Multicenter phase I dose escalation and expansion study of pyrotinib in combination with camrelizumab and chemotherapy as first-line treatment for HER2-positive advanced gastric and gastroesophageal junction adenocarcinoma

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Summary

Background Pembrolizumab plus trastuzumab and chemotherapy showed remarkable efficacy as first-line therapy for advanced HER2-positive gastric cancer. Pyrotinib is an irreversible pan-HER inhibitor. This single-arm, open-label phase 1 dose-escalation (1a) and expansion (1b) study investigated camrelizumab, an anti-PD-1 antibody, plus pyrotinib and chemotherapy as first-line treatment for advanced HER2-positive gastric and gastroesophageal junction (G/GEJ) adenocarcinoma.

Methods Between June 2020 and June 2022, 41 patients with previously untreated HER2-positive locally advanced unresectable or metastatic G/GEJ adenocarcinoma were enrolled. In phase 1a, patients underwent a 3 + 3 escalating dose design, receiving oral pyrotinib (240 mg, 320 mg, or 400 mg daily), intravenous camrelizumab (200 mg), and CapeOX (oxaliplatin 130 mg/m^2 on day 1 and capecitabine 1000 mg/m^2 twice daily for two weeks) every 3 weeks until progression, intolerable toxicity or consent withdrawal. The recommended phase 2 dose (RP2D) of pyrotinib was determined and used in the phase 1b. The primary endpoints were the safety, maximum tolerated dose (MTD), RP2D, and confirmed objective response rate (ORR). This trial was registered with chictr.org, number ChiCTR2000029717.

Findings Among 41 patients, 10 were in phase 1a (3 at 240 mg, 3 at 400 mg, and 4 at 320 mg due to one patient withdrawing consent), and 31 were in phase 1b. In phase 1a, the MTD of pyrotinib was 320 mg daily due to dose-limiting toxicities (diarrhea [n = 3] and vomiting [n = 1]) observed at 400 mg. Based on all available data, the RP2D of pyrotinib was set at 320 mg. Among 41 patients, 20 patients (48.8%) developed grade ≥3 treatment-emergent adverse events (TEAEs), and four patients (9.8%) had any grade serious adverse events. No deaths occurred due to TEAEs. Among 27 patients who received the RP2D of pyrotinib and had a post-baseline tumor assessment, two patients (7.4%) achieved a confirmed complete response, and 19 patients (70.4%) achieved a confirmed partial response, resulting in a confirmed ORR of 77.8% (95% CI: 57.7–91.4).

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Interpretation Pyrotinib plus camrelizumab and chemotherapy showed promising efficacy in the first-line treatment of advanced HER2-positive G/GEJ cancer. The safety profile was consistent with known toxicities of the agents, and no new or unexpected safety signals were identified.

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Keywords: Advanced gastric adenocarcinoma; Pyrotinib in combination with camrelizumab and chemotherapy; Phase 1 study; First-line therapy

Research in context

Evidence before this study

We conducted a PubMed search for first-line therapy trials for HER2-positive gastric cancer published until June 25, 2022. We identified three trials that combined pembrolizumab with trastuzumab and chemotherapy, including a phase 1b/2 trial, a single-arm phase 2 trial, and a double-blind, placebo-controlled phase 3 KEYNOTE-811 trial. The KEYNOTE-811 trial showed that pembrolizumab plus trastuzumab and chemotherapy significantly improved the objective response rate compared with placebo plus trastuzumab and chemotherapy at the first interim analysis, with similar rates of grade ≥3 adverse events between the two groups.

Added value of this study

To our knowledge, this is the first phase 1 study to evaluate the combination of pyrotinib, a small molecule irreversible pan-HER inhibitor, with camrelizumab, an anti-PD-1 antibody, and chemotherapy as first-line treatment for advanced HER2-positive gastric cancer. The recommended phase 2 dose of pyrotinib was set at 320 mg. We observed a high objective response rate and long survival outcomes with an acceptable safety profile.

Implications of all the available evidence

The combination of pyrotinib, camrelizumab and chemotherapy showed promising efficacy with manageable safety profile as first-line treatment for advanced HER2-positive gastric cancer.

Introduction

Gastric cancer is one of the most common malignancies in the world. There are an estimated one million new cases and eight hundred thousand related deaths. It is estimated that gastric cancer accounts for 5.6% of all cancer incidences and 7.7% of all cancer-related deaths worldwide.[1] Compared to other regions, Asia has a greater disease burden due to the higher number of new cases and deaths.[2] Approximately half of gastric cancer patients are diagnosed with advanced disease, resulting in a poor prognosis.[3,4]

Approximately 17–20% of patients with gastric cancer have HER2 positivity, which includes overexpression of HER2 protein and amplification of the HER2 oncogene.[3,4] In the TOGA trial, trastuzumab was added to first-line chemotherapy (cisplatin plus fluorouracil or capecitabine) in patients with HER-2-positive advanced gastric cancer. The median overall survival (OS) was significantly improved from 11 to 13.8 months, and the median progression-free survival (PFS) was significantly improved from 5.5 to 6.7 months.[5] Therefore, trastuzumab combined with chemotherapy has been recommended as a standard first-line treatment for HER-2 positive patients with advanced gastric cancer. However, the survival benefit was still not satisfactory.

In preclinical studies, synergistic antitumor activity was observed when targeting both PD-1 and HER2, and an increase in IFN-γ-producing effector T cells and a decrease in Treg cells were observed.[6,7] In a study of human gastric cancer organoids, knockdown of HER2 reduced expression of PD-L1, increased proliferation of cytotoxic T lymphocytes, and sensitized gastric carcinoids to PD-1/PD-L1 inhibitors.[8] Currently, the crosstalk between HER2 and PD-L1 pathways has not been clearly elucidated.

A single-arm, phase 2 trial demonstrated that pembrolizumab in combination with trastuzumab and chemotherapy was safe as the first-line treatment for advanced HER2-positive gastric cancer. 32 of 35 patients (91%) had an objective response, 70% were progression-free at 6 months, and 80% survived at 12 months.[9] After that, pembrolizumab versus placebo combined with trastuzumab and chemotherapy was evaluated in the phase 3 KEYNOTE-811 study. The first interim analysis was performed on 133 patients in the pembrolizumab group and 131 in the placebo group. The objective response rate (ORR) was 74.4% in the pembrolizumab group and 51.9% in the placebo group. The incidence of adverse events was similar in the two groups.[10] Based on the KEYNOTE-811 trial findings, the Food and Drug

Administration granted accelerated approval to the combination therapy for this indication.

Pyrotinib is a small molecule irreversible pan-ErbB tyrosine kinase inhibitor that inhibits EGFR/HER1, HER2, and HER4. The phase 3 PHOEBE trial showed that pyrotinib combined with capecitabine resulted in a significant improvement in PFS compared with lapatinib plus capecitabine in the second-line treatment of HER2-positive advanced breast cancer.[11] Furthermore, pyrotinib showed antitumor activity in gastric cancer in small studies.[12,13] A phase 2 study of camrelizumab in combination with chemotherapy showed promising results in the first-line treatment of advanced gastric cancer.[14] Camrelizumab combined with trastuzumab and chemotherapy led to a higher ORR than trastuzumab plus chemotherapy for HER2-positive advanced gastric cancer (75% versus 46.2% p = 0.032), according to a retrospective study.[15] Based on the above evidence, we conducted a phase 1 dose-escalation and expansion study of camrelizumab in combination with pyrotinib and chemotherapy as first-line treatment in patients with HER2-positive locally advanced unresectable or metastatic gastric and gastroesophageal junction adenocarcinoma. Furthermore, in the PHENIX study and the PHOEBE study, the incidence of all grades diarrhea was about 95%, and the incidence of grade ≥3 diarrhea was about 30%.[16,11] Therefore, diarrhea caused by pyrotinib was a major concern. For this reason, patients in this study received preventive diarrhea management.

Methods

Study design and participants

This was a multicenter single-arm, open-label phase 1 dose-escalation (1a) and expansion (1b) study of camrelizumab in combination with CapeOX and pyrotinib in patients with previously untreated HER2-positive locally advanced unresectable or metastatic gastric and gastroesophageal junction adenocarcinoma. The study protocol and all its amendments were reviewed and approved by Jiangsu Cancer Hospital (2020-014). All patients provided written informed consent for participation before enrollment.

Eligible patients were aged ≥18 years old with histologically or cytologically confirmed previously untreated HER2-positive locally advanced unresectable or metastatic gastric and gastroesophageal junction adenocarcinoma. Other inclusion criteria included measurable lesions according to the Response Evaluation Criteria for Solid Tumors (RECIST) version 1.1, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, a life expectancy of at least three months, adequate organ function (hematological: absolute neutrophil count ≥1.5 × 10^9 cells/L, platelets ≥90 × 10^9/L, hemoglobin concentration ≥90 g/L; hepatic: total bilirubin concentration ≤1 × upper limit of normal (ULN), alanine aminotransferase [ALT] concentration and aspartate aminotransferase [AST] concentration ≤1.5 × ULN [if there was liver metastasis, ALT and AST concentration ≤5 × ULN], alkaline phosphatase concentration ≤2.5 × ULN; renal: blood urea nitrogen concentration and creatinine concentration ≤1.5 × ULN and creatinine clearance rate ≥50 mL/min; cardiac: left ventricular ejection fraction ≥50%, Fridericia-corrected QT interval (QTcF) <450 ms in men and <470 ms in women; coagulation: international normalized ratio ≤1.5 × ULN, partially activated plasminogen time ≤1.5 × ULN). HER2-positivity was defined as immunohistochemistry (IHC) 3^+ or $IHC2^+$ and in situ hybridization $(ISH)^+$ (or fluorescence in situ hybridization $[FISH]^+$) assessed by a central laboratory. Patients who had received previous anti-HER2 therapy or anti-PD-1/PD-L1 therapy were excluded. Other key exclusion criteria included hypersensitivity to therapeutic drugs, or autoimmune disease, or uncontrolled symptomatic brain metastases.

Procedures

In phase 1a, a traditional 3 + 3 dose-escalation design was used. Patients received camrelizumab 200 mg intravenously on day 1 every 3 weeks, CapeOX regimen (oxaliplatin 130 mg/m^2 intravenously on day 1, capecitabine 1000 mg/m^2 orally during days 1–14) every 3 weeks, and pyrotinib orally once daily (240 mg or 320 mg or 400 mg) every 3 weeks. If ≤1 patient in each dose group experienced dose-limiting toxicity (DLT), the dose of pyrotinib could be increased. If ≥2 patients experienced DLT, the previous dose was defined as the maximum tolerated dose (MTD). DLT was defined as any grade 4 hematological toxicity or any ≥ grade 3 non-hematological toxicity occurring within the first 21 days of treatment, or any camrelizumab or pyrotinib toxicity that resulted in a delay in treatment of ≥21 days. The recommended phase 2 dose (RP2D) of pyrotinib was determined based on all available safety and efficacy data from phase 1a. Once established, the RP2D was used in the subsequent phase 1b, in which additional patients were enrolled and treated. Treatment continued until disease progression, intolerable toxicity, or consent withdrawal, while CapeOX was administered for up to 8 cycles. Furthermore, anti-diarrheal prophylaxis with loperamide was recommended during the first 2 cycles of pyrotinib (Supplementary Table S1).

Tumor response assessments were performed every 6 weeks (±7 days) for the first 4 months during treatment and every 9 weeks (±7 days) thereafter by investigators according to RECIST version 1.1. Adverse events were documented continuously throughout treatment and for 30 days after the last study dose and were graded according to the Common Terminology Criteria for Adverse Events (version 5.0). Survival was followed up every 2 months after treatment discontinuation until death or withdrawal of consent.

Outcomes

The primary endpoint was to determine the safety, MTD, RP2D, and confirmed ORR. Safety was descriptively summarized in terms of adverse events in the safety set (patients who received at least one dose of study treatment and had at least one post-baseline safety assessment). Confirmed ORR was defined as the percentage of patients with complete response or partial response, which was confirmed by two successive scans within a minimum interval of 4 weeks, in the response evaluable set (patients who received RP2D of pyrotinib, camrelizumab, and CapeOX and had at least one post-baseline tumor assessment). Secondary endpoints were disease control rate (DCR, defined as the percentage of patients with complete response, partial response, and stable disease), PFS (defined as the time from treatment initiation to the first documented progressive disease or death from any cause), and OS (defined as the time from treatment initiation until death from any cause). DCR was assessed in the response evaluable set. PFS and OS were assessed in the full analysis set (patients who received at least one dose of study treatment). Exploratory endpoints were associations between clinical outcomes and biomarkers.

DNA isolation and capture-based targeted DNA sequencing

Genomic DNA isolation and targeted sequencing were carried out in Burning Rock Biotech, a CLIA-certified commercial clinical laboratory, following optimized protocols as described previously.[17,18] In brief, tumor DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissues using a commercial kit (Qiagen) and cell-free DNA (cfDNA) was extracted from 4 to 5 mL of plasma samples using a circulating nucleic acid extraction kit (Qiagen) according to the manufacturer's instructions. DNA fragments between 200 and 400 bp from the sheared tissue and cfDNA samples were size-selected, hybridized to capture probes, selected with magnetic beads, and amplified.

Target capture of baseline samples utilized a commercial gene panel of 520 genes (OncoScreen Plus) covering 1.64 megabases of the genome. Target capture of follow-up samples employed a commercial gene panel of 168 genes (Lung Plasma) covering 0.273 megabases of the genome. The quality and size distribution of the fragments were evaluated using a bioanalyzer (Agilent Technologies). Barcoded libraries were sequenced on a next-generation sequencer (Illumina) with paired-end reads to an average depth of 1000× for tissue samples and 10,000× for liquid biopsy samples.

Sequence data analysis

Sequencing data were aligned to the human reference genome (hg19) using a sequence aligner (Burrows–Wheeler Aligner version 0.7.10).[19] Local realignment, PCR duplicate marking and variant calling were performed using a toolkit (Genome Analysis Tool Kit version 3.2) and variant caller (VarScan version 2.4.3).[20,21] Tumor and plasma samples were compared to matched white blood cell samples to identify somatic variants. Variants were filtered using VarScan, and loci with depth less than 100 were removed. Variants in plasma and tissue samples required at least 8 and 2 supporting reads for single nucleotide variants (SNVs) and insertion-deletion variants (indels), respectively. Variants with population frequency over 0.1% in public databases were excluded as single nucleotide polymorphisms (SNPs). Remaining variants were annotated using ANNOVAR (2016-02-01 release) and SnpEff version 3.6.[22,23] Structural variant (SV) analysis was performed using Factera version 1.4.3.[24]

Copy number variations (CNVs) were analyzed based on the depth of coverage of the capture regions. Coverage data were corrected for GC content and probe design bias. Average coverage of reference samples without CNVs was used to normalize coverage across samples. CNVs were called if the coverage of a gene region was statistically significantly different from the reference.[25] The limits of detection were 1.5-fold for deletions and 2.64-fold for amplifications. MSI status of tumor and plasma samples was determined using a previously published read count distribution-based method.[26,27]

Tumor mutation burden (TMB) calculation

TMB for each patient was calculated as the ratio of the total number of non-synonymous mutations detected to the total size of the coding region of the panel used, according to the following equation. The mutation count included non-synonymous SNVs and indels located within the coding region and ±2 bp upstream or downstream, excluding hotspot mutations, CNVs, SVs, and germline SNPs. Only mutations with an allelic fraction (AF) ≥2% for tumor samples and ≥0.2% for plasma samples were counted. For accurate TMB calculation, the maximum AF (MaxAF) should be ≥5% for tumor samples and ≥1% for plasma samples. The total coding region size used to estimate TMB was 1.003 Mb for the 520-gene OncoScreen Plus panel.

$$\mathrm{TMB} = \frac{\text{mutation count (except for CNVs, SVs, SNPs, and hot mutations)}}{1.003\ \mathrm{Mb}}$$

Statistics

The sample size for phase 1a was based on a 3 + 3 design. In phase 1b, the ORR of 43.7% from the trastuzumab plus chemotherapy arm of the ToGA study was considered as a historical control.[5] Assuming an improvement in ORR from 43.7% to 76%, 24 patients (including those who received RP2D of pyrotinib in phase 1a) would be required to achieve a power of 80% with a two-sided significance level of 0.05. Considering a 10% dropout rate, 27 patients need to be enrolled.

Patients baseline characteristics were summarized by descriptive statistics. The 95% confidence intervals (CIs) for ORR and DCR were calculated based on the Clopper–Pearson method. PFS and OS were estimated with the Kaplan–Meier method, and 95% CIs for the medians were calculated using the Brookmeyer–Crowley method. Categorical variables were compared using Fisher's exact test. Continuous variables were compared using the Mann–Whitney U test. The level of statistical significance was set at $p < 0.05$. Statistical analyses were performed with Power Analysis and Sample Size (PASS) version 15.

Both male and female patients were eligible. Sex data were extracted from the identity information provided by the patients.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Baseline patient characteristics

A total of 41 patients were enrolled between June 2020 and June 2021, and all patients received at least one cycle of pyrotinib combined with camrelizumab and CapeOX. The baseline characteristics of patients are summarized in Table 1. Twenty-three patients (56.1%) had the primary tumor located in the stomach and the others (43.9%) had the primary tumor located at the gastroesophageal junction. In addition, 75.6% of patients had a HER2 IHC score of 3^+. Ten patients were enrolled in phase 1a and 31 patients were enrolled in phase 1b. In phase 1a, six patients discontinued treatment due to disease progression (n = 2) and adverse events (n = 4). In phase 1b, two patients experienced disease progression, 13 patients experienced adverse events, and eight patients withdrew consent, resulting in 23 patients discontinuing treatment (Fig. 1).

MTD and RP2D

In phase 1a, three patients received 240 mg of pyrotinib, four patients received 320 mg of pyrotinib (one additional patient was included because one patient withdrew consent), and three patients received 400 mg of pyrotinib. No DLT was observed at the pyrotinib doses of 240 mg and 320 mg. However, at 400 mg, one patient experienced grade 3 diarrhea and vomiting, and two patients developed grade 3 diarrhea within the first 21 days of treatment. Therefore, the MTD was determined to be pyrotinib 320 mg once daily. Based on all available data, the RP2D of pyrotinib was set at 320 mg once daily.

Safety

A total of 41 patients were included in the safety set. At the cut-off date of July 8, 2022, the median follow-up time was 7.9 months (95% CI: 6.0–11.4). Among 41 patients, 97.6% of patients developed any grade treatment-emergent adverse events (TEAEs), and 48.8% of patients developed grade ≥3 TEAEs. The most common TEAEs of any grade were diarrhea (39 [95.1%]), vomiting (22 [53.7%]), decreased appetite (19 [46.3%]), and anemia (18 [43.9%]). Diarrhea (7 [17.1%]) was the most common grade ≥3 TEAEs, with other grade ≥3 TEAEs occurring in less than 10% of patients. Four patients (9.8%) had any grade serious adverse events. In addition, due to TEAEs, 23 patients (56.1%) experienced dose reductions, 15 patients (36.6%) experienced dose interruptions, and 13 patients (31.7%) discontinued treatment. In 35 patients who received RP2D of pyrotinib, 14 patients (40%) developed grade ≥3 TEAEs, and two (5.7%) developed serious adverse events of any grade (Table 2). No deaths occurred due to TEAEs.

Efficacy

Among 35 patients who received RP2D of pyrotinib, 27 had post-baseline tumor assessments available and were included in the response evaluable set. The remaining eight patients did not have post-baseline assessments for the following reasons: surgery (n = 1), withdrawal due to adverse events (grade 2 diarrhea [n = 1], grade 2 vomiting [n = 1], grade 3 vomiting [n = 1]), and consent withdrawal (n = 4). In the response evaluable set, the ORR was 92.6% (95% CI: 75.7–99.1), with three patients (11.1%) achieving a complete response and 22 (81.5%) achieving a partial response. In addition, two (7.4%) patients had stable disease with a DCR of 100% (95% CI: 87.2–100). Two patients (7.4%) had a confirmed complete response, and 19 (70.4%) had a confirmed partial response; the confirmed ORR was 77.8% (95% CI: 57.7–91.4). The confirmed ORR for patients with gastric adenocarcinoma was 80.0% (16/20, 95% CI: 57.9–93.4), while the confirmed ORR for patients with gastroesophageal junction adenocarcinoma was 69.2% (9/13, 95% CI: 38.7–90.9). Furthermore, the full analysis set included 41 patients who received at least one dose of study treatment. In the full analysis set, the confirmed ORR was 61.0% (25/41, 95% CI: 44.5–75.8).

The best overall responses in target lesions for 33 patients who had post-baseline tumor assessments and received three doses of pyrotinib are shown in Fig. 2A. All patients had a decrease in tumor size from baseline.

Characteristic	All (n = 41)	Pyrotinib 320 mg/d (n = 35)
Age, years, median (IQR)	67 (60–71)	66 (59–71)
Sex, n (%)		
Male	27 (65.8)	23 (65.7)
Female	14 (34.2)	12 (34.3)
ECOG performance status, n (%)		
0	18 (43.9)	16 (45.7)
1	23 (56.1)	19 (54.3)
Differentiation, n (%)		
Well differentiated	1 (2.4)	0
Moderately differentiated	12 (29.3)	11 (31.4)
Moderately-poorly differentiated	13 (31.7)	10 (28.6)
Poorly differentiated	6 (14.6)	6 (17.1)
Unknown	9 (22.0)	8 (22.9)
Primary site, n (%)		
Gastric	23 (56.1)	21 (60.0)
Gastroesophageal junction	18 (43.9)	14 (40.0)
Number of metastatic sites, n (%)		
1	9 (22.0)	6 (17.1)
2	26 (63.4)	24 (68.6)
3	6 (14.6)	5 (14.3)
Metastatic sites, n (%)		
Lung	4 (9.8)	4 (11.4)
Liver	27 (65.9)	24 (68.6)
Lymph node	37 (90.2)	31 (88.6)
Other	10 (24.4)	9 (25.7)
Prior surgery, n (%)		
No	29 (70.7)	27 (77.1)
Yes	12 (29.3)	8 (22.9)
HER2 status, n (%)		
$IHC3^+$/FISH no result	31 (75.6)	26 (74.3)
$IHC2^+$/$FISH^+$	10 (24.4)	9 (25.7)

IQR: interquartile range; ECOG: Eastern Cooperative Oncology Group; IHC: immunohistochemistry; FISH: fluorescence in situ hybridization.

Table 1: **Demographic and disease characteristics at baseline.**

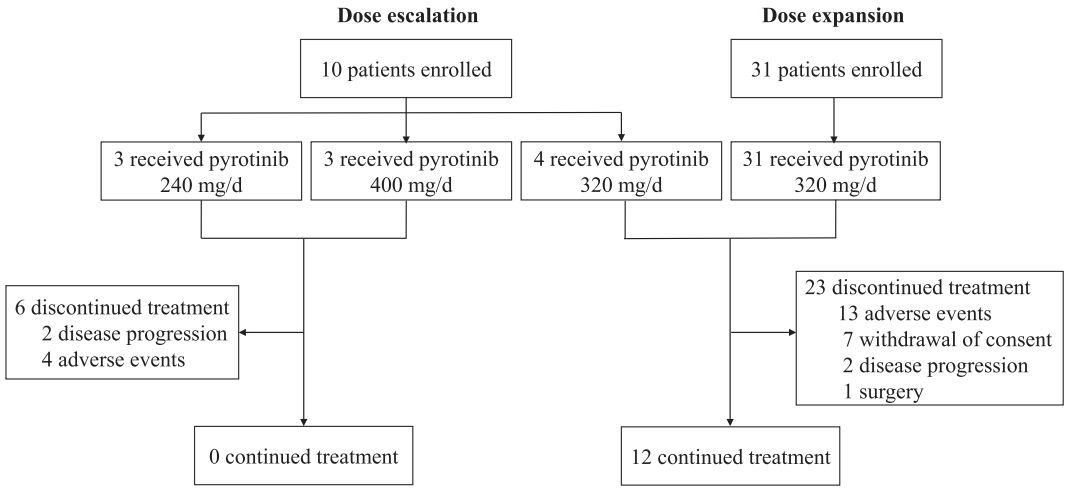

Fig. 1: Trial profile.

TEAEs, n (%)	All (n = 41)		Pyrotinib 320 mg/d (n = 35)	
	Any grade	Grade ≥3	Any grade	Grade ≥3
Diarrhea	39 (95.1)	7 (17.1)	33 (94.3)	3 (8.6)
Vomiting	22 (53.7)	3 (7.3)	19 (54.3)	2 (5.7)
Decreased appetite	19 (46.3)	1 (2.4)	16 (45.7)	0
Anemia	18 (43.9)	1 (2.4)	15 (42.9)	1 (2.9)
Decreased neutrophil count	16 (39.0)	3 (7.3)	11 (31.4)	0
Decreased platelet count	16 (39.0)	1 (2.4)	12 (34.3)	1 (2.9)
Nausea	16 (39.0)	0	14 (40.0)	0
Aspartate aminotransferase increased	16 (39.0)	4 (9.8)	14 (40.0)	3 (8.6)
Decreased white blood cell count	15 (36.6)	0	10 (28.6)	0
Reactive cutaneous capillary endothelial proliferation	15 (36.6)	2 (4.9)	12 (34.3)	1 (2.9)
Asthenia	15 (36.6)	0	12 (34.3)	0
Alanine aminotransferase increased	14 (34.1)	4 (9.8)	11 (31.4)	3 (8.6)
Gamma-glutamyltransferase increased	13 (31.7)	2 (4.9)	11 (31.4)	1 (2.9)
Hypokalemia	13 (31.7)	6 (14.6)	11 (31.4)	5 (14.3)
Hyperuricemia	10 (24.4)	0	9 (25.7)	0
Alkaline phosphatase increased	10 (24.4)	0	8 (22.9)	0
Hypoalbuminemia	10 (24.4)	1 (2.4)	9 (25.7)	1 (2.9)
Hyperbilirubinemia	9 (22.0)	2 (4.9)	8 (22.9)	1 (2.9)
Hypocalcemia	8 (19.5)	1 (2.4)	6 (17.1)	1 (2.9)
Hyponatraemia	8 (19.5)	1 (2.4)	8 (22.9)	1 (2.9)
Peripheral sensory neuropathy	6 (14.6)	0	6 (17.1)	0
Blood creatinine increased	6 (14.6)	1 (2.4)	6 (17.1)	1 (2.9)
Constipation	4 (9.8)	0	4 (11.4)	0
Oral mucositis	3 (7.3)	0	3 (8.6)	0
Abdominal distention	3 (7.3)	0	3 (8.6)	0
Dizziness	3 (7.3)	0	3 (8.6)	0

	All (n = 41)	Pyrotinib 240 mg/d (n = 3)	Pyrotinib 320 mg/d (n = 35)	Pyrotinib 400 mg/d (n = 3)
Any TEAEs	40 (97.6)	3 (100.0)	34 (97.1)	3 (100.0)
Grade ≥3 TEAEs	20 (48.8)	3 (100.0)	14 (40.0)	3 (100.0)
TEAEs leading to dose interruption	15 (36.6)	2 (66.7)	12 (34.3)	1 (33.3)
TEAEs leading to dose reduction	23 (56.1)	0	20 (57.1)	3 (100.0)
TEAEs leading to discontinuation	13 (31.7)	2 (66.7)	9 (25.7)	2 (66.7)

Table 2: **Treatment-emergent adverse events (TEAEs) occurring in ≥5% of treated patients.**

Five patients treated with 240 mg dose (n = 2) and 400 mg (n = 3) dose of pyrotinib achieved a partial response, and one patient treated with 240 mg dose achieved a complete response. Most of patients responded to treatment within 2 months, and the median treatment duration was 4.3 months (95% CI: 3.0–7.6). Among 25 patients who responded to 320 mg dose of pyrotinib, 12 remained on treatment after 6 months, and seven had a duration of response longer than 6 months (Fig. 2B).

In the full analysis set, seven PFS events were reported and the median PFS was not reached. PFS at 6 and 12 months were 81.1% and 67.0%, respectively. Five deaths occurred with the median OS of 22.1 months (95% CI: 2.2–42.1). OS at 6 and 12 months were 94.3% and 86.4%, respectively (Fig. 3). In patients who received RP2D of pyrotinib, at 6 and 12 months, PFS was 91.8% and 84.2%, respectively; OS was 96.6% and 91.5% (Supplementary Figure S1).

Biomarker

Baseline tissue samples from 12 patients were subjected to second-generation sequencing of the 520-gene panel. Among them, one patient had a complete response, nine were partial response, and two were stable disease. The median TMB was 6.98 mut/Mb, and two patients (16.7%) had a TMB greater than 10. Gene analysis revealed that TP53 (11/12, 92%) and ERBB2 (7/12, 58%) had the highest mutation frequencies in the tissue samples (Supplementary Figure 2A). Patients with complete response and partial response were classified into the responder group (n = 10), while patients with stable disease were classified into the non-responder group (n = 2). Among the ten responders, seven had ERBB2 mutations (7/10, 70%), while two non-responders were ERBB2 wild-type (2/2, 100%, nominal p = 0.045, Fig. 4A). Notably, all seven patients had ERBB2 copy number amplification. Moreover, one patient also had a large ERBB2 genomic rearrangement

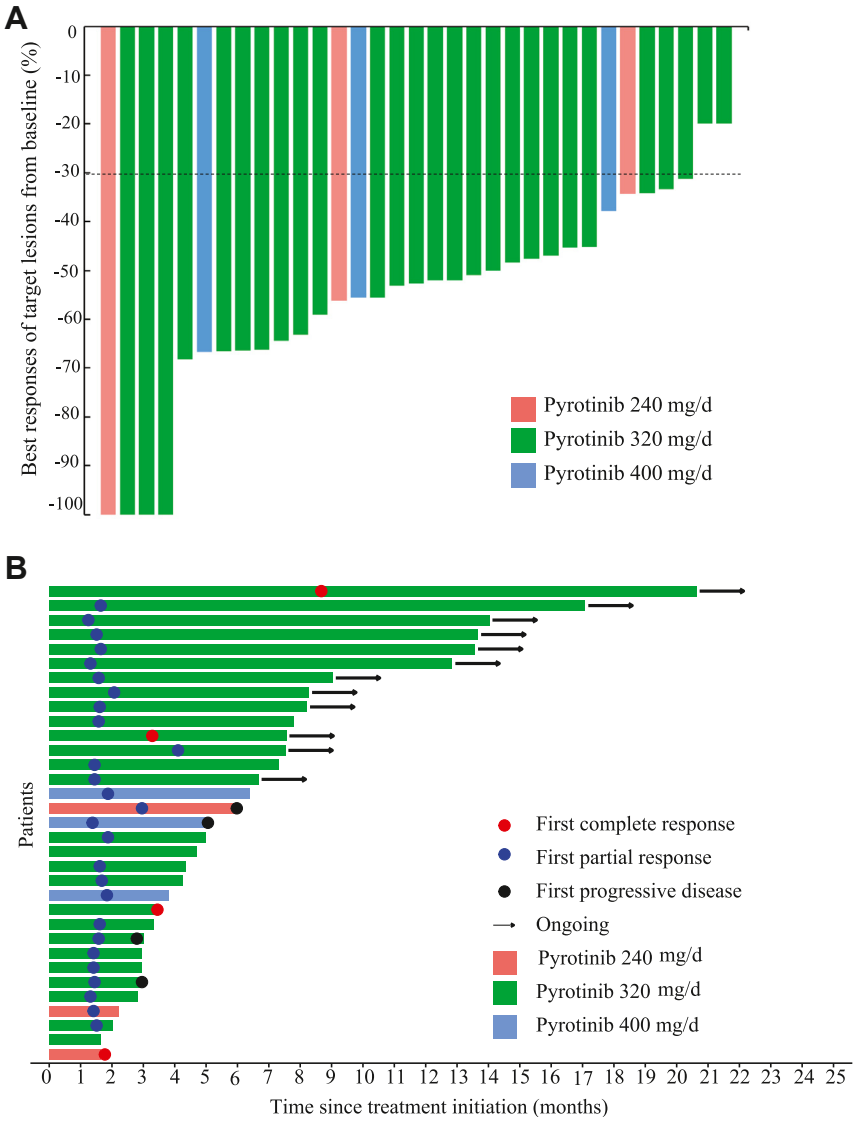

Fig. 2: Best change from baseline in sum of diameters in target lesions per patient (A) and Duration of treatment per patient (B). Each bar in (A) and each horizontal line in (B) represents a patient.

(exon1-8del), and another patient also had an MED24-ERBB2 gene fusion. These results suggest a potential association between ERBB2 mutation and the efficacy of camrelizumab in combination with pyrotinib and chemotherapy.

Blood samples were collected from 14 patients at baseline and after two cycles of combination therapy for the evaluation of circulating tumor DNA (ctDNA) using the 168-gene panel, including one patient with complete response, 12 with partial response, and one with stable disease. Among 28 samples, TP53 (17/28, 57%) and ERBB2 (12/28, 43%) had the highest mutation frequencies (Supplementary Figure S2B). Among the 13 responders, there was a significant reduction in the detection rates of ERBB2 and TP53 gene mutations. The number of patients with ERBB2 gene mutations decreased from ten (10/13, 76.9%) at baseline to two (2/13, 15.4%) after treatment (nominal $p = 0.005$), while the number of patients with TP53 gene mutations decreased from 12 (12/13, 92.3%) at baseline to two (2/13, 15.4%) after treatment (nominal $p < 0.001$, Fig. 4B and C). All ten patients with ERBB2 mutations at baseline had ERBB2 copy number amplification. In addition, three patients had other ERBB2 alterations: one patient had a frameshift mutation (p.C220fs) and a missense mutation (p.A890E); another patient had an ERBB2-MSL1 gene fusion; and the third patient had two ERBB2 gene fusions with IKZF3 and JUP. Furthermore, all 14 patients tested

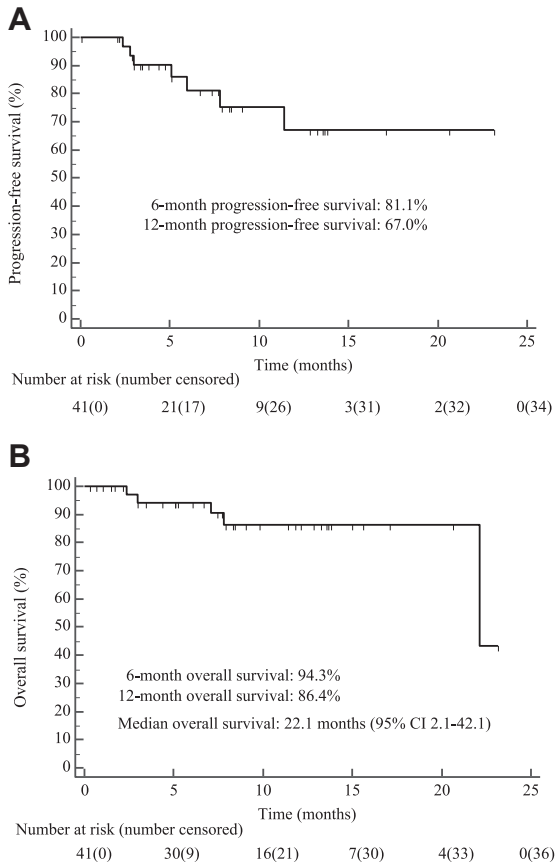

Fig. 3: Progression-free survival (A) and overall survival (B) in the full analysis set.

positive for ctDNA at baseline. However, after treatment, six patients (42.9%), all with partial response, tested negative for ctDNA. Among the remaining seven responders, both the mean variant allele frequency and maximum variant allele frequency showed a significant decrease after treatment (both nominal p = 0.031, Supplementary Figure S3).

Discussion

This is the first study reporting camrelizumab in combination with small molecular pyrotinib and chemotherapy as first-line treatment for advanced HER2-positive gastric and gastroesophageal junction adenocarcinoma. Based on the benefit-risk ratio for patients, the RP2D of pyrotinib was set at 320 mg once daily when combined with camrelizumab and chemotherapy. The combination therapy showed encouraging efficacy and no new safety signals were identified.

Pembrolizumab in combination with trastuzumab and chemotherapy has been approved by the Food and Drug Administration for the first-line treatment of HER2-positive advanced gastric cancer. This regimen has shown efficacy in phase 1–3 studies.[9,10,28] In phase 1, the ORR was 76.7%, the median PFS was 8.6 months, and the median OS was 19.3 months.[28] In phase 2, the ORR was 91%, the median PFS was 13 months, and the median OS was 27.3 months.[9] In phase 3 (first interim analysis), the ORR was 74.4%, which was significantly higher than that of trastuzumab and chemotherapy (51.9%).[10] In contrast, this study showed that camrelizumab combined with chemotherapy and pyrotinib results in comparable efficacy to the standard pembrolizumab, trastuzumab, and chemotherapy regimen. Camrelizumab combined with chemotherapy and 320 mg of pyrotinib reported an ORR of 92.6% and a confirmed ORR of 77.8%. The median PFS was not reached, and the median OS was 22.1 months.

Although both regimens showed comparable efficacy, pyrotinib and trastuzumab had different mechanisms of action, which may affect their clinical application. Trastuzumab is a recombinant humanized IgG1 monoclonal antibody that inhibits HER2 by binding to its extracellular domain. Trastuzumab resistance mechanisms may involve HER2 mutations or heterodimerization with other receptors, such as HER1-HER2 or HER2-HER3.[29] For example, ERBB2/4 mutations were associated with rapid progression under trastuzumab therapy in HER2-positive metastatic gastric cancer.[30] On the other hand, pyrotinib is an oral, irreversible pan-ErbB inhibitor that covalently binds to the ATP binding sites within the intracellular kinase regions of EGFR/HER1, HER2, and HER4. This suggests that pyrotinib may overcome trastuzumab resistance by targeting multiple ErbB receptors and their mutations. In patients with HER2-positive metastatic breast cancer who had previously received trastuzumab and taxanes or had primary trastuzumab resistance, pyrotinib plus capecitabine resulted in ORR of 67% and 70%, respectively.[11,31] In conclusion, pyrotinib combined with camrelizumab and chemotherapy may be effective in patients with HER2-positive advanced gastric cancer who are intolerant or resistant to trastuzumab.

In terms of safety, this study was consistent with previous studies of pyrotinib in advanced gastric cancer and camrelizumab plus chemotherapy in advanced gastric cancer.[12–14] No new safety signals were identified, with 97.6% of patients experiencing all grades TEAEs and 48.8% experiencing grade ≥3 TEAEs. The incidences of adverse events were similar to those reported in phase 2 and 3 studies of pembrolizumab plus trastuzumab and chemotherapy.[9,10]

Diarrhea was the most common adverse event in this study, occurring in 95.1% of patients with all grades and in 17.1% of patients with grade ≥3. These incidences of diarrhea were higher than those observed in phase 2 and 3 trials of pembrolizumab plus trastuzumab and chemotherapy (73% for all grades and 0% for grade ≥3 in phase 2 trial; 52.5% for all grades and 7.4% for grade ≥3 in phase 3 trial).[9,10] However, the incidences were

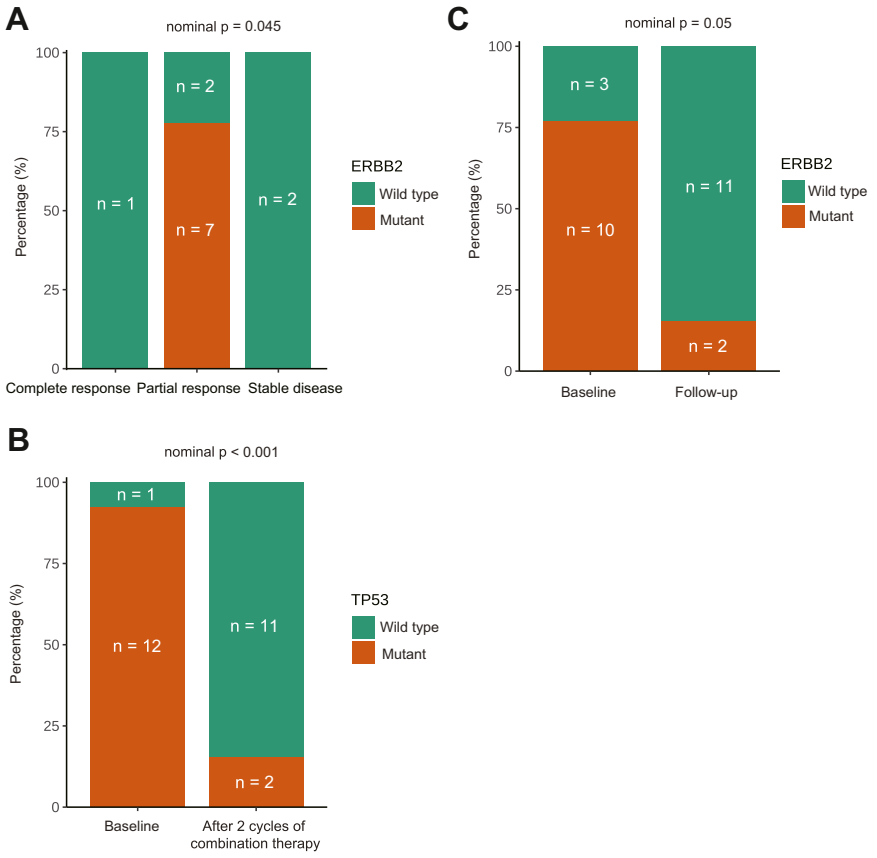

Fig. 4: (A) Comparison of treatment response in patients with wild-type ERBB2 versus those with ERBB2 mutations in 12 tissue samples at baseline. (B) Comparison of the percentage of plasma ERBB2 wild-type and mutant status and (C) TP53 wild-type and mutant status at baseline (n = 13) versus after 2 cycles of pyrotinib combined with camrelizumab and chemotherapy (n = 13). p values were calculated using Fisher's exact test.

consistent with other phase 3 trials of lapatinib plus chemotherapy in HER2-positive advanced gastric adenocarcinoma (58% for all grades and 12% for grade ≥3 in the TRIO-013/LOGiC trial; 77% for all grades and 18% for grade ≥3 in the TyTAN trial).[32,33] Diarrhea was related to chemotherapy and pyrotinib in this study. In the ToGA trial and the KEYNOTE-811 trials, the chemotherapy group observed diarrhea; the incidence of all grades diarrhea was 28% and 44.45%, with grade ≥3 at 4% and 8.3%.[5,10] Additionally, pyrotinib plus capecitabine group had the highest incidence of treatment-related grade ≥3 diarrhea (30.8%), compared to pyrotinib monotherapy group (22.5%) and placebo plus capecitabine group (12.8%) in the phase 3 PHENIX study.[16] Based on these data, we hypothesized that the grade ≥3 diarrhea attributable to chemotherapy was lower than that caused by pyrotinib. HER2 and EGFR are expressed on intestinal epithelial cell membranes and regulate chloride secretion through the PI3K and PKC pathways. Excessive chloride secretion can lead to secretory diarrhea observed with EGFR tyrosine kinase inhibitors.[34,35] However, the mechanism of pyrotinib-induced diarrhea remains unclear, with only one study suggesting a potential role for gut microbiome imbalance and related metabolite changes.[36] Regardless of the underlying mechanisms, close monitoring and management of diarrhea are essential during pyrotinib treatment.

This study also explored potential predictive biomarkers for response to combination therapy. We performed second-generation sequencing on 12 baseline tissue samples and 14 baseline blood samples, and detected ERBB2 mutations in seven tissue samples and ten blood samples. All these samples had ERBB2 copy number amplification, and most had an IHC score of 3^{+}. Previous studies showed that patients with ERBB2 amplification had longer PFS and OS than those without ERBB2 amplification when treated with first-line pembrolizumab, trastuzumab, and chemotherapy.[9,28] Consistent with these findings, we found an association between ERBB2 mutation and treatment response in tissue samples; we also observed a decrease in the

ERBB2 mutation percentage in the blood of responding patients after treatment. In addition to ERBB2 mutation, we found that responders had a higher prevalence of TP53 mutation than non-responders. Moreover, we observed a decrease in TP53 mutation in the blood of responding patients after treatment. TP53 mutation was reported to be positively correlated with PD-L1 expression and associated with shorter PFS in immune checkpoint inhibitor treated patients with gastric and esophageal adenocarcinomas.[37] This study suggests that TP53 mutation may be an initial predictor of efficacy of camrelizumab plus chemotherapy and pyrotinib.

This study had several limitations that should be considered. First, this study had a phase 1 study design and a small sample size. Second, this study lacked pharmacokinetic and pharmacodynamic analyses, which impeded the understanding the combination's pharmacological properties. Third, biomarker analyses were conducted on a limited number of patients. Among the patients included in the biomarker analyses, all non-responders were in the stable disease status due to the high ORR in the study. Therefore, the results of this study in terms of clinical outcomes and biomarker analyses should be interpreted with caution. Further research is needed to validate these findings. Fourth, the follow-up period in this study was limited, preventing a comprehensive assessment of the treatment's long-term efficacy and safety.

In conclusion, this study showed promising results with the first-line treatment of camrelizumab combined with chemotherapy and pyrotinib in patients with HER2-positive advanced gastric cancer. The regimen achieved a high ORR and a long OS. Most adverse events were mild or moderate in severity; diarrhea was the most common one. Diarrhea should be closely monitored and managed during treatment. However, further clinical trials are needed to confirm the efficacy and safety of this regimen in a large population.

Contributors

LZ, SL, JB, and XL contributed to the design of the study. LZ, SL, XL, QY, JX, SC, GF, CG, LF, BL, MM, XN, GW, LY, JY, RZ, and HC contributed to data collection. LZ and SL contributed to data analysis.

LZ, SL, XL, QY, and JX contributed to data interpretation. CG and LF contributed to the literature search.

LZ and SL contributed to the supervision of the study. JX, SC, GF, and CG provided resources necessary for the study. LF, BL, and MM contributed to the preparation of the figures. LZ, JX, SC, GF, CG, LF, BL, MM, XN, GW, LY, and WS contributed to the writing of the manuscript. All authors reviewed and approved the final version of the manuscript. LZ and SL accessed and verified the underlying data in the study.

Data sharing statement

Data are available from the corresponding author on reasonable request.

Declaration of interests

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102314.

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