Clinical Cancer Research

Safety, Efficacy, and Biomarker Analysis of Pyrotinib in Combination with Capecitabine in HER2-Positive Metastatic Breast Cancer Patients: A Phase I Clinical Trial

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Abstract

Purpose: This phase I study assessed the safety, tolerability, MTD, pharmacokinetics, antitumor activity, and predictive biomarkers of pyrotinib, an irreversible pan-ErbB inhibitor, in combination with capecitabine in patients with HER2-positive metastatic breast cancer (MBC).

Patients and Methods: Patients received oral pyrotinib 160 mg, 240 mg, 320 mg, or 400 mg once daily continually plus capecitabine 1,000 mg/m^2 twice daily on days 1 to 14 of a 21-day cycle. Pharmacokinetic blood samples were collected on days 1 and 14. Next-generation sequencing was performed on circulating tumor DNA to probe for predictive biomarkers.

Results: A total of 28 patients were enrolled, 22 patients were treated at the two top-level doses. Among 17 (60.7%) trastuzumab-pretreated patients, 11 received trastuzumab for metastatic disease and 6 received adjuvant trastuzumab. No dose-limited toxicity was observed. Grade 3 treatment-related adverse events (AE) occurred in 12 (42.9%) patients; anemia (14.3%) and diarrhea (10.7%) were the most common grade 3 AEs. The overall response rate (ORR) was 78.6% [95% confidence interval (CI): 59.0%–91.7%], and the clinical benefit rate was 85.7% (95% CI: 67.3%–96.0%). The median progression-free survival (PFS) was 22.1 months (95% CI: 9.0–26.2 months). ORR was 70.6% (12/17) in trastuzumab-pretreated patients and 90.9% (10/11) in trastuzumab-naïve patients. Analysis of all genetic alterations in HER2-related signaling network in baseline blood samples suggested that multiple genetic alterations were significantly associated with poorer PFS compared with none or one genetic alteration (median, 16.8 vs. 29.9 months, $P = 0.006$).

Conclusions: In a population largely naïve to HER2-targeted therapy, pyrotinib in combination with capecitabine was well-tolerated and demonstrates promising antitumor activity in patients with HER2-positive MBC.

Introduction

Overexpression of HER2 in breast cancer leads to more aggressive disease and a poorer prognosis (1–4). The introduction of trastuzumab and other anti-HER2 agents, such as pertuzumab, ado-trastuzumab emtansine, lapatinib, and neratinib, have greatly improved the survival and prognosis of patients with HER2-positive metastatic breast cancer (MBC) (5–12). However, patients frequently acquire resistance within 12 to 18 months of HER2-directed therapy (1). Thus, the continued development of novel anti-HER2 agents and the unraveling mechanisms of resistance are important.

Pyrotinib is an oral, irreversible pan-ErbB tyrosine kinase inhibitor (TKI) that potently inhibits EGFR/HER1, HER2, and HER4 (13). Preclinical data of pyrotinib demonstrate effective proliferation inhibition of HER2-overexpressing cells both *in vivo* and *in vitro* (14, 15). In a phase I pyrotinib monotherapy study (16), the MTD of pyrotinib was determined to be 400 mg daily. The study also suggests that pyrotinib is safe and highly effective in patients with HER2-positive MBC, with an overall response rate (ORR) of 50.0% and a median progression-free survival (PFS) of 35.4 weeks in the dosage range of 80 to 400 mg per day. The most common adverse events with pyrotinib monotherapy are diarrhea, nausea, oral ulceration, asthenia, and leukopenia. This study revealed that PIK3CA and TP53 mutations in circulating tumor DNA (ctDNA) are associated with a worse efficacy of pyrotinib monotherapy (16).

This phase I trial evaluated the combination of pyrotinib and capecitabine in patients with HER2-positive MBC to determine

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Translational Relevance

The irreversible pan-ErbB inhibitor pyrotinib has demonstrated promising single-agent efficacy and acceptable tolerability in HER2-positive metastatic breast cancers (MBC). To extend its activity and to explore novel therapeutic strategies, combinatorial approach with cytotoxic agent capecitabine is tested. In this phase I study for HER2-positive MBC, patients received oral pyrotinib 160 to 400 mg per day in combination with capecitabine 2,000 mg/m^2 per day, and 39.3% (11/28) patients were naïve to trastuzumab. No dose-limited toxicity was observed in any cohort. The overall response rate was 78.6% with a median progression-free survival of 22.1 months. The combination of pyrotinib suggested promising antitumor activity and was well tolerated in patients with pretreated HER2-positive MBC. Furthermore, genetic analysis of HER2-related signaling network in circulating tumor DNA suggested multiple genetic alterations are significantly associated with shorter progression-free survival compared with none or one genetic alteration.

the MTD and safety. The pharmacokinetics profile and preliminary antitumor activity of this combination were also evaluated. In addition, all genetic alteration statuses of HER2 bypass signaling pathway, PI3K/Akt/mTOR pathway, and TP53 were analyzed to detect potential predictive or prognostic biomarkers for the combination of pyrotinib and capecitabine.

Patients and Methods

Patient eligibility

Patients were eligible if they (i) had a confirmed histologic/cytologic diagnosis of MBC for which standard therapy failed or for which standard treatment was not available, (ii) are HER2 positive (IHC 3+, or IHC2+ confirmed by FISH), (iii) aged between 18 and 70 years, (iv) had a measurable lesion defined by revised Response Evaluation Criteria in Solid Tumors guidelines version 1.1 (RECIST 1.1), (v) had an Eastern Cooperative Oncology Group performance status < 2, and (vi) had adequate hematologic, hepatic, and renal function.

Patients were excluded if they had received capecitabine within 1 year before study entry, or if they had received prior treatment with small-molecule anti-HER2 TKIs. Other exclusive criteria included: chemotherapy, radiotherapy, hormone therapy, immunotherapy, or investigational agents within 4 weeks before treatment day 1; resistant (disease progression within 12 weeks after initiation of capecitabine) or intolerable to prior capecitabine exposure; intracranial lesions or history of brain metastases; a history of clinically significant cardiac disease, including congestive heart failure, myocardial infarction, and significant arrhythmia; and evidence of significant medical illness including severe/uncontrolled hypertension, diabetes, or thyroid disease. No limits on number of prior cytotoxic regimens for metastatic disease were required.

Study design

This single-center, open-label, phase I study was designed to determine the safety, MTD, pharmacokinetics, and preliminary antitumor activity of pyrotinib plus capecitabine in patients with HER2-positive MBC. A 3+3 dose-escalation scheme was used to define the MTD of pyrotinib plus capecitabine. Eight patients were added to the dose cohorts of the candidate dose of pyrotinib plus capecitabine in the following phase II study. A total of 22 patients were treated at the two top-level doses (Fig. 1).

This study was conducted in accordance with the International Conference on Harmonization Guideline for Good Clinical Practice and the ethical principles in the Declaration of Helsinki. The study protocol was approved by an Institutional Review Board and all patients provided written informed consent before enrollment in this study (NCT02361112).

Study treatment and dose-escalation protocol

During dose escalation, 3 patients in each cohort received oral pyrotinib (160, 240, 320, or 400 mg) once daily continuously in combination with oral capecitabine 1,000 mg/m^2 twice daily on days 1 to 14 of a 21-day cycle. Pyrotinib was administered within 30 minutes after breakfast in the morning; the capecitabine dose was split into two equal doses and administered every 12 hours. The initial dose of pyrotinib was based on preclinical data (14, 15) and on the results of a phase I monotherapy study in patients with breast cancer (16) in China. The study reported an MTD for pyrotinib monotherapy as 400 mg daily. Each patient was treated at one dose level unless there was disease progression, unacceptable toxicity, informed consent withdrawal, or termination by the investigator.

If no patients experienced a dose-limited toxicity (DLT) within the first cycle, then 3 patients were enrolled at the next dose level; if one patient experienced a DLT, then an additional 3 patients were treated at the same dose level. The dose escalated if <33% of evaluable patients had a DLT. If ≥33% of patients experienced a DLT by day 21, dose escalation stopped and the previous dose level was considered the MTD. If a patient in any dose cohort had a toxicity that met the definition of DLT, then the patient's dose was reduced by one dose level.

According to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.0, a DLT is defined as follows: any treatment-related hematologic grade 4 adverse events (AE); grade 2 heart failure; grade 3 or grade 4 other nonhematologic AEs, with the exception of manageable diarrhea relieved within 3 days, alopecia, nausea, or vomiting (unless the patient was receiving appropriate medical therapy), and increased alkaline phosphatase. No prophylaxis for diarrhea was given, and management of diarrhea was provided in Supplementary Table S1.

Evaluation of patients

Safety evaluations were conducted at screening; on days 7, 14, and 21 of cycle 1; on days 7 and 21 of cycle 2; on day 21 of cycle 3 to 4; on day 63 of every 3 cycles of cycle 5 to 16; on day 84 of every 4 cycles of cycle 17 to 32; and on day 126 of every 6 cycles after cycle 33. Safety assessments included laboratory variables, vital signs, interim medical history, radiographs, and ECGs. All patients were required to keep the patient diaries. An efficacy evaluation was performed in accordance with Response Evaluation Criteria in Solid Tumors guidelines version 1.1 (RECIST 1.1; ref. 17). A best response of complete response (CR) or partial response (PR) had to be confirmed at least 4 weeks after initial evaluation. The efficacy evaluation was performed once every 2 cycles in the first 4 cycles, once every three cycles in cycle 5 to 16, once every 4 cycles in cycle 17 to 32, and once every 6 cycles after cycle 33.

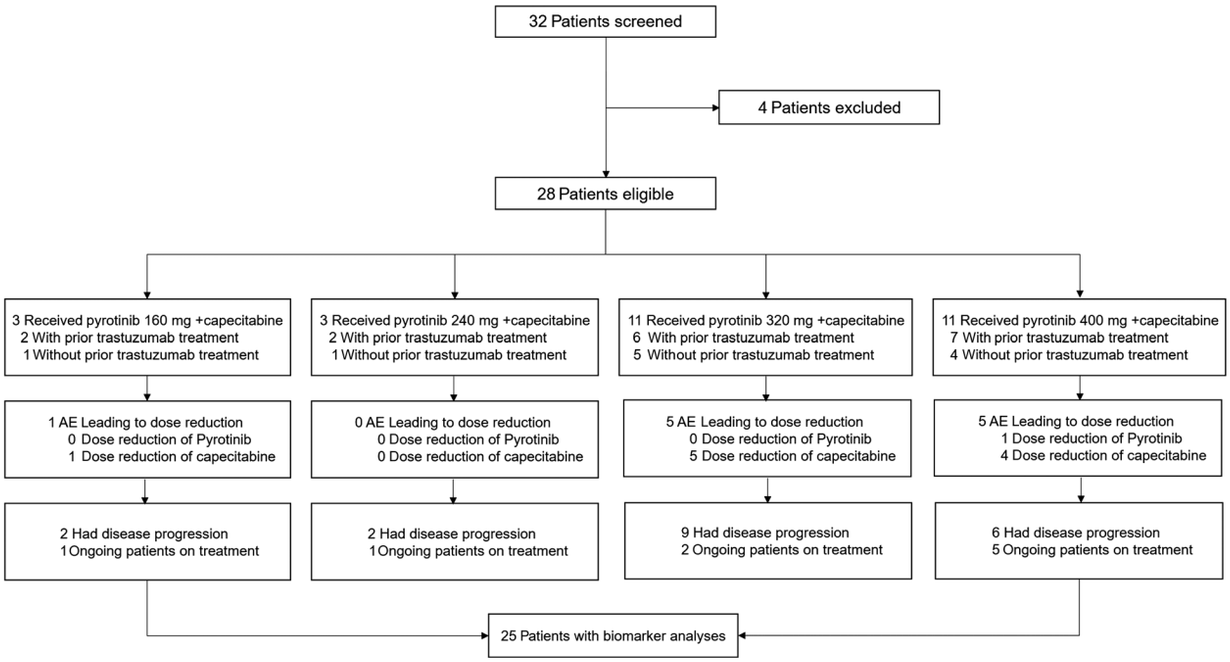


Figure 1.
Study flowchart.

Pharmacokinetic assessments

The pharmacokinetics of pyrotinib, capecitabine, and 5-fluorouracil (5-FU) were assessed in HER2-positive patients receiving pyrotinib in combination with capecitabine. Blood samples were collected in Heparin-Li anticoagulant tubes for the analysis of pyrotinib and in Heparin-Na anticoagulant tubes containing tetrahydrouridine as a stabilizing agent for capecitabine and 5-FU before dosing and 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after the morning dose on days 1 and 14. Plasma concentrations of pyrotinib (lower limit of quantification of 0.429 ng/mL), capecitabine, and 5-FU (lower limit of quantification of 20.0 ng/mL and 5.00 ng/mL, respectively) were determined using validated LC/MS-MS methods developed at WuXi AppTec. The main pharmacokinetic parameters of pyrotinib, capecitabine, and 5-FU were estimated using noncompartmental analysis (WinNonlin version 6.4; Pharsight).

Biomarker analyses

All biomarker analyses were prospectively planned, and informed consents for blood collection were obtained from 25 patients. Low-coverage whole-genome sequencing to analysis gene copy number variants (CNV) and targeted gene sequencing panel (1,021 genes) to detect single-nucleotide variants (SNV) were performed on ctDNA of baseline samples by next-generation sequencing (NGS). Detailed protocols for ctDNA sequencing are provided in the Supplementary Materials.

Results

Patients

From August 2014 to April 2017, 28 patients (median age: 48; range: 24–59) received at least one dose of the study treatment. Patient baseline characteristics are summarized in Table 1. Eight (28.6%) patients received three or more prior chemotherapy regimens. A total of 17 (60.7%) patients received prior trastuzumab treatment, and 11 (39.3%) patients have had trastuzumab for metastatic disease. Among the 6 (21.4%) patients who received trastuzumab only in adjuvant/neoadjuvant setting, 3 relapsed during adjuvant trastuzumab treatment (Table 1). None of the patients have had pertuzumab or TDM1 before enrollment. Twenty-one (75%) patients presented with visceral metastasis on entry of this study.

Dose escalation

During dose escalation, no patients in each dose cohort experienced a DLT within 21 days of dosing. Given that pyrotinib 400 mg daily was determined as MTD in previous phase I study, dose escalation stopped at the pyrotinib 400 mg plus capecitabine 2,000 mg/m^2 daily cohort. Both pyrotinib 320 mg daily and pyrotinib 400 mg daily plus capecitabine cohorts were considered candidate dosages for the subsequent phase II study.

Safety

At the time of data cutoff (January 31, 2018), the median duration of treatment with continual daily pyrotinib plus capecitabine was 78 weeks (range: 1.9–132.0 weeks). The relative dose intensity was 98.7% and 89.8% for pyrotinib and capecitabine, respectively.

During the continual dosing period of pyrotinib plus capecitabine, all 28 (100%) patients experienced at least one treatment-related AE. The most common treatment-related AEs of any grade observed in $\geq$10% of patients included diarrhea (85.7%), leukopenia (53.6%), neutropenia, and palmar–plantar erythrodysesthesia syndrome (PPE; each at 50.0%), hyperbilirubinemia (46.4%), nausea (32.1%), vomiting, and anemia (each at 28.6%), oral ulceration, and hypercreatininemia (each at 25.0%),

Table 1. Demographic and baseline clinicopathologic characteristics of the enrolled patients

	160 mg ($n = 3$)	240 mg ($n = 3$)	320 mg ($n = 11$)	400 mg ($n = 11$)	Total ($n = 28$)
Median age, years (range)	52 (39–55)	51 (42–58)	43 (24–52)	49 (29–59)	48 (24–59)
ECOG Performance status, *n* (%)					
0	3 (100.0)	3 (100.0)	8 (72.7)	8 (72.7)	22 (78.6)
1	0	0	3 (27.3)	3 (27.3)	6 (21.4)
HR					
Positive	0	2 (66.7)	5 (45.5)	8 (72.7)	15 (53.6)
Negative	3 (100.0)	1 (33.3)	5 (45.5)	3 (27.3)	12 (42.9)
Unknown	0	0	1 (9.1)	0	1 (3.6)
No. of metastatic organs, median (range)	2 (2–4)	3 (1–3)	1 (1–4)	3 (1–5)	2 (1–5)
No. of patients with visceral metastasis, *n* (%)					
w/o	2 (66.7)	0	4 (36.4)	1 (9.1)	7 (25.0)
w	1 (33.3)	3 (100.0)	7 (63.6)	10 (90.9)	21 (75.0)
No. of prior metastatic cytotoxic regimens, *n* (%)					
<3	3 (100.0)	1 (33.3)	7 (63.6)	9 (81.8)	20 (71.4)
≥3	0	2 (66.7)	4 (36.4)	2 (18.2)	8 (28.6)
Prior taxane treatment, *n* (%)	3 (100.0)	3 (100.0)	10 (90.9)	11 (100.0)	27 (96.4)
Prior anthracycline treatment, *n* (%)	2 (66.7)	1 (33.3)	11 (100.0)	10 (90.9)	24 (85.7)
Prior trastuzumab treatment, *n* (%)	2 (66.7)	2 (66.7)	6 (54.5)	7 (63.6)	17 (60.7)
Trastuzumab-pretreated for metastatic disease only	0	1 (33.3)	1 (9.1)	6 (54.5)	8 (28.6)
Trastuzumab-pretreated in the adjuvant/neoadjuvant setting only	2 (66.7)	1 (33.3)	2 (18.2)	1 (9.1)	6 (21.4)
Trastuzumab-pretreated in both adjuvant/neoadjuvant setting and metastatic setting	0	0	3 (27.3)	0	3 (10.7)

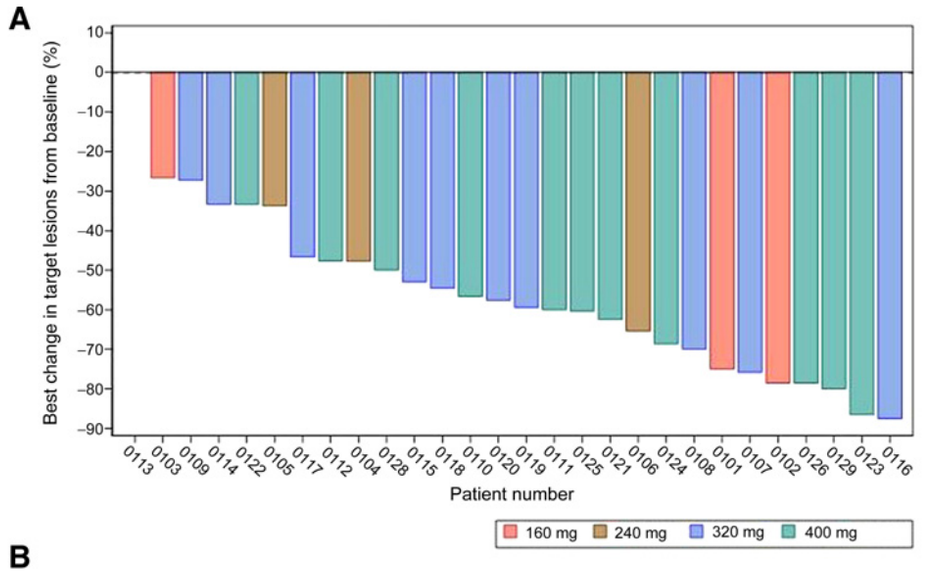
hypertriglyceridemia, and rash (each at 17.9%), dyspigmentation (14.3%), alanine aminotransferase (ALT) elevation, and dizziness (each at 10.7%). Grade 3 treatment-related AEs occurred in 12 (42.9%) patients, and anemia (14.3%) and diarrhea (10.7%) were the most common grade 3 AEs. The incidence of grade 2 diarrhea (17.9%), PPE (28.6%), oral ulceration (7.1%), and rash (3.6%) were demonstrated in Supplementary Table S5. No grade 4 or grade 5 pyrotinib-related AEs were reported. (Table 2; Supplementary Table S5).

Most diarrheal events were grade 1 (151/168, 89.9%), and only 3 patients experienced grade 3 diarrhea (Supplementary Table S2–4). The median onset of diarrhea was 3 days after start of treatment and the median duration was 2 days. Most diarrhea events were reported during the first cycle of treatment and the frequency persistently declined in the following cycles (Supplementary Fig. S1). Diarrhea was managed by appropriate diet adjustment, loperamide (start at 4 mg followed by 2 mg after each episode of diarrhea), or isotonic solution (1–1.5 L/day) plus intravenous fluids if necessary (Supplementary Table S1). A patient who had diarrhea tended to have repeated episodes and the median number of diarrheal events per patient was 3 (Supplementary Table S2). All grade 2 or 3 diarrheal events resolved to ≤grade 1 within 3 days. One patient (3.6%) required dose interruption and dose reduction of pyrotinib due to grade 3 diarrhea, one patient required dose interruption of capecitabine, and one required discontinuation of capecitabine.

PPE occurred in 50% of enrolled patients, which was similar to other study that included capecitabine in a treatment cohort (9, 18). Anemia was not common (28.6%) in total population, but grade 3 anemia was the most common grade 3 treatment-related AE (4 patients, 14.3%). No cardiovascular AE was reported in this study.

Table 2. Incidence of treatment-related AEs (≥10%) and grade 3/4 treatment-related AEs in patients during the continual dosing period of pyrotinib plus capecitabine

	Pyrotinib dose cohorts (mg)									
	Pyrotinib 160 mg + capecitabine ($n = 3$)		Pyrotinib 240 mg + capecitabine ($n = 3$)		Pyrotinib 320 mg + capecitabine ($n = 11$)		Pyrotinib 400 mg + capecitabine ($n = 11$)		Total ($n = 28$)	
AE	Any grade, *n* (%)	Grade 3/4, *n* (%)	Any grade, *n* (%)	Grade 3/4, *n* (%)	Any grade, *n* (%)	Grade 3/4, *n* (%)	Any grade, *n* (%)	Grade 3/4, *n* (%)	Any grade, *n* (%)	Grade 3/4, *n* (%)
Diarrhea	3 (100)	0	3 (100)	0	10 (90.9)	1 (9.1)	8 (72.7)	2 (18.2)	24 (85.7)	3 (10.7)
Leukopenia	3 (100)	0	2 (66.7)	0	4 (36.4)	1 (9.1)	6(54.5)	0	15 (53.6)	1 (3.6)
Neutropenia	3 (100)	0	1 (33.3)	0	4 (36.4)	1 (9.1)	6 (54.5)	0	14 (50.0)	1 (3.6)
PPE	3 (100)	0	2 (66.7)	0	3 (27.3)	0	6 (54.5)	1 (9.1)	14 (50.0)	1 (3.6)
hyperbilirubinemia	1 (33.3)	0	1 (33.3)	0	5 (45.5)	0	6 (54.5)	0	13 (46.4)	0
Nausea	0	0	1 (33.3)	0	4 (36.4)	0	4 (36.4)	0	9 (32.1)	0
Vomiting	0	0	1(33.3)	0	3 (27.3)	1 (9.1)	4 (36.4)	0	8 (28.6)	1 (3.6)
Anemia	1 (33.3)	1 (33.3)	1 (33.3)	0	2 (18.2)	2 (18.2)	4 (36.4)	1 (9.1)	8 (28.6)	4 (14.3)
Oral ulceration	2 (66.7)	0	0	0	2 (18.2)	0	3 (27.3)	0	7 (25.0)	0
hypercreatininemia	2 (66.7)	0	1 (33.3)	0	0	0	4 (36.4)	0	7 (25.0)	0
hypertriglyceridemia	1 (33.3)	0	0	0	3 (27.3)	0	1 (9.1)	0	5 (17.9)	0
Rash	0	0	0	0	2 (18.2)	0	3 (27.3)	1 (9.1)	5 (17.9)	1 (3.6)
dyspigmentation	0	0	0	0	2 (18.2)	0	2 (18.2)	0	4 (14.3)	0
ALT elevation	0	0	0	0	2 (18.2)	0	1 (9.1)	0	3(10.7)	0
Dizziness	0	0	1 (33.3)	0	2 (18.2)	0	0	0	3 (10.7)	0



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	Pyrotinib 160 mg+ capecitabine (*n* = 3)			Pyrotinib 240 mg+ capecitabine (*n* = 3)			Pyrotinib 320 mg+ capecitabine (*n* = 11)			Pyrotinib 400 mg+ capecitabine (*n* = 11)			Total (*n* = 28)		
	No	%	95% CI	No	%	95% CI	No	%	95% CI	No	%	95% CI	No	%	95% CI
Best overall response															
CR	0	0		0	0		0	0		0	0		0	0	
PR	2	66.7		2	66.7		8	72.7		10	90.9		22	78.6	
SD	1	33.3		1	33.3		2	18.2		1	9.1		5	17.9	
PD	0	0		0	0		0	0		0	0		0	0	
NE	0	0		0	0		1	9.1		0	0		1	3.6	
ORR (%)	2	66.7	(9.4–99.2)	2	66.7	(9.4–99.2)	8	72.7	(39.0–94.0)	10	90.9	(58.7–99.8)	22	78.6	(59.0–91.7)
CBR (%)	3	100.0	(29.2–100.0)	2	66.7	(9.4–99.2)	9	81.8	(48.2–97.7)	10	90.9	(58.7–99.8)	24	85.7	(67.3–96.0)
PFS (months)															
median (95% CI)	26.2		(11.1–NE)	6.8		(4.9–NE)	17.9		(2.8–26.2)	22.1		(6.2–NE)	22.1		(9.0–26.2)

Figure 2.

Antitumor clinical activity of the combination of pyrotinib and capecitabine. **A,** Maximum reduction of target lesion from baseline in patients of each cohorts. **B,** The best overall response rate, clinical benefit rate, and progression-free survival in different pyrotinib dose cohorts. *In the 240 mg, 320 mg, 400 mg pyrotinib combination cohort, one patient from each group had a best response of SD for less than 24 weeks after study entry, who were not calculated in the numerator of clinical benefit rate.

A total of 6 (21.4%) patients experienced AEs requiring dose interruptions of pyrotinib. One (3.6%) patient required pyrotinib dose reduction due to AE (grade 3 diarrhea). No patient discontinued pyrotinib due to AEs.

Capecitabine was interrupted in 14 (50.0%) patients due to AEs. Ten (35.7%) patients experienced dose reduction of capecitabine due to AEs and 3 (10.7%) patients discontinued capecitabine administration due to AEs.

Antitumor activity

A summary of best overall response based on investigator review is provided in Fig. 2. A total of 22 patients (78.6%) achieved a best response of PR, 5 patients (17.9%) had a best response of stable disease (SD), and none had progressive disease (PD). One patient withdrew informed consent before the first efficacy evaluation. The ORR (CR + PR) was 78.6% (95% CI: 59.0%–91.7%) for all the 28 patients, and was 66.7% (2/3), 66.7% (2/3), 72.7% (8/11), and 90.9% (10/11) for each dose cohort (160, 240, 320, and 400 mg, respectively). The clinical benefit rate (CBR, CR + PR + SD over 24 weeks) was 85.7% (95% CI: 67.3%–96.0%) for all 28 patients. The median time of response was 8.0 weeks. The median duration of response for the 22 PR patients was 98 weeks (95% CI: 45.3–not arrived). The best ORR was 70.6% (12/17) in the 17 patients who were previously treated with trastuzumab and 90.9% (10/11) in 11 trastuzumab-naïve patients. For the 11 patients who had had trastuzumab for metastatic disease, the best ORR was 72.7% (8/11). As it is depicted in Supplementary Table S6, the best ORR was 66.7% (4/6) in 6 patients who received trastuzumab only in adjuvant setting.

The median PFS was 22.1 months (95% CI: 9.0–26.2 months) for all 28 patients. At the time of data cutoff (January 31, 2018), there were 9 ongoing patients on pyrotinib treatment.

Pharmacokinetics

After coadministration of 160–400 mg pyrotinib once a day with capecitabine 2,000 mg/m^2/day (twice a day), T_{max} and $t_{1/2}$ of pyrotinib was similar on days 1 and 14, and was independent of dose level. The accumulation ratio based on AUC was approximately 1, indicating there was no obvious accumulation after repeated administration. In the dose range of 160–400 mg, $AUC_{0\text{-}24h}$ and C_{max} at steady state increased proportionately with increasing dose, demonstrating the linear pharmacokinetic characteristics of pyrotinib (Table 3).

Table 3. Pharmacokinetic parameters of pyrotinib and capecitabine in Chinese patients with HER2-positive MBC

		Day 1		Day 14			Day 1				Day 14			
		Pyrotinib		Pyrotinib			Capecitabine		5-FU		Capecitabine		5-FU	
Dose	Parameter	Mean	CV%	Mean	CV%	Dose	Parameter	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
160 mg	$t_{1/2z}$ (h)	9.86	13.1	15.5	56.1	160 mg	$t_{1/2z}$ (h)	0.387	6.2	0.616	30.9	0.422	13.9	0.573	12
($n = 3$)	T_{max} (h)	3	2.00–24.0	5	2.00–5.00	($n = 3$)	T_{max} (h)	1	1.00–2.00	2	1.00–2.00	1	0.500–2.00	2	1.00–2.00
	C_{max} (ng/mL)	93.9	10.9	76.8	58.1		C_{max} (ng/mL)	3,720	25.9	179	51.2	2,550	23.3	278	32.1
	$AUC_{0\text{-}24h}$ (h*ng/mL)	1050	39.7	1,050	40		$AUC_{0\text{-}t}$ (h*ng/mL)	5,040	34.3	343	44.8	4,490	12.6	551	37.1
	Rac (AUC)	/	/	1	59.1		$AUC_{0\text{-}\infty}$ (h*ng/mL)	5,080	33.9	353	44.1	4,530	11.8	559	36.1
240 mg	$t_{1/2z}$ (h)	13	4	16.4	10.4	240 mg	$t_{1/2z}$ (h)	0.375	46.4	0.666	15.8	0.609	4.5	1.06	2.8
($n = 2$)	T_{max} (h)	3.48	1.97–5.00	4.5	3.00–6.00	($n = 2$)	T_{max} (h)	2.48	0.967–4.00	2.48	0.967–4.00	0.75	0.500–1.00	1.25	0.500–2.00
	C_{max} (ng/mL)	109	1.9	121	42.1		C_{max} (ng/mL)	2,600	92.5	191	79.6	4,620	90.4	360	41.5
	$AUC_{0\text{-}24h}$ (h*ng/mL)	1350	4.5	1,850	45.9		$AUC_{0\text{-}t}$ (h*ng/mL)	3,850	69.3	278	63.2	4,980	41.1	607	17.1
	Rac (AUC)	/	/	1.37	50.1		$AUC_{0\text{-}\infty}$ (h*ng/mL)	3,910	67.8	294	58.9	5,030	41.1	630	19.2
320 mg	$t_{1/2z}$ (h)	18.8	74	17.2	39.9	320 mg	$t_{1/2z}$ (h)	0.438	22.8	0.744	23.1	0.565	96.5	0.837	52.4
($n = 9$)	T_{max} (h)	4	3.00–8.00	4	2.00–6.03	($n = 9$)	T_{max} (h)	1	0.500–2.00	2	0.500–2.00	1.17	0.500–4.00	1.17	1.00–4.00
	C_{max} (ng/mL)	107	34.3	112	43.1		C_{max} (ng/mL)	3,440	39	113	32.2	3,020	39	174	52.9
	$AUC_{0\text{-}24h}$ (h*ng/mL)	1480	42.3	1,540	50.7		$AUC_{0\text{-}t}$ (h*ng/mL)	5330	38.7	236	33.7	4,950	33.6	409	54.4
	Rac (AUC)	/	/	1.04	37.5		$AUC_{0\text{-}\infty}$ (h*ng/mL)	5370	38.6	248	32.5	5,070	37.2	424	58.9
400 mg	$t_{1/2z}$ (h)	14.9	32.9	18.2	45.5	400 mg	$t_{1/2z}$ (h)	0.578	131.8	0.727	23.2	0.659	55.4	0.905	47.4
($n = 9$)	T_{max} (h)	4	2.00–6.00	5	2.00–12.0	($n = 9$)	T_{max} (h)	1	0.500–2.00	2	1.00–3.00	2	0.500–5.00	2.22	1.00–5.00
	C_{max} (ng/mL)	176	35.8	170	50.1		C_{max} (ng/mL)	3350	55.3	138	35.6	2,260	44.7	186	42.2
	$AUC_{0\text{-}24h}$ (h*ng/mL)	2310	42.1	2,830	60.4		$AUC_{0\text{-}t}$ (h*ng/mL)	5350	41.3	287	37.4	5,210	50.2	492	49.2
	Rac (AUC)	/	/	1.22	24.3		$AUC_{0\text{-}\infty}$ (h*ng/mL)	5670	58.7	304	36.3	5,310	50.3	522	47.5

Capecitabine was rapidly absorbed and biotransformed into its bioactive metabolite 5-FU after coadministration at 2,000 mg/m^2/day (twice per day) with pyrotinib. The main pharmacokinetic parameters (AUC and C_{max}) of capecitabine and 5-FU showed no significant difference between 320 mg and 400 mg pyrotinib dose cohorts.

Biomarker analyses

All genetic alterations, including both SNVs and CNVs of HER2 bypass signaling pathway, PI3K/Akt/mTOR pathway and TP53 were analyzed for baseline blood samples of 25 patients. These alterations included amplification, mutation, or deletion of EGFR, FGFR, IGFR, ERBB2, PIK3CA and AKT, mTOR, PTEN, and TP53 (Fig. 3). PFS of patients with multiple (two or more) genetic alterations was significantly shorter than that of patients with none or one genetic alteration (median, 16.8 vs. 29.9 months, $P = 0.006$ by log-rank test; see Fig. 3). However, the status of multiple genetic alterations was not correlated with objective responses ($P = 1.000$). Meanwhile, no single alteration was correlated with significant survival and response difference.

Discussion

This phase I study investigated the safety of pyrotinib 160 to 400 mg per day in combination with capecitabine 2,000 mg/m^2 per day, and no DLT was observed in any cohort. According to the definition of DLT in this protocol, two events of grade 3 diarrhea that continued for 2 and 3 days in each respective case in the first cycle of pyrotinib 400 mg cohort were not determined as DLT. Thus, the MTD of pyrotinib in combination with capecitabine in patients with HER2-positive MBC was determined as 400 mg per day.

The incidence of AEs observed in this combination regimen phase I study was higher than that in pyrotinib monotherapy study (16). Diarrhea, leukopenia, neutropenia, PPE, hyperbilirubinemia, nausea, vomiting, and anemia were more frequent when capecitabine was added to pyrotinib. For instance, the incidence of diarrhea increased from 41.7% in monotherapy to 85.7% in combined treatment. The grade 3/4 AEs were also more often in this study compared with previous monotherapy study (50% vs. 11.1%). The most common grade 3/4 treatment-related AEs were anemia (14.3%) and diarrhea (10.7%). The incidence of grade 2 AEs was lower than that of grade 1 AEs, and grade 2 diarrhea and PPE occurred in 17.9% and 28.6% patients, respectively. Most of the increased AEs were common in capecitabine-based regimens (9), and overlapping of AE spectrums between pyrotinib and capecitabine also contributed to elevation of AEs. No unexpected AEs were observed and all AEs were relieved or disappeared after dose reduction or drug discontinuation.

The IC_{50} (nmol/L) for EGFR and HER2 of pyrotinib versus neratinib are 13 versus 23 and 38 versus 43 (15), respectively, which indicate a similar inhibition of EGFR family between these two agents. In this study, the incidence of diarrhea, PPE, and vomiting in pyrotinib plus capecitabine were 85.7%, 50.0%, and 28.6%, respectively; while in the neratinib combination study, the incidence were 88%, 48%, and 29%, respectively (19). Cardiac events were rare in both studies. Two patients experienced elongation of QTcF to over 480 ms in pyrotinib plus capecitabine, which were determined of no

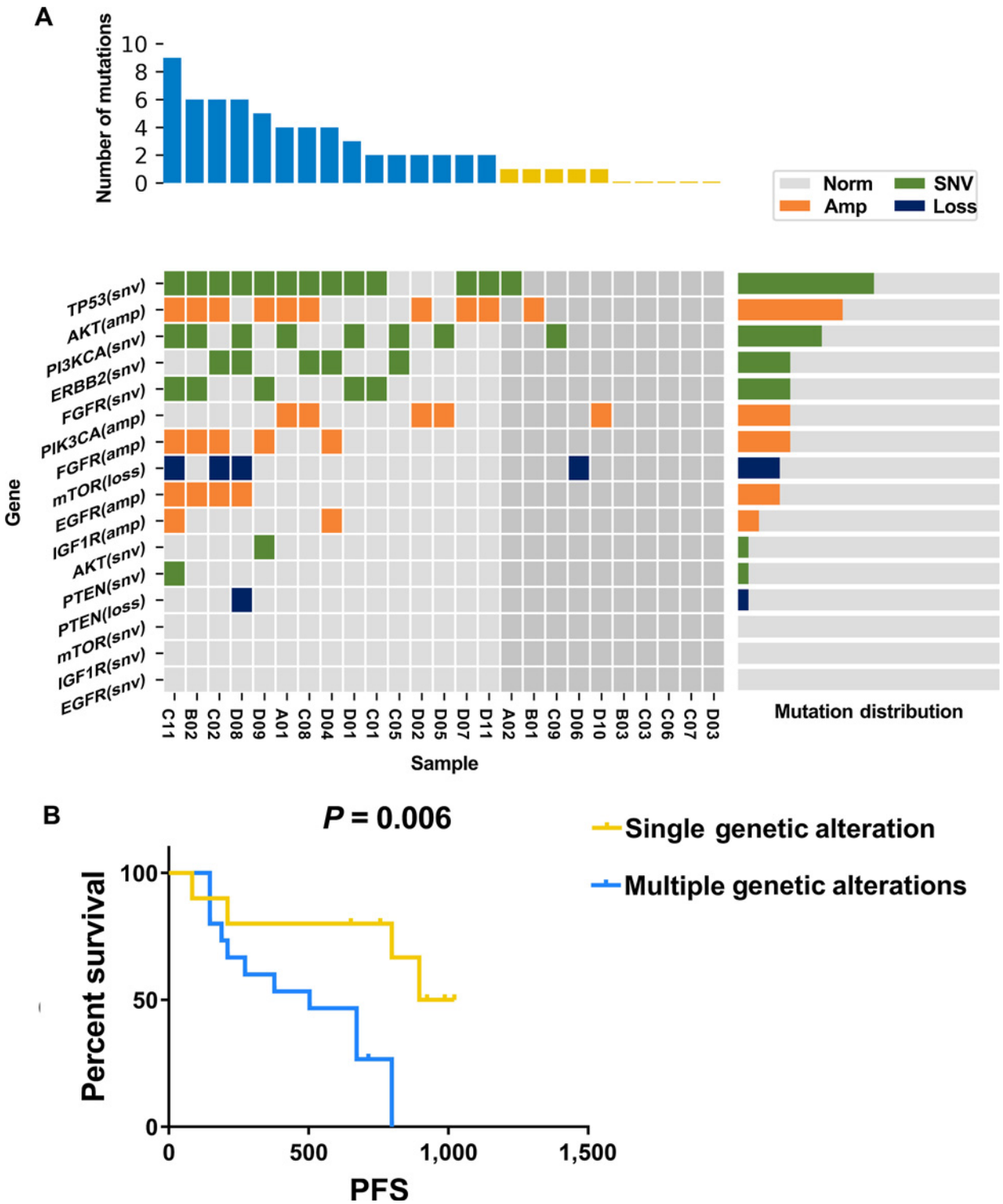

Figure 3.

Mutation distribution of all genetic alteration statuses of HER2 bypass signaling pathway, PI3K/Akt/mTOR pathway and TP53 in ctDNA of baseline samples and its association with progression-free survival. **A,** Mutation distribution of all genetic alteration statuses of HER2 bypass signaling pathway, PI3K/Akt/mTOR pathway, and TP53 in ctDNA of baseline samples. **B,** Kaplan–Meier analysis-based estimation of probabilities of progression-free survival in enrolled patients in accordance with all genetic alteration statuses of HER2 bypass signaling pathway, PI3K/Akt/mTOR pathway, and TP53 in ctDNA of baseline samples. Multiple genetic alteration, patients with two or more genetic alterations; single genetic alteration, patients with none or one genetic alteration.

clinical significance by investigators. Reduction in left ventricular ejection fraction of ≥10% occurred in 2 (1.9%) patients in the neratinib combination study (19). However, toxicities of neratinib and pyrotinib could not be compared directly in these two trials with totally different populations and sample size. Further study with additional data is required to investigate the comparative toxicity of these two agents.

The combination of pyrotinib and capecitabine demonstrated very promising efficacy results in patients with HER2-positive MBC. In other studies of anti-HER1/HER2 TKIs (9, 19, 20), when combined with capecitabine, lapatinib demonstrated an ORR of 23.7% (95% CI, 18.0%–30.3%) and a median TTP of 27.1 weeks, and neratinib was associated with an ORR of 64% (95% CI, 51%–76%) and a median PFS of 40.3 weeks for patients with no prior lapatinib treatment. In this study, pyrotinib plus capecitabine demonstrated a promising ORR of 78.6% (95% CI, 59.0%–91.7%) and a TTP of 22.1 months (95.8 weeks). The 400 mg pyrotinib combination cohort demonstrated an even higher ORR of 90.9%. In 17 (60.7%) trastuzumab-pretreated patients, the ORR was 70.6%. In 11 patients who were pretreated with trastuzumab for metastatic disease, the ORR was 72.7% (Supplementary Table S6). These data suggested promising efficacy of pyrotinib plus capecitabine in trastuzumab-pretreated patients. However, this study included more trastuzumab-naïve patients (11 patients, 39.3%), and no patient had previous pertuzumab or T-DM1 exposure, which may lead to overestimation of efficacy results. Because of different study designs and relatively small sample size, efficacy results of these studies could not be compared directly. A randomized phase II trial comparing pyrotinib plus capecitabine and lapatinib plus capecitabine was reported at the San Antonio Breast Cancer Symposium 2017 (21), which demonstrated a significantly improved ORR (78.5% vs. 57.1%, $P = 0.010$) and PFS (18.1 months vs. 7.0 months, $P < 0.0001$) with pyrotinib plus capecitabine. However, 46.2% patients were trastuzumab-naïve in this phase II trial. The toxicity profile indicated higher rates of grade 3–4 diarrhea (15.4% vs. 4.8%), neutropenia (9.2% vs. 3.2%), and vomiting (4.6% vs. 1.6%) in pyrotinib combination group. Hyperbilirubinemia was more frequent in lapatinib plus capecitabine (30.8% vs. 49.2%). A phase III trial comparing pyrotinib plus capecitabine and lapatinib plus capecitabine in trastuzumab-pretreated patients (NCT03080805) was ongoing.

In the exploratory biomarker analysis, no single genetic alteration was observed to have predictive or prognostic value. However, when all genetic variations, including both SNVs and CNVs of HER2 bypass signaling pathway, PI3K/Akt/mTOR pathway, and TP53 were analyzed, it demonstrated that multiple (two or more) genetic variations were associated with significantly worse survival compared with one or none genetic variation. Similar results were observed with concomitant genetic alterations in EGFR-mutant advanced non–small cell lung cancer (22).

According to the evidence of significant molecular heterogeneity in breast cancer (23, 24), multiple mechanisms involved in signal transduction molecules are demonstrated to be associated with the primary or acquired resistance of anti-HER2 therapy (25), including upregulation of PI3K/Akt/mTOR pathway (26, 27), loss of PTEN (28, 29), amplification of EGFR genes (30, 31), and overexpression of IGF1R (32–34). In addition, both SNVs and CNVs contribute to the evolution of breast cancer progression (35). Previous retrospective studies suggested that genome-wide copy number profiles in ctDNA samples are correlated with significantly worse survival ($P < 0.001$) and remained significant independent of clinicopathologic factors (HR, 2.14; 95% CI, 1.4–3.8; $P < 0.001$; ref. 36). The biomarker analysis in this study suggested potential predictive and prognostic significance of multiple genetic variations including both SNVs and CNVs within all genetic variations in HER2 bypass signaling pathway, PI3K/Akt/mTOR pathway, and TP53 via ctDNA for anti-HER2 therapy in MBC.

In the biomarker analysis of previous pyrotinib monotherapy trial (16), PIK3CA and/or TP53 mutations predicted a poorer response from pyrotinib. Because this correlation is not observed in this combination study, this inconsistency is probably due to the addition of capecitabine. A single biomarker is no longer predictive for the chemotherapy and target therapy combination. Sample size of this study also limited its power to achieve statistical significance. Biomarker analysis of the further phase II and III studies would probably bring us more information for the prediction of anti-HER2 resistance.

In summary, in a population largely naïve to HER2-targeted therapy, pyrotinib 400 mg plus capecitabine 2,000 mg/m^2 per day has promising antitumor activity and may be safely administered in pretreated patients with HER2-positive MBC.

Disclosure of Potential Conflicts of Interest

X. Zhu is a senior medical director at Jiangsu Hengrui Medicine Co., Ltd. J. Zou is an employee of Jiangsu Hengrui Medicine Co., Ltd. No potential conflicts of interest were disclosed by the other authors.

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