Evaluation of ctDNA in Predicting Response to Neoadjuvant Therapy and Analysis of Residual Disease in Local Advanced Gastric Cancer: Protocol of A Single-Arm Multicenter Prospective Observational Study

Jiangpeng Wei

Department of Digestive Surgery, Xi Jing Hospital, Air Force Military Medical University

Xin Guo

Department of Digestive Surgery, Xi Jing Hospital, Air Force Military Medical University

Weiming Duan

The Medical Department, 3D Medicines Inc., Shanghai, China

Xisheng Yang

Department of Digestive Surgery, Xi Jing Hospital, Air Force Military Medical University

Pengfei Yu

Department of Digestive Surgery, Xi Jing Hospital, Air Force Military Medical University

Jinqiang Liu

Department of Digestive Surgery, Xi Jing Hospital, Air Force Military Medical University

Hushan Zhang

The Medical Department, 3D Medicines Inc., Shanghai, China

Depei Huang

The Medical Department, 3D Medicines Inc., Shanghai, China

Zhengqing Yan

The Medical Department, 3D Medicines Inc., Shanghai, China

Feilong Zhao

The Medical Department, 3D Medicines Inc., Shanghai, China

Xiaohua Li

Department of Digestive Surgery, Xi Jing Hospital, Air Force Military Medical University

Gang Ji (✉ jigang@fmmu.edu.cn)

Department of Digestive Surgery, Xi Jing Hospital, Air Force Military Medical University

Study protocol

Abstract

Background

In gastric cancer, there is a controversial finding regarding the high recurrence rate after neoadjuvant therapy. The use of ctDNA detection for minimal residual disease (MRD) with plasma genotyping has shown higher sensitivity and specificity compared to imaging and serum markers for predicting recurrence. Therefore, we aim to investigate the perioperative dynamic changes in ctDNA in surgical II-III GS patients and assess the efficacy of neoadjuvant chemotherapy.

Methods

In detail, we will collect blood samples from each patient before neoadjuvant chemotherapy (NAC), after NAC, and one month after surgery. We will also collect tumor tissue before and after NAC. Gene mutations will be detected using a 733-gene NGS panel, and DNA concentrations will be measured. To evaluate the effectiveness of the treatment, we will use RECIST Version 1.1 (RECIST 1.1).

Discussion

This study aims to assess the correlation between perioperative changes in ctDNA levels and the response to chemotherapy in patients with gastric cancer. It will provide evidence that perioperative ctDNA detection may predict early recurrence, highlighting the potential clinical utility of ctDNA in guiding therapeutic decision-making. Compared to tumor tissue biopsy, ctDNA can non-invasively detect more comprehensive genomic information from a limited amount of plasma.

Trial registration:

Registered prospectively in the Chinese Clinical Trials Registry with registration number ChiCTR2200060842 on June 12st, 2022.

Background

According to data from 234 registries issued by the China Tumor Registration Center, China registered 679,100 new cases of gastric cancer in 2015 [1]. The incidence of this cancer is second only to lung cancer, which is comparable to the epidemiological findings of gastric cancer in Japan and South Korea [1]. In China, the proportion of new cases and deaths attributed to gastric cancer stands at 42.6% and 45.0%, respectively, in the worldwide total[1]. While urban populations have maintained a relatively stable gastric cancer mortality rate over the past decade, the growth rate among women in rural regions has been significantly lower than that of males[1].

The current therapy for locally advanced gastric cancer (LAGC) involves a multimodal approach centered on surgical treatments. Surgical resection is currently the only therapy that can cure early-stage GC, as the prognosis for patients with GC after surgical resection is better than without [2]. Long-term survival in patients with GC is associated with surgical resection. Currently, the tumor stage offers the best prognostic insight into patient survival [3]. However, challenges associated with GC treatment include developing new strategies for detecting GC early and providing precision treatment [4]. In China, the 5-year survival rate of patients with GC is 36.9% due to the advanced stage of the disease at the time of diagnosis[1].

Due to the high likelihood of postoperative recurrence and metastasis, expanding the extent of the resection would not enhance cure chances for patients with LAGC. Before surgery, chemotherapy may enhance the survival rate of patients with locally advanced stomach cancer. Preoperative chemotherapy may increase R0 resection rates by inducing tumor downstaging, controlling both local recurrence and distant metastasis, and improving disease-free and overall survival, while ensuring patient safety. Patients with resectable GC benefit significantly more from perioperative chemotherapy than surgery alone[5, 6]. Based on these studies, perioperative chemotherapy has been recommended as the first-line treatment (IA) for GS according to the NCCN Guideline (version 2022). The recommendation of ECF (epirubicin, cisplatin, and 5-fluorouracil) has been downgraded to class 2B and replaced by FP (5-fluorouracil, leucovorin, and cisplatin). These studies, however, have some limitations. In particular, only 42.5% of patients in the MAGIC study underwent D2 surgery, and 42% completed only 3 weeks of chemotherapy. Ji et al. conducted a randomized controlled, non-inferiority trial (RESOLVE study) and showed that patients with locally advanced gastric cancer who underwent surgery and were treated with SOX (S-1 combined with oxaliplatin)in the perioperative period demonstrated better efficacy than those treated with CapOx(capecitabine and oxaliplatin) in the postoperative period[7]. Therefore, SOX therapy is effective as a perioperative treatment for advanced gastric tumors.

Despite curative resection, 70% of GC patients still develop recurrence or metastasis within 2 years [8]. Preoperative chemotherapy, surgical resection, and postoperative chemotherapy are common treatments for GC. However, fewer studies focus on postoperative surveillance, and most research focuses on imaging, hematological, and biochemical parameters with low sensitivity and specificity[9, 10]. To improve patient selection, it is necessary to find tissue and blood biomarkers with predictive significance. Circulating tumor DNA (ctDNA) is highly tumor-specific and can detect metastatic disease and minimal residual disease (MRD) across a wide range of solid tumors [11]. ctDNA is derived from somatic tumor DNA fragments released into the systemic circulation upon cell death, and these cells are referred to as "minimal residual disease" (MRD)[12]. Noninvasive and repeatable tumor information can be obtained from plasma DNA, which may be used to guide specific treatments and monitor treatment efficacy. A 2020 study identified ctDNA alteration between plasma and matched cfDNA and white blood cells from the same patient[13]. According to research, the presence of detectable ctDNA following surgery for early-stage malignancies is directly related to recurrence probabilities. Yang et al. detected ctDNA in 46 gastric cancer patients who underwent surgery, with a ctDNA detection rate of 45% (19/42) in preoperative GS patients, 21% (4 of 19) for stages I-II, and 68% (15 of 22) for stage III. ctDNA was detected with a median

of 179 days before radiographic recurrence[14]. Therefore, ctDNA may be a useful, low-cost, high-sensitivity, and noninvasive prognostic biomarker for GC patients undergoing perioperative treatment.

The predictive significance of ctDNA in stage II-III gastric cancer has only been studied in a small number of cases. In this study, we aim to examine the possible uses of ctDNA and evaluate the efficacy of treating resected gastric cancer with targeted deep sequencing to identify MRD and micrometastatic recurrences. We will explore the perioperative dynamic alterations of ctDNA in surgical II-III GS patients and assess the effectiveness of neoadjuvant chemotherapy with SOX based on various fundamental and preclinical research.

Methods

This is a single-center, prospective observational study in which surgical GS patients will be recruited. Blood samples will be taken before and after neoadjuvant chemotherapy and in the month following surgery, while tumor-punctured tissue and surgical tissue will be collected before neoadjuvant chemotherapy and surgery, respectively. Patients included in the study will be followed for at least 6 months. The workflow of our study is presented in Fig. 1.

Primary aims: Our study aims to explore the correlation between blood ctDNA response before and after neoadjuvant therapy and the effect of neoadjuvant therapy.

Secondary aims:

1. To evaluate the advantages and disadvantages of maximum mutation frequency (VAF) or average VAF on ctDNA response after neoadjuvant therapy for advanced gastric cancer;

2. The consistency of ctDNA and tissue DNA detection before operation and the detection rate of ctDNA in gastric cancer were evaluated;

3. To assess the prognostic value of minimal residual disease (MRD) detection methods on the likelihood of recurrence in patients receiving radical surgery for stage II or stage III gastric cancer following neoadjuvant treatment;

4. The correlation between ctDNA before and after the operation with clinical progress-free survival (PFS) and overall survival (OS);

Study Population

The study population will be prospectively recruited from the Xijing Hospital of Digestive Diseases, affiliated with the Air Force Military Medical University (the Fourth Military Medical University). The participants are eligible for the current study if they meet the following criteria before neoadjuvant chemotherapy: 1. It is expected to complete radical D2 lymph node dissection (the number of lymph

nodes examined must be at least 15 to ensure the quality of the operation). 2. Physical condition and organ function allow large abdominal surgery. 3. Be willing and able to follow the protocol during the study. 4. Provide written informed consent before entering the study screening, and the patient has learned that he can withdraw from the study at any time without any loss. 5. Patients aged 18–70 years with gastric adenocarcinoma confirmed by pathology before the operation

The total course of chemotherapy and adjuvant chemotherapy was 6 months, and the survival period was expected to be > 6 months. 7. No other serious concomitant diseases and good organ function. 8. There are no medical contraindications that seriously affect anesthesia and surgery. 9. Have not received anti-tumor treatment (such as surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy); 10. The 8th Edition American Joint Committee on Cancer(AJCC) stage is ct3-4an1-3m0 gastric cancer patients. 11. The blood routine examination standard shall meet the following standards: white blood cell (WBC) > 4.0 × 10/L; b. ANC > 1.5 × 10/L   c. absolute neutrophil count(ANC) ≥ 1.5 × 109/L   d. platelet count(HB) ≥ 80 g/L   e. platelet count(PLT) ≥ 100 × 109/L 12. The biochemical examination shall meet the following standards: a total bilirubin (TBIL) ≤ 1.5 ×upper limit of normal (ULN)   b. alanine aminotransferase (ALT) and aspartate aminotransferase (AST) < 2.5 × ULN   c. blood urea nitrogen (Bun) and creatinine (Cr) ≤ 1.5 × The clearance rate of ULN or endogenous creatinine ≥ 50 ml/min (Cockcroft Gault formula). 13. No history of upper abdominal surgery (except laparoscopic cholecystectomy); No history of peritonitis and pancreatitis. 14. There are no other serious diseases that make the survival time < 5 years. 15. Karnofsky Performance Status (KPS) > 60; Eastern Cooperative Oncology Group (ECOG)score: 0–2. 16. No history of other tumors

Exclusion criteria: 1. Pregnant or lactating women who are in the reproductive period and do not take effective contraceptive measures. 2. Complicated with serious medical diseases or conditions: such as clinically serious (i.e. active) heart disease, serious and uncontrolled medical diseases, infections, serious uncontrollable digestive system disorders, serious electrolyte disorders, active disseminated intravascular coagulation, major organ failure, such as decompensated heart, lung, liver, kidney failure, peripheral neuropathy, etc., unable to tolerate D2 radical gastrectomy. 3. Organ transplantation requires immunosuppressive therapy. 4. Serious uncontrolled repeated infection or other serious uncontrolled concomitant diseases. 5. History of other malignant tumors within 5 years from the start of the trial, except cured skin basal cell carcinoma and cervical carcinoma in situ. 6. Patients without self-knowledge ability and mental disorders. 7. Have a history of organ transplantation (including bone marrow autotransplantation and peripheral stem cell transplantation). 8. Use other test drugs at the same time or in other clinical trials. 9. Those who have received biological therapy or other anti-cancer traditional Chinese medicine with an interval of fewer than 4 weeks. 10. HER-2 test is positive or esophageal gastric junction adenocarcinoma, patients who are willing to receive Herceptin treatment or patients with indications and willingness to receive immunotherapy; 11. Postoperative chemotherapy is not acceptable. 12. Patients with peripheral neuropathy nct-ctcae ≥ grade 2; 13. Tumor recidivism surrounding organs (T4b) or combined with distant metastasis. 14. For those who are allergic to the drugs in this study protocol, the researcher determines that they are not suitable for participating in this clinical study.

A follow-up examination will be performed on the 3rd month, 6th month, 1 year, and 2 years following the CT scan by an experienced surgeon. Tumor markers were examined every 3 months.

Clinical and demographic data will be collected, such as age, sex, smoking status, past medical history, family history of cancer, radiological reports, tumor markers, pathology reports, and postoperative TNM staging.

Intervention

Neoadjuvant chemotherapy

Preoperative SOX chemotherapy consists of three-week cycles, including intravenous administration of oxaliplatin at a dose of 130 mg/m^2 on day 1, and oral administration of S-1 at a dose of 40–60 mg twice a day (BID) from day 1 to day 14. The dose of S-1 is dependent on the patient's body surface area (BSA): 40 mg BID for BSA < 1.25 m^2, 50 mg BID for 1.25 m^2 < BSA < 1.5 m^2, and 60 mg BID for BSA > 1.5 m^2. Day 15 to day 21 is the rest period.

Tumor Response And Toxicity Criteria

Lesions will be evaluated using enhanced Computer tomography(CT), Endoscopic ultrasonography(EUS), and Magnetic resonance imaging(MRI)as needed according to the RECIST 1.1 criteria after the third cycles of SOX. Toxicities will be measured using the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTC AE), version 4.0, and recorded in the AE report form. Serious adverse events (SAE) will be defined according to the rules of good clinical practice (GCP) and reported to the lead center within one working day, followed by prompt notification to other centers.

Follow-up

After the last cycle of adjuvant therapy, subjects will enter survival follow-up to collect disease progression (date of progression) and subsequent anti-tumor treatment information until the subject's death, loss to follow-up, withdrawal of informed consent, or termination of the study by the sponsor. During this period, follow-up will be conducted every 3 months (± 7 days) in the first 1–2 years, every 6 months (± 14 days) in the third to fifth years, and annually thereafter, to collect survival information and information on subsequent treatment.

Sample Collection And Dna Isolation

Blood sample processing and cfDNA isolation

All sampling will be completed in the Department of Digestive Surgery at Xijing Hospital. A blood collection protocol was followed according to guidelines and regulations. 20 ml of peripheral blood will be collected and processed into an EDTA anticoagulation tube. If the blood sample was not processed immediately, it would be prepared and stored at 28°C for no more than 8 hours. Plasma should be centrifuged from whole blood during the shelf-life period. Plasma samples should be stored at -25° to -15°C for no longer than 2 weeks. Methods for collecting, processing, and analyzing blood samples are detailed in blood collecting standard operational procedures.

Tissue Processing And Genomic Dna Extraction

Tissue samples will be obtained from local tumors during diagnosis. Both surgical samples and punctured tissue are acceptable. Tissue processing and genomic DNA extraction Formalin-fixed paraffin-embedded (FFPE) tissue sections will be evaluate for tumor cell content using hematoxylin and eosin (H&E) staining. Only samples with a tumor content of ≥ 20% are eligible for subsequent analyses. FFPE tissue sections will be place in a 1.5 microcentrifuge tube and deparaffinized with mineral oil. Samples will be incubate with lysis buffer and proteinase K at 56°C overnight until the tissue was completely digested. The lysate will be subsequently incubate at 80°C for 4 hours to reverse formaldehyde crosslinks. Genomic DNA will be isolate from tissue samples using the ReliaPrep™ FFPE gDNA Miniprep System (Promega) and quantify using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific) following the manufacturer's instructions.

Sample Size Calculation

Based on a previous study, we assume the response proportion for ctDNA to neoadjuvant treatment to be 30%, with a non-response rate of 70%. Our study will focus on cT3-4aN1-3M0 GS patients, with a non-response rate and response rate for 2-year DFS of 40% and 70%, respectively[7]. Given this information, we plan to recruit 84 individuals for the final analysis. A result will be considered significant when the P value is < 0.05.

Data analysis

Full Analysis Set: An analysis set based on the intent-to-treat population (ITT), which includes randomized patients who have taken at least one dose of medication and have undergone at least one primary effectiveness evaluation of the treatment. If a patient does not observe the entire treatment process, the last observation data is used to calculate the final result based on the last observation carried forward (LOCF).

Using Fisher's exact test for categorical variables and the Mann-Whitney (rank-sum) test for continuous variables, we will compare preoperative ctDNA-positive and ctDNA-negative patients' clinical characteristics. To determine the correlation between ctDNA maximum VAF and tumor size, tumor

volume, or efficacy of neoadjuvant chemotherapy, the Spearman correlation was used. The log-rank method was used to compare ctDNA detection with pathologic response and tumor regression grade (TRG). Overall survival (OS) and progression-free survival (PFS) were compared between different ctDNA statuses using log-rank tests.

Data analysis will be performed using SPSS version 20.0 and GraphPad Prism version 6.0. All statistical comparisons will be performed with two-sided tests; P values of < 0.05 will be considered statistically significant, and parameter estimates will be included the 95% confidence interval (95% CI).

Discussion

Curative surgery is the primary method of treating gastric cancer, which has a high mortality and morbidity rate worldwide[15]. More than 60% of gastric cancer patients experience recurrence after surgery [16]. Early recurrence detection is crucial for improving the prognosis of GC. Routine imaging size criteria of less than 1 cm cannot reliably identify MRD[17]. The serum markers CEA, CA 19 – 9, and CA 125 have poor sensitivity and specificity, predicting only about 40% of recurrences [18, 19]. Delayed diagnosis and treatment of early recurrent metastases results in patients not receiving optimal treatment. The rate of pathological complete response (pCR) in gastric cancer varies from 3–15%[20]. Patients who achieve pCR after preoperative chemotherapy have a favorable prognosis[21]. However, in a study by Fields et al., a patient who achieved pCR after neoadjuvant chemotherapy had a recurrence rate of 36%, with CNS recurrence as their first site of recurrence, with a mean duration of recurrence at 12.6 ± 7.7 months[20]. New biomarkers are urgently needed to predict recurrence risk in patients with GC. ctDNA can serve as a noninvasive biomarker to monitor tumor progression throughout the treatment process with high sensitivity and specificity in colon cancer[22–24]. In gastric cancer, postoperative ctDNA detection identified patients at high risk of relapse 179 days earlier than imaging[14]. In 2020, Alessandro et al. conducted a study and found that preoperative ctDNA status reflects the efficacy of neoadjuvant therapy in gastric cancer[13]. Tumor-derived DNA contains tumor-specific epigenetic information, which is detectable in liquid biopsies, such as blood samples [25]. However, there are limited studies investigating the prognostic utility of ctDNA in gastric cancer.

This is a first-of-its-kind prospective observational study that investigates whether changes in plasma ctDNA during three weeks of neoadjuvant chemotherapy can predict the effectiveness of SOX. All patients will receive five sample detections in total. Tissue specimens obtained before and after neoadjuvant chemotherapy will be used as baseline controls. Peripheral blood samples will be collected before and after neoadjuvant therapy to assess tumor response to neoadjuvant therapy. Peripheral blood tests one month after surgery will be developed to predict recurrences. There are currently two strategies to monitor tumor MRD: tumor-informed (fixed or personalized) assays and tumor-agnostic approaches (also called tumor-naive or plasma-only approaches)[26]. There are no uniform standards for monitoring tumor MRD in the field of neoadjuvant therapy for patients with gastric cancer. Our study was designed to address this challenge. With 2-year follow-up data, our biomarker reliability tests will provide sensitivity and specificity as outcomes of the two strategies.

The combination of liquid biopsy with current clinical practice in our study is expected to have multiple effects on clinical practice. Our study sheds new light on the monitoring efficacy of neoadjuvant chemotherapy and the prediction of recurrence after surgery. However, further large-scale, multicenter studies are needed before this strategy can be used clinically. This study is limited to a single institution with only 84 enrolled participants, which may introduce selection bias. Future investigations may provide complementary information on different populations.

Abbreviations

MRD	minimal residual disease
NAC	neoadjuvant chemotherapy
LAGC	locally advanced gastric cancer
ECF	epirubicin, cisplatin, and 5-fluorouracil
FP	5-fluorouracil, leucovorin, and cisplatin
SOX	S-1 combined with oxaliplatin
CapOx	capecitabine and oxaliplatin
ctDNA	Circulating tumor DNA
GS	gastric cancer
PFS	progress-free survival
OS	overall survival
WBC	white blood cell
ANC	absolute neutrophil count
HB	hemoglobin
PLT	platelet count
TBIL	total bilirubin
ULN	limit of normal
ALT	alanine aminotransferase
AST	aspartate aminotransferase

Bun	blood urea nitrogen
Cr	creatinine
KPS	Karnofsky Performance Status
ECOG	Eastern Cooperative Oncology Group
FFPE	Formalin-fixed paraffin-embedded
H&E	hematoxylin and eosin
LOCF	last observation carried forward
TRG	tumor regression grade
pCR	pathological complete response
CT	Computer tomography
EUS	Endoscopic ultrasonography
MRI	Magnetic resonance imaging
NCI-CTC AE	National Cancer Institute Common Toxicity Criteria for Adverse Events
SAE	Serious adverse events
GCP	good clinical practice

Declarations

Ethics approval and consent to participate: This study was approved by the Ethics Committee of Xi Jing Hospital, The Fourth Military Medical University(Ethics approval number: KY20212226-C-1) and conducted in accordance with the principles of the Helsinki Declaration.

Informed Consent: Written informed consent was obtained from all study participants before the start of the study.

Registry and the Registration No. of the study/trial: Clinical trial registration has been completed on the website of Chinese clinical trial registry(ChiCTR2200060842).

Consent for publication: Written informed consent for publication will be obtained from all participants before inclusion in the study.

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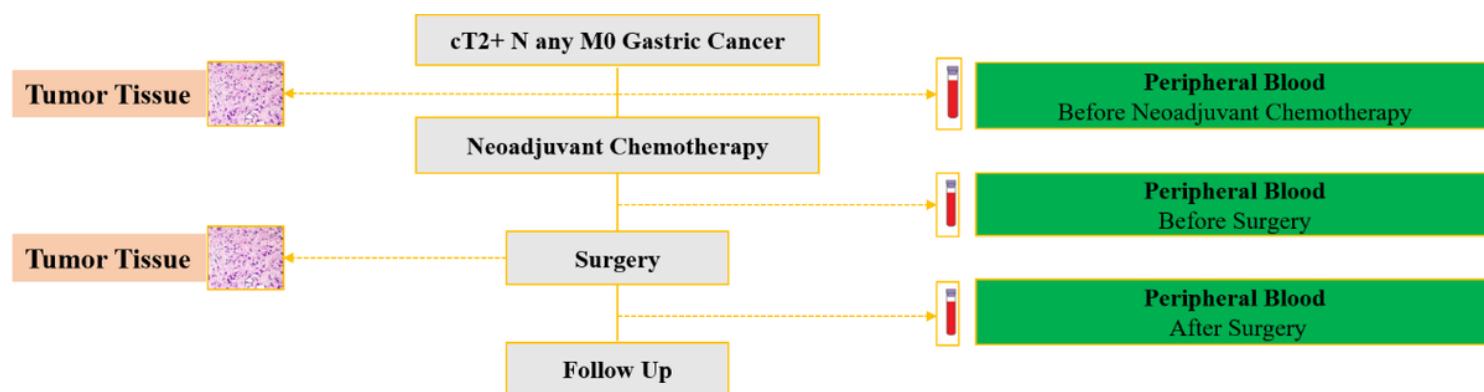
Figures

Figure 1

Workflow of the study.