "generalisability" and applicability of the study

findings to Western patients. Secondly, the results of this study may not apply to all gastric cancer patients. Finally, longer survival or recurrence status is not assessed in this study.

In conclusion, the positive results of this multicenter clinical trial will further stimulate larger international randomized trials, which can improve the quality of supportive care for cancer patients and increase access to patients who may benefit from nutritional support in the nonsurgical oncological setting.

Authors' contributions During the study, DZ and YL contributed equally as first authors. DZ, YL, LZ, XG and XW developed the study concept and drafted the manuscript. DZ, ML, and YL are responsible for the randomization of patients. DZ, XX, HX, GL, KY, JB-Z, YW, JQ, JZ, KD, YW, ZT, CJ, WW, ZS, and GL-L are responsible for recruiting, managing the treatment of the patients and collecting data. All authors have read and approved of the final manuscript.

Funding This study is funded by the National Natural Science Foundation of China (81770531) and the Jiangsu Province Key Research and Development Project (BE2022822).

Competing interests There are no conflicts of interest to declare. There is no competing interest in the iVital EnergyTM. FRESENIUS KABI does not participate in data collection, analysis and interpretation of results.

Patient consent for publication Patients or their next of kin consent obtained.

Provenance and peer review Not commissioned; externally peer reviewed

ORCID iDs

Da Zhou http://orcid.org/0000-0003-3062-5952

Mengjie Lu http://orcid.org/0000-0003-4788-7668

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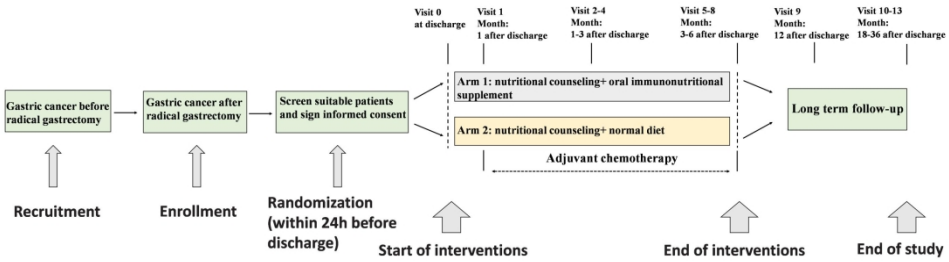
54. Scislo L, Pach R, Nowak A*, et al.* The Impact of Postoperative Enteral Immunonutrition on Postoperative Complications and Survival in Gastric Cancer Patients - Randomized Clinical Trial. *Nutr Cancer* 2018; 70: 453-59.

55. Klek S, Scislo L, Walewska E*, et al.* Enriched enteral nutrition may improve short-term survival in stage IV gastric cancer patients: A randomized, controlled trial. *Nutrition* 2017; 36: 46-53.

Figure legend:

Figure 1 Flowchart of the CRUCIAL trial



248x80mm (300 x 300 DPI)

Version 2.0 (1/12/2021)

Jinling Hospital, Nanjing University

CONSENT FORM

TITLE OF STUDY: Effects of oral immunonutritional supplement on 3-year disease-free survival in gastric cancer patients with pathologic stage III after total gastrectomy

PRINCIPAL INVESTIGATOR: Xinying Wang, MD, PhD

We are conducting a clinical trial (a type of research study). Clinical trials include only patients who choose to take part in the study. This consent form will provide information on the procedures and risks involved in the clinical trial. At the same time, this consent form will ask for your permission to use and release the medical information that we will get from you during this study. Please read this informed consent carefully before deciding whether to participate in this study. You can also discuss it with your family and friends. If you have any questions, you can ask the study doctor for more explanation.

This study is being sponsored by Jinling Hospital of Nanjing University. You are being asked to take part in this study because you were admitted to Jinling Hospital with gastric cancer.

WHY IS THIS STUDY BEING DONE?

The nutritional status of patients with gastric cancer (GC) after total gastrectomy continues to deteriorate and lasts a long time after discharge, which is an independent risk factor for mortality. Several studies have found that oral nutritional supplement (ONS) can reduce the incidence of sarcopenia and delay weight loss after total gastrectomy. However, the duration of ONS is usually shorter (6-12 weeks), and the evidence on the efficacy of oral immunonutritional supplement and its effect on long-term disease-free survival (DFS) in patients with GC is limited. Therefore, we conduct a multicenter, randomized study to evaluate the efficacy and safety of oral immunonutritional supplement in treating GC patients with pathologic stage III after total gastrectomy.

It is planned that a total of 696 people will take part in this study from 14 hospitals in Jiangsu province China.

WHAT WILL HAPPEN IN THE STUDY?

You meet the inclusion criteria for this study, so you will be "randomly" assigned by the computer program to either of the two groups described below. This means that you will have the same probability of being assigned to any group and that you or the doctor will not be able to actively choose the outcome of the assignment.

Group 1: the immunonutrition supplement (INS) group. In the INS group, you will consume two bottles per day of a high-calorie, high-protein ONS [iVital Energy™ (vanilla, 200 mL per bottle, 1.5 kcal per mL), FRESENIUS KABI, Germany] and three capsules of marine fish oil [webber naturals (1.425 g of fish oil per capsule, containing 0.6 g of EPA and 0.3 g of DHA), Canada] per day after discharge for 6 months, in addition to the diet. Group 2: the control (C) group. In the C group, you will receive nutritional counseling and dietary modifications; the intake of protein-rich foods will increase. Before intervention and at the end of intervention, 10ml of your venous blood will be collected to measure the level of ω-3 PUFA in red blood cells. At the same time, you will be followed up for 3 years.

You can stop being a part of this study at any time. If you decide to stop being in the study, please talk to the study doctor first.

WHAT ARE THE RISKS OF THIS STUDY?

The ONS and marine fish oil used in this study are widely used in the nutritional treatment of various diseases. The possible side effects of ONS are typical adverse reactions of enteral nutrition, such as abdominal distension, diarrhea, nausea, vomiting, etc. The possible side effects of fish oil capsules include nausea, belching, nosebleed, and diarrhea. The above side effects are a low incidence and will gradually disappear after drug withdrawal. Furthermore, the combined dose of the EPA and DHA supplement was up to 5 g/day, which did not increase the risk of spontaneous bleeding episodes or bleeding complications. All adverse complications and events attributed to interventions, including unplanned hospitalizations, will be recorded.

There also may be other side effects that we cannot predict. These side effects are often manageable and reversible. If you experience any adverse reactions, talk to the study doctor immediately, and all medically appropriate efforts will be made to prevent

and/or control them by the study doctor.

WHAT ARE THE BENEFITS OF THIS STUDY?

If you agree to take part in this study, you are free to use the iVital Energy™ provided by FRESENIUS KABI (Germany) and marine fish oil provided by WEBBER NATURALS (Canada). Furthermore, you will receive an early nutritional assessment and counseling. And your nutritional status will be regularly monitored.

WHAT ARE THE COSTS?

When you have enrolled in this study, you may receive tests, chemotherapy, and exams, which are standard medical care after surgery. The sponsor will not pay for the routine costs required. However, taking part in this study will not lead to added costs to you or your insurance company. The iVital Energy™ and marine fish oil will be provided free of charge, as is the cost of ω-3 PUFA measurement.

COMPENSATION?

You will receive no payment for taking part in this study.

WHAT ARE YOUR RIGHTS?

Your participation in this study is completely voluntary. You can withdraw from the study at any time without any reason, which will not affect your treatment. All your data and observation records are confidential and for this study only; During the trial, you will have access to relevant information at any time. If you have any problems during the trial or need to consult the relevant questions, you can contact the doctor in charge.

WHAT ABOUT CONFIDENTIAL CONTENT?

The written informed consent stated that the study data would be stored in a computer database and kept confidential under national laws. Patients can only be identified in the database by their initials or patient numbers. The principal investigator is responsible for maintaining the patient identification form or the inclusion form for all patients, including the inclusion code, patient number, full name, and latest address.

STATEMENT OF CONSENT AND AUTHORIZATION

Version 2.0 (1/12/2021)

I have read and fully understood the introduction of "Effects of oral immunonutritional support on long-term prognosis and nutritional status of patients with Stage III gastric Cancer during Chemotherapy after total gastrectomy", and I have been aware of the background, purpose, and research process of this study. The relevant information in this informed consent has also been explained by the study doctor. I agree to participate in this study and to allow the relevant researchers to use my research information for the foregoing purposes. All my questions were satisfactorily answered. When I sign this informed consent, I will not give up any of my rights. As a patient, I am willing to participate in this study and fully cooperate with doctors after understanding the purpose, method, possible benefits, and possible side effects of this study.

Patient Signature: _____

Contact information of Patient: _____

Date of Signature: _____

(If the patient's informed consent capacity is deficient or insufficient, add or replace the following methods)

- Next of Kin
- Parent (patient is a minor)
- Other relationship: _____

Signature of Patient's Legally Authorized Representative: _____

Contact information of Patient's Legally Authorized Representative: _____

Witness to consent process (if applicable): _____

Date of Signature: _____

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title Page 2	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration Page 4	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version Page 6	3	Date and version identifier
Funding Page 17	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Page 15 and 17
	5b	Name and contact information for the trial sponsor Page 3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities Page 15
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) Page 14 and 15
Introduction		
Background and rationale Page 5 and 6	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses Page 6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Page 6

Methods: Participants, interventions, and outcomes

Study setting Page 6 and 15	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria Page 7	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered Page 9 and 10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) Page 10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) Page 10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Page 10 and 11
Outcomes Page 11	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline Page 27	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size Page 13	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment n/a	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation Page 6	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
Allocation concealment mechanism Page 6	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation Page 17	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking) n/a	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Page 7-9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols n/a
Data management Page 15	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Page 13-14
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) Page 14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) n/a

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Page 14-15
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Page 14
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Page 10, 11, 13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor n/a

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Page 6
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) n/a
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Page 7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Page 7
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Page 7
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Page 18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Page 16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation n/a

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Page 4
	31b	Authorship eligibility guidelines and any intended use of professional writers n/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code Page 18
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates Page 7
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Page 11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.