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ORIGINAL ARTICLE

Dual targeted therapy with pyrotinib and trastuzumab for HER2-positive advanced colorectal cancer: A phase 2 trial

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Abstract

This trial was initiated to evaluate the efficacy and safety of pyrotinib in combination with trastuzumab in patients with human epidermal growth factor receptor 2 (HER2)-positive recurrent/metastatic colorectal cancer (CRC). In this single-arm, open-label, multicenter, phase 2 trial patients with HER2-positive recurrent/metastatic CRC were enrolled and received oral pyrotinib 400 mg once a day plus intravenous trastuzumab 8 mg/kg loading dose followed by 6 mg/kg once every 3 weeks. The primary endpoint was the objective response rate (ORR). Disease control rate (DCR), progression-free survival (PFS), duration of response, and safety were assessed as secondary endpoints. From December 2019 to October 2021, a total of 20 patients were enrolled and 18 of them were evaluable for response. All patients were B-rapidly accelerated fibrosarcoma (*BRAF*) wild type. Four patients achieved partial response, with an ORR of 22.2% (4/18, 95% confidence interval [CI] 6.4–47.6) and DCR of 61.1% (11/18, 95% CI 35.8–82.7), while the ORR and DCR were 33.3% (4/12, 95% CI 13.8–60.9) and 83.3% (10/12, 95% CI 51.6–97.9), respectively, in *RAS* wild-type patients. At the time of cut-off day, median follow-up was 10.7 months (range 3.8–13.8). The median PFS was 3.4 months (95% CI 1.8–4.3) in the overall population and 4.3 months (95% CI 3.2–8.5) in the *RAS* wild-type group. The most common adverse event of grade ≥3

Abbreviations: CI, confidence interval; CISH, chromogenic ISH; CRC, colorectal cancer; CTCAE, Common Terminology Criteria for Adverse Events; DCR, disease control rate; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; FISH, fluorescent ISH; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; ITT, intention-to-treat; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RAS, rat sarcoma; RECIST, response criteria evaluation in solid tumors.

was diarrhea (13/20, 65.0%). Pyrotinib combined with trastuzumab showed promising antitumor activity and a manageable safety profile in patients with *RAS/BRAF* wild-type HER2-positive advanced CRC.

KEYWORDS
colorectal cancer, human epidermal growth factor receptor 2, pyrotinib, RAS mutation, trastuzumab

1 | INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) is a member of the HER/ERBB family that can be activated through gene amplification and gene mutation to trigger a cascade of subcellular signal transduction pathways that control epithelial cell growth, differentiation, and motility.[1] *HER2* amplification and/or overexpression have been found in multiple tumor types, including breast cancer, gastric cancer, colon cancer, ovarian cancer, and lung cancer,[2] and especially HER2 is an important therapeutic target for breast cancer and gastric cancer.[3,4] Contrary to breast cancer and gastric cancer where HER2-positive disease accounts for about 20% and 16%, respectively,[5] only about 5% of colorectal cancer (CRC) patients harbor HER2 alterations.[6] While HER2 alterations appeared more frequent in *RAS/BRAF* wild-type CRC with the incidence rate reported to be 5% to 14%, usually in the left colon or rectum.[7]

The recurrence rate and survival of HER2-positive CRC are similar to those of CRC as a whole. There is no evidence that HER2 alterations contribute prognostic value in CRC. Remarkably, progression-free survival (PFS) with cetuximab is shorter in *HER2*-amplificated patients, averaging less than 3 months, which is only a third of that for *HER2* non-amplificated patients in *RAS/RAF* wild-type CRC, suggesting that HER2 may be a predictive marker of epidermal growth factor receptor (EGFR)-targeted drug resistance.[8,9]

Due to the low incidence of HER2 alterations in CRC, studies regarding treatment of HER2-positive CRC have been limited. The phase II HERACLES clinical study[10] and the phase II basket MyPathway study[11] preliminarily proved the efficacy and safety of anti-HER2, chemotherapy-free therapy in HER2-positive advanced CRC, indicating HER2 as a potential therapeutic target for CRC. Thus, trastuzumab plus lapatinib or pertuzumab has been written into the National Comprehensive Cancer Network (NCCN) and Chinese Society of Clinical Oncology (CSCO) guidelines. The treatment of CRC has go one step further in the era of precision medicine. However, trials for anti-HER2 therapy in HER2-positive CRC are lacking in China, with no Chinese population involved in currently published global studies. As such, an unmet need has emerged in HER2-positive CRC patients in China.

Pyrotinib, a novel irreversible pan-HER tyrosine kinase inhibitor agent targeting HER2, EGFR, and HER4, was approved for breast cancer treatment in China in 2018.[12] The antitumor effect of pyrotinib also has been reflected in other solid tumors, such as lung cancer and gastric cancer.[13–16] In addition, pyrotinib combined with trastuzumab and docetaxel as neoadjuvant treatment presented clinical efficacy in HER2-positive early or local breast cancer with an acceptable manageable safety profile in a phase 3 trial.[17] This study was designed to explore the efficacy and safety of pyrotinib plus trastuzumab in patients with HER2-positive advanced CRC.

2 | PATIENTS AND METHODS

2.1 | Study design and patients

This study is an investigator-initiated, multicenter, open-label, single-arm, phase 2 trial recruiting patients from six hospitals and cancer institutes in China. Eligible patients had a histologically confirmed diagnosis of metastatic colorectal cancer with HER2 positivity established by immunohistochemistry (IHC) and in situ hybridization (ISH). To be HER2 eligible, the original tumor or the biopsied metastasis (whichever is most recent) must be IHC 3+ or IHC 2+ in more than 50% of cells, confirmed by chromogenic ISH (CISH) or fluorescent ISH (FISH) with a HER2:CEP17 ratio ≥2.0. For IHC, a positive staining (3+) is defined as an intense membrane staining which can be circumferential, basolateral, or lateral of the tumor cells. At least one measurable lesion, as defined by the Response Criteria Evaluation in Solid Tumors (RECIST) version 1.1, was required. Other major inclusion criteria were an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, age 18 years or older, progression on or intolerance to standard therapy, or refusing chemotherapy, and adequate hematological, renal, and hepatobiliary functions.

All patients provided written informed consent. The study was done in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization and Good Clinical Practice guidelines.

2.2 | Procedure

Trastuzumab was started intravenously at a loading dose of 8 mg/kg (day 1), followed by 6 mg/kg once per 3 weeks, and pyrotinib was given orally at 400 mg per day in 21-day treatment cycles. The dose of pyrotinib was permitted to be reduced to 240 mg per day

due to serious adverse events; patients who needed further dose reductions were withdrawn from the study. Treatment was continued until disease progression, adverse events requiring treatment cessation, withdrawal of consent, or investigator decision to terminate treatment. Tumor assessments were done at each center by local investigators within 4 weeks before the start of treatment and were repeated every 6 weeks in accordance with RECIST version 1.1. Safety was continuously assessed and graded with the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Laboratory testing (hematology, serum chemistry, urine analysis, and electrocardiograms) was done at baseline (days −28 to 0), at day 1 of every treatment cycle, and at the end of treatment. Left ventricular ejection fraction was assessed at baseline (days −28 to 0), every 6 weeks thereafter, and at the end of treatment.

Blood samples were taken at participating institutions and the samples were sent to the AcornMed Biotechnology Co., Ltd. for in vitro diagnosis at baseline.

In vitro testing to confirm *HER2* alteration and *RAS/BRAF* status in baseline include somatic gene mutation detection, microsatellite instability analysis, genetic tumor gene detection, and single nucleotide polymorphism detection, involving a total of 34 genes such as *KRAS*, *NRAS*, *HRAS*, *BRAF*, *ERBB2*, etc.

2.3 | Outcomes

The primary endpoint was objective response rate (ORR) based on investigator-assessed tumor responses per RECIST version 1.1, defined as the proportion of patients achieving a confirmed objective response consisting of complete or partial response to treatment as best response. Secondary endpoints were disease control rate (DCR), overall survival (OS), progression-free survival (PFS), duration of response, and safety.

2.4 | Statistical analysis

Assuming an ORR of 25%, 18 patients would provide a 95% confidence interval of [8%, 50.6%], the width of which is below 42.6%. Considering a dropout rate of 10%, a sample size of 20 is needed. Statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc). Continuous variables were summarized using medians and ranges, and categorical variables were described using frequency and percentage. The confidence intervals (CIs) of ORR, DCR, and PFS were established using the Clopper and Pearson method. PFS, OS, and DOR were estimated using the Kaplan–Meier method. Baseline and safety analyses were performed for all enrolled patients (intention-to-treat [ITT] population), and efficacy analyses were conducted for those who administered at least one dose of study treatment and was evaluated for efficacy (efficacy analysis population).

3 | RESULTS

3.1 | Patients

Between December 2019 and October 2021, a total of 20 patients with HER2 positive metastatic CRC were enrolled. At the time of the cut-off date on October 18, 2021, five patients remained on treatment, 11 patients had discontinued treatment due to progressive disease but remained in follow-up, two patients had withdrawn informed consent within 6 weeks, and two patients had died (Figure 1). Eighteen patients were evaluable for efficacy, including 12 patients with *RAS* wild-type status, five patients with *KRAS* mutations, and one patient with *NRAS* mutations. The demographic and patient characteristics of all included patients at baseline are summarized in Table 1. Most patients endured extensive metastatic disease

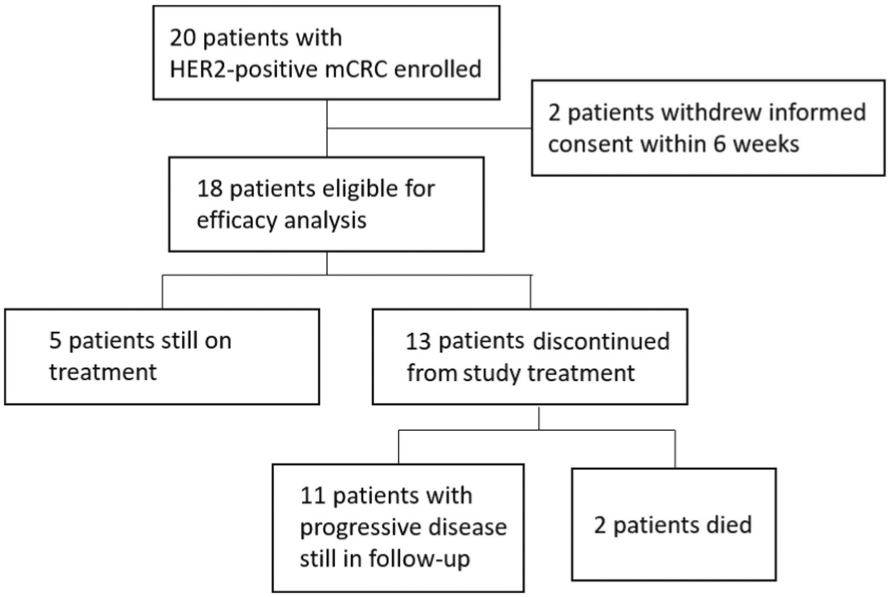

FIGURE 1 Trial profile. HER2, human epidermal growth factor receptor 2; mCRC, metastatic colorectal cancer.

TABLE 1 Baseline characteristics

	Patients given trastuzumab and pyrotinib (n = 20)
Ages (years), median (IQR)	59 (54.3–65.5)
Sex	
Male	12 (60.0%)
ECOG PS 0–1	19 (95.0%)
HER2 positivity	
Protein overexpression with no gene alteration	1 (5.0%)
Gene amplification and overexpression	14 (70.0%)
Gene mutation and overexpression	5 (25.0%)
HER2 expression by immunohistochemistry score	
IHC 3+	14 (70.0%)
IHC 2+, FISH+	4 (20.0%)
Unknown[a]	2 (10.0%)
RAS status	
Wild-type	14 (70.0%)
KRAS mutation	5 (25.0%)
NRAS mutation	1 (5.0%)
BRAF status	
Wild-type	100 (100.0%)
Site of primary tumor	
Rectum	6 (30.0%)
Colon	14 (70.0%)
Metastatic disease in multiple sites	16 (80.0%)
Number of previous lines of therapy, median (range)	2 (2–3)
Patients with ≥3 previous lines of therapy	9 (45.0%)
Previous anti-EGFR treatment	9 (45.0%)

Abbreviations: BRAF, B-rapidly accelerated fibrosarcoma; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; IQR, interquartile range; KRAS, Kirsten-RAS; NRAS, neuroblastoma-RAS; RAS, rat sarcoma.

[a]Two patients were detected *HER2* amplification by next-generation sequencing in a hospital pathology laboratory or a qualified gene testing institution.

(Table 1). Among all patients, 14 (70.0%) had *HER2* gene amplification, five (25.0%) had *HER2* mutation, and one (5.0%) did not have any *HER2* gene alteration. Patients were mostly pretreated: nine (45.0%) of 20 had received at least three previous regimens (median 2, range 2–3), including oxaliplatin, fluorouracil, irinotecan, bevacizumab, regorafenib, and EGFR-targeted antibodies. Wild-type BRAF was detected in all patients and the majority of patients (14, 70.0%) were *RAS* wild type.

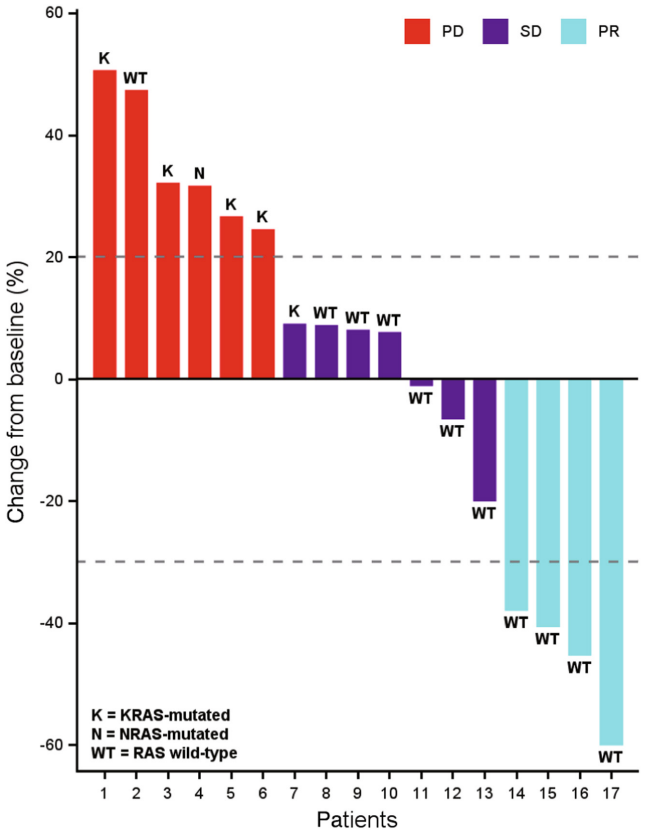

FIGURE 2 Change in tumor size of patients with HER2-positive metastatic colorectal cancer treated with pyrotinib + trastuzumab. One patient is excluded from this plot due to loss of detailed radiologic data as the computed tomography image was performed in a local hospital during the Covid-19 pandemic with an assessment of stable disease by the local doctor. RAS, rat sarcoma; KRAS, Kirsten-RAS; NRAS, neuroblastoma-RAS; K, KRAS-mutated; N, NRAS-mutated; PD, progressive disease; PR, partial response; SD, stable disease; WT, RAS wild type.

3.2 | Efficacy

Of the 18 evaluable patients, four (22.2%) achieved a confirmed partial response and seven (38.8%) had stable disease (Figure 2). The ORR and DCR were 22.2% (95% CI 6.4–47.6) and 61.1% (95% CI 35.8–82.7), respectively (Table 2). Seven (38.8%) patients developed clinically detected tumor progression at the first radiological assessment at the sixth week (Figure 3). One patient who lacked tumor diameter evaluation because the computed tomography imaging was performed in a local hospital during the Covid-19 pandemic, with clinical assessment of stable disease by the local doctor, was excluded from the waterfall plot but included in the calculation of DCR and survival analysis. There were 12 *RAS* wild-type patients, and all (33.3%) partial responses occurred in them. Overall, the ORR was 33.3% (95% CI 13.8–60.9) and the DCR was 83.3% (95% CI 51.6–97.9) of patients with wild-type *RAS/BRAF*. Three (75.0%) of four patients who achieved an objective response had tumors with *HER2* gene amplification and the rest had *HER2* mutation. At the time of

TABLE 2 Responses to treatment

	Patients given trastuzumab and pyrotinib ($n = 18$)	Patients with RAS wild type given trastuzumab and pyrotinib ($n = 12$)
Confirmed ORR by investigator, % (95% CI)	22.2 (6.4–47.6)	33.3 (13.8–60.9)
Complete response	0	0
Partial response	4 (22.2%)	4 (33.3%)
Stable disease	7 (38.8%)	6 (50.0%)
Disease control	11 (61.1%)	10 (83.3%)

Abbreviations: CI, confidence interval; ORR, objective response rate.

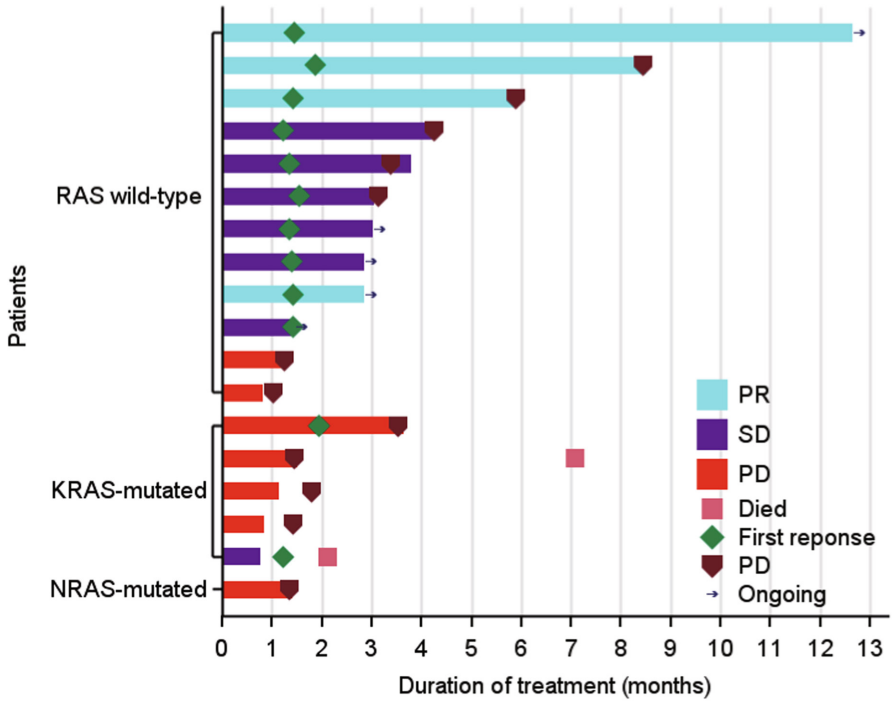

FIGURE 3 Duration of treatment of patients with HER2-positive metastatic colorectal cancer with pyrotinib plus trastuzumab. The red square symbol for PD represents that the treatment efficacy is evaluated as PD; while the brown symbol for PD refers to the time when the event of progressive disease happened. KRAS, Kirsten-RAS; NRAS, neuroblastoma-RAS; PD, progressive disease; PR, partial response; RAS, rat sarcoma; SD, stable disease.

the cut-off day, median follow-up was 10.7 (range 3.8–13.8) months. The median PFS was 3.4 (95% CI 1.8–4.3) months (Figure 4). The OS was not yet reached, with 16 (88.9%) patients alive at the cut-off date censored, and eight (44.4%) of 18 patients were alive at 1 year.

3.3 | Side effects

Table 3 shows treatment-related adverse events that occurred in at least 10.0% of patients or all that were CTCAE grade 3 or worse. The most common adverse event in the 20 enrolled patients was diarrhea (17, 85.0%), followed by fatigue (7, 35.0%), hand-foot syndrome (5, 25.0%), and anemia (5, 25.0%). No treatment-related grade 4 and 5 adverse events occurred. Fifteen (75.0%) patients had grade 3 adverse events: 13 patients had diarrhea, one patient had a urine output decreased, and one patient had anorexia. No treatment-related cardiotoxicity was observed. Treatment suspension or dose reduction of pyrotinib occurred in 13 (65.0%) patients, resulting from diarrhea in seven (35.0%) patients.

4 | DISCUSSION

Previous studies applying anti-HER2 therapy in HER2-positive CRC patients generally capitalized on dual-HER2 targeted therapies and focused on those who failed standard therapy or progressed after ≥2 prior regimens.[11,18–20] The phase II HERACLES clinical study exhibited 28% (9/32) ORR, 4.7 months (95% CI 3.7–6.1) median PFS and 10.0 months (95% CI, 7.9–15.8) median OS with trastuzumab plus lapatinib in patients with HER2-positive and *KRAS* exon 2 (codons 12 and 13) wild-type metastatic CRC.[20] MyPathway, a phase II basket study of trastuzumab plus pertuzumab in patients with HER2-positive metastatic CRC regardless of *RAS* mutation status, reported an ORR of up to 32% and also found that patients with the *KRAS* gene mutation had a shorter PFS (*KRAS* mutated:*KRAS* wild, 1.4 months:5.3 months) and OS (*KRAS* mutated:*KRAS* wild, 8.5 months:14.0 months).[11] Based on their achievements, the 2019 V2 edition of the NCCN guidelines recommended anti-HER2 therapy of trastuzumab combined with lapatinib or pertuzumab for HER2-positive colorectal cancer patients who failed standard treatment.[21]

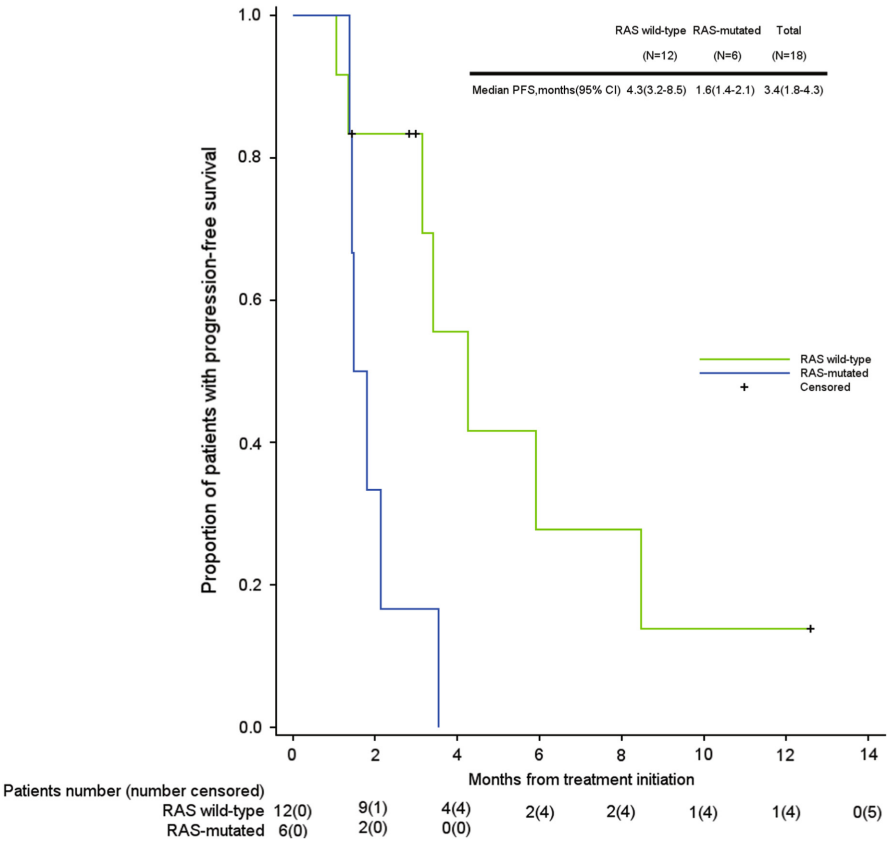

FIGURE 4 Progression-free survival by RAS mutation status. *RAS* mutated includes *KRAS* mutated and *NRAS* mutated. KRAS, Kirsten-RAS; NRAS, neuroblastoma-RAS; RAS, rat sarcoma.

TABLE 3 Adverse events in all enrolled HER2-positive metastatic CRC patients treated with pyrotinib plus trastuzumab

Adverse event	Grades 1–2	Grade 3
Gastrointestinal		
Diarrhea	4 (20.0%)	13 (65.0%)
Nausea	4 (20.0%)	0
Vomiting	3 (15.0%)	0
Metabolic and nutritional disorders		
Fatigue	7 (35.0%)	0
Anorexia	1 (5.0%)	1 (5.0%)
Headache	4 (20.0%)	0
Hand-foot syndrome	5 (25.0%)	0
Urine output decreased	0	1 (5.0%)
Hematological toxicity		
Anemia	5 (25.0%)	0
GGT increased	3 (15.0%)	0
Hypoalbuminemia	2 (10.0%)	0
Hyponatremia	3 (15.0%)	0
Platelet count decreased	3 (15.0%)	0

Abbreviations: CRC, colorectal cancer; HER2, human epidermal growth factor receptor 2; GGT, gamma glutamyl transferase.

A phase II, TRIUMPH study in Japan of trastuzumab and pertuzumab in patients with central tissue and/or ctDNA confirmed *RAS* wild-type, *HER2* amplificated CRC and obtained primary efficacy with an ORR of 35% in the tissue-positive group, ORR 33% in the ctDNA positive group, and median PFS of 4.0 months for both groups.[22] However, data on anti-HER2 therapy for HER2-positive CRC are absent in China. Referring to NCCN guidelines, the 2020 edition of the CSCO Guidelines for the Diagnosis and Treatment of Colorectal Cancer added recommendations for anti-HER2 treatment in the third-line or posterior-line treatment of CRC.[23] The randomized phase 3 PHOEBE trial reported that the efficacy of pyrotinib was better than that of lapatinib, with an improved ORR (79% vs. 57%), longer PFS (18.1 months vs. 7.0 months), and a 64% lower risk of death from disease when in combination with capecitabine in HER2-positive local relapsed or metastatic breast cancer,[24] indicating that pyrotinib may have more antitumor potential than lapatinib.

The present study is the first study exploring the antitumor activity of pyrotinib combined with trastuzumab in HER2-positive metastatic CRC patients, and the first reported study on dual-HER2 targeted therapy in this population in China. The results illustrate that 33.3% of patients with RAS wild-type achieved an objective response, consistent with the effects of other dual-HER2 therapies in previous studies where *RAS* wild-type, HER2-positive metastatic CRC patients reached an ORR of 30–40%.[10,11] On the contrary, for six patients with the RAS mutation in our study, none obtained an objective response and only one patient remained stable disease, suggesting that *RAS* is a predictor for negative clinical response to dual-HER2 targeted therapy.

The adverse events in our study are similar to the prior-reported toxicity profiles of pyrotinib. Grade 3 diarrhea was observed in 13 (65.0%) patients, much higher than that of previous trials,[15,24,25] presumably because CRC is more likely to cause diarrhea than lung

adenocarcinoma and breast cancer. Most patients tolerated pyrotinib after dose reduction. In addition, unlike the high incidence of rash with lapatinib,[10] only two patients developed grade 1 rash in our study.

Recently, trastuzumab combined with tucatinib in the treatment of HER2-positive CRC has shown meaningful antitumor activity with a confirmed ORR of 38.1% (95% CI 27.7–49.3), median PFS of 8.2 months (95% CI 4.2–10.3), and median OS of 24.1 months (95% CI 20.3–36.7), but has not yet been marked in China.[19] Enhertua (also known as DS-8201), a new antibody-drug conjugate of irinotecan combined with trastuzumab which is on the market in the United States, shows a prominent effect, with an ORR of more than 40% in HER2-positive CRC, but its side effects, especially interstitial pneumonia, require careful monitoring and intervention as needed.[26] In China, a number of one-arm exploratory studies are ongoing to provide more effective anti-HER2 combination therapies for advanced CRC.[27,28]

Previous studies have shown that those patients who benefit from anti-HER2 therapy are mainly *RAS/BRAF* wild-type, with *HER2* amplification.[10,11,29] However, these anti-HER2 targeting therapies are generally for third-line or posterior treatment. Nevertheless, in the future, with further research, anti-HER2 targeting therapy has the opportunity to progress from third-line to second-line or even first-line therapy. In addition to the combinations of dual-HER2 targeted drugs, the combination of anti-HER2 targeting drugs with traditional chemotherapy or antivascular therapy or immune checkpoint inhibitors may also be an option.

In terms of limitations, the single-arm design in our study lacks a control group and randomization, and the sample size is too small to allow subgroup analysis to compare therapeutic effects across HER2 levels, HER2 alterations, and status of other proto-oncogenes. Large-sample studies are required to verify the efficacy of pyrotinib combined with trastuzumab in HER2-positive metastatic CRC, and the population of precise for this therapy also needs to be further explored.

In conclusion, the present study reveals the potential of pyrotinib and trastuzumab in patients with *RAS/BRAF* wild-type HER2-positive advanced CRC and might provide a novel treatment option.

AUTHOR CONTRIBUTIONS

X.F. and Y.Y. were involved in the study conception and design, provision of study materials and patient recruitment, data analysis and interpretation, manuscript writing, and manuscript approval. S.Z. was involved in the study conception and design, data analysis and interpretation, manuscript writing, and manuscript approval. J.Y., L.Y., W.F., W.H., and H.H. were involved in the provision of study materials as well as patient recruitment and manuscript approval.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: Ethics Committee of Second Affiliated Hospital of Zhejiang University School of Medicine.

Informed Consent: N/A.

Registry and the Registration No. of the study/trial: ClinicalTrials.gov (identifier: NCT04380012).

Animal Studies: N/A.

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