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ORIGINAL REPORT

Phase I Study and Biomarker Analysis of Pyrotinib, a Novel Irreversible Pan-ErbB Receptor Tyrosine Kinase Inhibitor, in Patients With Human Epidermal Growth Factor Receptor 2–Positive Metastatic Breast Cancer

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ABSTRACT

Purpose
This phase I study assessed the safety, tolerability, pharmacokinetics, antitumor activity, and predictive biomarkers of pyrotinib, an irreversible pan-ErbB inhibitor, in patients with human epidermal growth factor receptor 2 (HER2)–positive metastatic breast cancer.

Patients and Methods
Pyrotinib was administered continuously, orally, once per day to patients who did not have prior exposure to tyrosine kinase inhibitors of HER2. Planned dose escalation was 80, 160, 240, 320, 400, and 480 mg. For pharmacokinetic analysis, timed blood samples were collected on day 1 and day 28. Next-generation sequencing was performed on circulating tumor DNA and genomic DNA from tumor samples.

Results
Thirty-eight patients were enrolled. The dose-limiting toxicity was grade 3 diarrhea, which occurred in two patients administered 480 mg of pyrotinib; thus, the maximum tolerated dose was 400 mg. Common pyrotinib-related adverse events included diarrhea (44.7% [17 of 38]), nausea (13.2% [five of 38]), oral ulceration (13.2% [five of 38]), asthenia (10.5% [four of 38]), and leukopenia (10.5% [four of 38]). The only grade 3 adverse event was diarrhea. Pharmacokinetic analyses indicated that pyrotinib exposure was dose dependent. The overall response rate was 50.0% (18 of 36), and the clinical benefit rate (complete response + partial response + stable disease ≥ 24 weeks) was 61.1% (22 of 36). The median progression-free survival was 35.4 weeks (95% CI, 23.3 to 40.0 weeks). The overall response rate was 83.3% (10 of 12) in trastuzumab-naive patients and 33.3% (eight of 24) in trastuzumab-pretreated patients. Preliminary results suggest that *PIK3CA* and *TP53* mutations in circulating tumor DNA (P = .013) rather than in archival tumor tissues (P = .474) may predict the efficacy of pyrotinib.

Conclusion
Continuous once-per-day pyrotinib was well tolerated and demonstrated promising antitumor activity in HER2-positive patients with metastatic breast cancer. The maximum tolerated dose was established as 400 mg. Diarrhea was the dose-limiting toxicity. The promising antitumor activity and acceptable tolerability of pyrotinib warrant its further evaluation in a phase II study.

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ASSOCIATED CONTENT

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Appendix

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Data Supplement

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INTRODUCTION

Overexpression of human epidermal growth factor receptor 2 (HER2) is observed in 15% to 30% of patients with breast cancer and is associated with a more aggressive phenotype, a shorter time to recurrence, and a poorer prognosis.[1-3] The treatment and outcome of patients with HER2- positive breast cancer have been significantly improved by the introduction of anti-HER2 agents, such as trastuzumab,[4] lapatinib,[5] pertuzumab,[6] ado-trastuzumab emtansine,[7] and neratinib.[8] However, these agents have limitations with respect to cardiac[9,10] or GI[5,8] adverse effects (AEs), which may hamper continuous administration. In addition, some patients tend to acquire resistance shortly after initial response to HER2-directed

therapy.[6,11] Thus, continued development of new anti-HER2 strategies is needed to offer alternative treatment options for patients who are intolerant to or resistant to standard therapies.

Pyrotinib is an oral, irreversible pan-ErbB receptor tyrosine kinase inhibitor (TKI) with activity against epidermal growth factor receptor (EGFR)/HER1, HER2, and HER4.[12] Preclinical data suggest that pyrotinib can irreversibly inhibit multiple ErbB receptors and effectively inhibit the proliferation of HER2-overexpressing cells both in vivo and in vitro (see Appendix, online only).

Mutations in certain genes, such as *PIK3CA*, *TP53*, *PTEN*, and *AKT*, have been reported to correlate with the outcome of anti-HER2 treatment. However, previous studies evaluating the predictive value of gene mutations on the basis of primary tumor samples are controversial.[13,14] One possible explanation is the heterogeneity of intratumor genes.[15,16] Biopsies of the primary tumor could not reflect the whole picture of gene mutations for the patient, especially for patients with different mutation status between metastatic and primary tumors. Circulating tumor DNA (ctDNA) has been suggested as an alternative to tumor biopsies for mutational analysis because of its noninvasive and real-time[17,18] applications. We hypothesized that mutational analysis of ctDNA would overcome intratumor heterogeneity and would be more precise in identifying predictive biomarkers compared with identifying mutations in archival tumor samples.

PATIENTS AND METHODS

Study Design

This was a single-center, open-label, phase I ascending multiple oral dose study conducted in China to determine the safety, tolerability, pharmacokinetics (PK), and primary antitumor activity of pyrotinib in patients with HER2-positive metastatic breast cancer (MBC). This study was conducted in accordance with the International Conference on Harmonization Guideline for Good Clinical Practice and the ethical principles originating in the Declaration of Helsinki. The study protocol was approved by an institutional review board, and written informed consent was obtained from all patients before their enrollment in this study.

Patient Eligibility

Patients were eligible for enrollment if they were between 18 and 70 years of age, had a histologic or cytologic diagnosis of MBC, were HER2 positive (immunochemistry 3+, or immunochemistry 2+ confirmed by fluorescent in situ hybridization), had a performance status of 0 to 1 on the Eastern Cooperative Oncology Group scale, and had adequate bone marrow and organ function. Patients were excluded if they had received prior treatment with small molecular anti-HER2 TKIs.

Dose Escalation

A traditional 3+3 design was used for dose escalation. Patients received oral doses of pyrotinib. Dosing consisted of 80, 160, 240, 320, 400, and 480 mg continuously administered once per day in 28-day cycles. Three patients were initially assigned to a starting dose level of 80 mg. If no patients experienced a dose-limiting toxicity (DLT) by day 28 of continuous daily dosing, then three patients were enrolled at the next dose level. If one patient experienced a DLT, then an additional three patients were treated at the same dose level. The dose was escalated if no more than one of the six patients had a DLT. If two patients at a dose level experienced a DLT, dose escalation stopped, and the previous dose level was considered the maximum tolerated dose (MTD).

According to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0, a DLT was defined as any pyrotinib-related hematologic grade 4 AE, grade 3 neutropenia with fever $\geq$ 38.5°C, grade 3 thrombocytopenia with bleeding tendency, grade 2 heart failure, or grade 3 or 4 other nonhematologic AE.

Evaluation of Patients

Safety evaluations were conducted at screening on days 7, 14, and 28 of cycle 1; on days 14 and 28 of cycle 2; on day 28 of cycles 3 to 12; on day 84 of every three cycles of cycles 13 to 24; and on day 168 of every six cycles after cycle 24. Safety assessments included laboratory variables, vital signs, interim medical history, radiographs, and echocardiograms. An efficacy evaluation was performed in accordance with Response Evaluation Criteria in Solid Tumors (RECIST) guidelines version 1.1[19] every two cycles from the first dose to cycle 12, then every three cycles from cycles 13 to 24, and every six cycles after cycle 24. Complete response (CR) or partial response (PR) had to be confirmed at least 4 weeks after initial response.

PK Analyses

Blood samples for PK analyses of pyrotinib were collected on days 1 and 28. Samples were obtained at predose and at 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after the dose. Plasma concentrations were measured by using a validated liquid chromatography-tandem mass spectrometry method. The bioanalytical method used 0.100 mL of plasma and was linear over the range of 0.500 (the lower limit of quantification) to 250 ng/mL. The plasma samples were stored at –70°C until analysis. PK analyses were performed by using Phoenix WinNonLin (Pharsight, Mountain View, CA) version 6.3. A standard noncompartmental method was used to calculate the peak plasma concentration (C_{max}), the time to C_{max} (t_{max}), the total area under the concentration-time curve ($AUC_{0\ to\ \infty}$), the area under the concentration-time curve to the 24-hour postdose ($AUC_{0\text{-}24\ hours}$) or at steady state (AUC_{ss}), the elimination half-life ($t_{1/2}$), and the accumulation ratio ($R = AUC_{ss}/AUC_{0\text{-}24\ hours}$).

Biomarker Analyses

All biomarker analyses were prospectively planned. Informed consent for collection of blood and archival tumor samples was obtained from 18 and 19 patients, respectively. Next-generation sequencing of 368 genes was performed on ctDNA and genomic DNA of baseline blood and archival tumor samples. Detailed protocols for ctDNA and genomic DNA sequencing are provided in the Appendix.

RESULTS

Patients

Thirty-eight female patients (median age, 46.9 years; range, 29 to 67 years) were enrolled from August 2013 to March 2015. The baseline characteristics of the 38 patients are presented in Table 1. Most of the patients were heavily pretreated; 50.0% (19 of 38) received three or more previous chemotherapy regimens in the metastatic setting. Twenty-five (65.8%) received prior trastuzumab-containing treatment, including seven patients who received trastuzumab in the adjuvant setting, 12 in the metastatic setting, and six in both. In addition, 81.6% of the patients (31 of 38) presented with visceral metastasis on study entry.

Dose Escalation of Pyrotinib

Grade 3 diarrhea was the only reported DLT for one (11.1%), two (25.0%), and two (100%) patients in the 320 mg, 400 mg, and

Table 1. Patients' Demographic and Baseline Characteristics

Characteristic	Pyrotinib Dose Cohorts (mg)													
	80 (n = 3)		160 (n = 8)		240 (n = 8)		320 (n = 9)		400 (n = 8)		480 (n = 2)		Total (n = 38)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Median age, years (range)	44.5 (32-55)		45.2 (29-65)		50.0 (38-62)		44.9 (30-56)		48.8 (39-67)		53.5 (52-55)		46.9 (29-67)	
ECOG performance status														
0	1	33.3	6	75.0	8	100.0	9	100.0	8	100.0	2	100.0	34	89.5
1	2	66.7	2	25.0	0		0		0		0		4	10.5
HR														
Positive	0		6	75.0	5	62.5	8	88.9	5	62.5	1	50.0	25	65.8
Negative	3	100.0	1	12.5	3	37.5	1	11.1	3	37.5	1	50.0	12	31.6
Unknown	0		1	12.5	0		0		0		0		1	2.6
Median No. of metastatic organs (range)	2.0 (1-4)		2.0 (1-4)		2.0 (1-4)		2.0 (1-4)		1.5 (1-2)		2.0 (2-2)		2.0 (1-4)	
No. of patients with visceral metastasis	3	100.0	5	62.5	7	87.5	6	66.7	8	100.0	2	100.0	31	81.6
No. of prior metastatic anticancer regimens														
< 3	1	33.3	6	75.0	4	50.0	1	11.1	6	75.0	1	50.0	19	50.0
≥ 3	2	66.7	2	25.0	4	50.0	8	88.9	2	25.0	1	50.0	19	50.0
Prior taxane treatment	3	100.0	8	100.0	8	100.0	9	100.0	8	100.0	2	100.0	38	100.0
Prior anthracycline treatment	3	100.0	7	87.5	7	87.5	8	88.9	7	87.5	2	100.0	34	89.5
Prior trastuzumab therapy	2	66.7	3	37.5	8	100.0	5	55.6	6	75.0	1	50.0	25	65.8

Abbreviations: ECOG, Eastern Cooperative Oncology Group; HR, hormone receptor.

480 mg dose cohorts, respectively, in this study (Appendix Table A1, online only). Because more than 33.3% of patients (two of two) in the 480-mg dose group experienced a DLT, pyrotinib 400 mg once per day was determined to be the MTD.

Safety

All 36 patients in the 80- to 400-mg dose cohorts completed the continual daily dose period of the first 28 days. The median duration of treatment was 32.0 weeks (range, 6.1 to 120.0 weeks). The median relative dose intensity was 100% for each dose level (range in the 400-mg MTD cohort, 79.5% to 100%). Two patients in the 480-mg cohort experienced grade 3 diarrhea and discontinued the study before day 28.

Pyrotinib-related AEs of any grade were observed in 28 patients (73.7%; Table 2). Pyrotinib-related AEs of any grade observed in more than 10% of patients included diarrhea (44.7% [17 of 38]), nausea (13.2% [five of 38]), oral ulceration (13.2% [five of 38]), asthenia (10.5% [four of 38]), and leukopenia (10.5% [four of 38]). Diarrhea was the only grade 3 pyrotinib-related AE (13.2% [five of 38]). No grade 4 or 5 pyrotinib-related AEs were reported.

The incidence and characteristics of diarrheal events are summarized in Appendix Table A2 (online only). The median onset of diarrhea was 7.5 days after start of treatment and the median duration was 4 days. Most diarrhea events were reported during the first cycle of treatment and the frequency declined in the subsequent cycles (Appendix Fig A1, online only). All diarrheal AEs were considered pyrotinib-related. Grade 3 diarrhea was managed by appropriate medication (such as loperamide). In the 80- to 400-mg dose cohorts, only one patient in the 400-mg cohort discontinued pyrotinib for 1 day because of grade 3 diarrhea and restarted without dose reduction; all other patients who experienced diarrhea resolved without dose interruption or dose reduction (Appendix Table A2). Detailed management of diarrhea is described in Appendix Table A3 (online only).

Increased liver function tests considered to be of potential clinical importance included increased AST in two (5.3%) and increased ALT in three (7.9%) of 38 patients during treatment. One patient (2.6%) presented with increased grade 3 AST, one (2.6%) with increased grade 1 AST, and three (7.9%) with increased grade 1 ALT. At the final visit, increased AST and ALT levels were present in one (2.6%) and two (5.3%) patients, respectively. No cardiovascular AE was reported in this study.

One death resulting from progression of liver metastases was reported in the 240-mg cohort 9 days after the last dose of study drug was administered. A total of 4 patients (10.5%) experienced AEs requiring treatment interruptions (one in the 400-mg dose group as a result of grade 1 acute febrile pharyngitis, one in the 400-mg dose group, and two in the 480-mg dose group as a result of grade 3 diarrhea).

Antitumor Activity

A summary of the confirmed best overall response on the basis of investigator review is provided in Table 3 and Figure 1. Given that two patients in the 480-mg cohort discontinued the study before evaluation, the remaining 36 patients (in the 80- to 400-mg dose cohorts) were considered evaluable for efficacy. A total of 18 patients (50%) achieved a best response of PR, four (11.1%) had stable disease (SD) ≥ 24 weeks, and seven (19.4%) had progressive disease (PD). The overall response rate (ORR: CR + PR) was 50.0% (95% CI, 32.9% to 67.1%) for all 36 patients and was 87.5% (95% CI, 47.3% to 99.7%) for the 400-mg dose cohort (n = 8). The clinical benefit rate (CR + PR + SD ≥ 24 weeks) was 61.1% (95% CI, 43.5% to 76.9%) for all 36 patients and 100.0% (95% CI, 63.1% to 100.0%) for the 400-mg dose cohort. The median time to response was 8.0 weeks. The median duration of response for the 18 PR patients was 32.4 weeks (95% CI, 27.7 to 52.1 weeks).

Of 36 patients with evaluable efficacy, 24 had previously received trastuzumab (Fig 2). The best ORR was 33.3% (eight of

Table 2. Pyrotinib-Related AEs of All Grades That Occurred in All Patients and of Grade ≥ 3 That Occurred in One or More Patients From Screening Visit Until 28 Days After Last Dose of Pyrotinib

	Pyrotinib Dose Cohorts (mg)													
	80 (n = 3)		160 (n = 8)		240 (n = 8)		320 (n = 9)		400 (n = 8)		480 (n = 2)		Total (n = 38)	
AE	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Diarrhea	0		0		4	50.0	4	44.4	7	87.5	2	100.0	17	44.7
Grade ≥ 3	0		0		0		1	11.1	2	25.0	2	100.0	5	13.2
Nausea	0		2	25.0	1	12.5	0		1	12.5	1	50.0	5	13.2
Oral ulceration	0		0		0		1	11.1	3	37.5	1	50.0	5	13.2
Asthenia	0		3	37.5	1	12.5	0		0		0		4	10.5
Leukopenia	0		2	25.0	1	12.5	0		1	12.5	0		4	10.5
PPE	0		1	12.5	0		0		2	25.0	0		3	7.9
Increased ALT	0		0		1	12.5	1	11.1	1	12.5	0		3	7.9
Increased creatinine	0		0		0		3	33.3	0		0		3	7.9
Rash	0		0		1	12.5	0		1	12.5	0		2	5.3
Abdominal pain	0		0		0		0		2	25.0	0		2	5.3
Dyspepsia	0		0		1	12.5	1	11.1	0		0		2	5.3
Neutropenia	0		1	12.5	0		0		1	12.5	0		2	5.3
Increased AST	0		0		1	12.5	1	11.1	0		0		2	5.3
Decreased hemoglobin	0		0		0		0		2	25.0	0		2	5.3
Vomiting	0		1	12.5	0		0		0		1	50.0	2	5.3
Constipation	0		0		0		0		1	12.5	0		1	2.6
Stomatitis	0		0		0		0		1	12.5	0		1	2.6
Toothache	0		1	12.5	0		0		0		0		1	2.6
Skin lesion	0		0		0		1	11.1	0		0		1	2.6
Decreased appetite	0		0		0		0		1	12.5	0		1	2.6
Dizziness	1	33.3	0		0		0		0		0		1	2.6
Muscle spasms	0		0		0		0		1	12.5	0		1	2.6
Dyspnea	0		0		0		0		1	12.5	0		1	2.6
Anemia	0		0		0		0		1	12.5	0		1	2.6

NOTE. Pyrotinib-related adverse events (AEs) includes definitely related AEs and probably related AEs.
Abbreviation: PPE, palmoplantar erythrodysesthesia.

24) in patients who previously received trastuzumab and 83.3% (10 of 12) in trastuzumab-naive patients (χ^2 test $P = .001$). In seven patients who received trastuzumab in the adjuvant setting, the ORR of pyrotinib was 42.9% (three of seven). In 11 patients who received trastuzumab in the metastatic setting, the ORR was 36.4% (four of 11), and in patients with trastuzumab exposure in both adjuvant and metastatic settings, the ORR was 16.7% (one of six). In addition, in 16 patients with PD who were receiving trastuzumab (in either the adjuvant or the metastatic setting), the ORR was 31.3% (five of 16).

The median progression-free survival was 35.4 weeks (95% CI, 23.3 to 40.0 weeks) for all patients in the 80- to 400-mg dose cohorts and 59.7 weeks (95% CI, 35.4 to 71.9 weeks) for the 400-mg dose cohort. At the time of data cutoff (December 31, 2015), there were four patients ongoing with pyrotinib treatment.

PK

Plasma samples for PK analyses were available for all 36 patients who received pyrotinib doses ranging from 80 to 400 mg. The PK parameters are summarized in Table 4.

After the first dose of pyrotinib from 80 to 400 mg on study day 1, the absorption of pyrotinib was relatively slow with a median t_{max} of 3.00 to 5.00 hours; mean $t_{1/2}$ ranged from 11.4 to 15.9 hours. Multiple-dose plasma concentration reached a steady state on day 8, and the exposure was 1.22- to 1.57-fold greater than single-dose exposure across 80- to 400-mg doses, as assessed by the mean accumulation ratio (R: AUC_{ss} on study day 28 to $AUC_{0\text{-}24\ hours}$ on study day 1; Table 4). These results suggest that there is no major accumulation of pyrotinib after repeated daily administration.

A linear regression model was adopted to assess the relationship between dose and exposure at steady state (Appendix Fig A2, online only). The results indicate that C_{max} ($\beta = 0.8092$) and AUC_{ss} ($\beta = 0.9066$) both increased in a dose-dependent manner, suggesting a linear relationship for C_{max} and AUC_{ss} versus dose.

Biomarker Analyses

PIK3CA and *TP53* were the two most frequently mutated genes detected in the ctDNA of 18 patients whose blood samples were available. In ctDNA mutational analysis, all eight patients with *PIK3CA* or *TP53* mutations were relatively insensitive to pyrotinib, with a best response of SD or PD. In contrast, a significantly higher response rate was seen in patients with wild-type *PIK3CA* and *TP53* (six of 10 [60.0%]; $P = .013$; Appendix Table A4, online only). Radiologic progression-free survival was significantly longer in patients with wild-type *PIK3CA* and *TP53* than in patients with mutated *PIK3CA* or *TP53* (median, 46.1 *v* 14.9 weeks; log-rank test $P = .015$). In mutational analysis of 19 patients with archival primary tumor samples, the combination of *PIK3CA* and *TP53* mutation status was not correlated with objective responses ($P = .474$; Appendix Table A4). A total of 12 patients had mutational data in both ctDNA and archival tumor tissues, and five of

Table 3. Best ORR in the Evaluable Population (cutoff date: December 31, 2015)

Response	Pyrotinib Dose Cohorts (mg)												Total (n = 36)		
	80 (n = 3)			160 (n = 8)			240 (n = 8)			320 (n = 9)			400 (n = 8)					
	No.	%	95% CI	No.	%	95% CI	No.	%	95% CI	No.	%	95% CI	No.	%	95% CI	No.	%	95% CI
CR	0			0			0			0			0			0		
PR	0			4	50.0		2	25.0		5	55.6		7	87.5		18	50.0	
SD, weeks																		
8-24	1	33.3		3	37.5		2	25.0		1	11.1		0			7	19.4	
≥ 24	1	33.3		1	12.5		1	12.5		0			1	12.5		4	11.1	
PD	1	33.3		0			3	37.5		3	33.3		0			7	19.4	
ORR		0	0.0 to 70.8		50.0	15.7 to 84.3		25.0	3.2 to 65.1		55.6	21.2 to 86.3		87.5	47.3 to 99.7		50.0	32.9 to 67.1
DCR		66.7	9.4 to 99.2		100.0	63.1 to 100.0		62.5	24.5 to 91.5		66.7	29.9 to 92.5		100.0	63.1 to 100.0		80.6	64.0 to 91.8
CBR		33.3	0.8 to 90.6		62.5	24.5 to 91.5		37.5	8.5 to 75.5		55.6	21.2 to 86.3		100.0	63.1 to 100.0		61.1	43.5 to 76.9
Median PFS, weeks (minimum-maximum)	23.71 (7.86-119.86)			31.71 (12.14-95.43)			14.64 (7.43-96.00)			31.86 (7.57-60.00)			59.43 (35.43-71.86)			35.43 (7.43-119.86)		

Abbreviations: CBR, clinical benefit rate (CR + PR + SD ≥ 24 weeks); CR, complete response; DCR, disease control rate (CR + PR + SD); ORR, objective response rate (CR + PR); PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

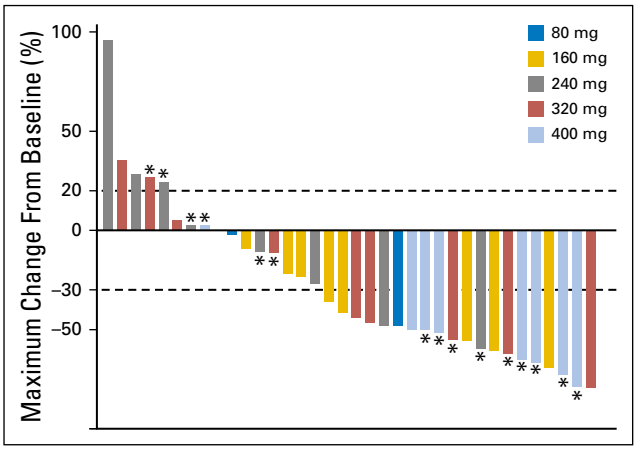

Fig 1. Maximum reduction of target lesions from baseline for patients in the 80- to 400-mg dose cohorts. The best response for target lesions per patient was determined on the basis of RECIST criteria. Dashed lines at 230 and 20 indicate RECIST criteria for progressive disease and partial response, respectively. (*) Patients who reported diarrheal events.

them had different results between ctDNA and tumor samples according to *PIK3CA* and *TP53* mutation status. These five patients had *PIK3CA* and/or *TP53* mutations in tumor samples, but were *PIK3CA* and *TP53* wild-type in ctDNA tests. Meanwhile, these five patients experienced a best response of PR or SD ≥ 24 weeks, which was consistent with the mutation results of ctDNA (Appendix Table A5, online only).

DISCUSSION

In this phase I study, pyrotinib was orally administered to patients with HER2-positive MBC. One patient in each of the 320-mg and 400-mg dose cohorts and two patients in the 480-mg dose cohort experienced grade 3 diarrhea; therefore, the MTD of pyrotinib was determined to be 400 mg once per day. Although diarrhea was reported by 17 patients (44.7%), no patient reported a grade ≥ 4 event. All pyrotinib-related diarrhea events were effectively controlled by use of antidiarrheal agents, and only one patient experienced dose interruption for 1 day because of diarrhea. The characteristics of diarrhea were similar to those reported for neratinib[20] (an irreversible pan-ErbB inhibitor) with a median onset of 7.5 days after study entry and a median duration of 4 days.

Other less frequently reported pyrotinib-related events (in 10% to 15% of patients) such as nausea, oral ulceration, asthenia, and leukopenia were also reported in neratinib monotherapy studies with numerically higher incidences.[20,21] Incidences of all grades and grade 3 diarrhea seem to be dose related. However, because of the small number of patients at each level, no significant relationships between dose level and AE severity could be defined.

Pyrotinib demonstrated very promising efficacy in patients with breast cancer. In this heavily pretreated cohort with 65.8% of patients who relapsed after trastuzumab therapy and with half the patients who received three or more chemotherapeutic regimens for advanced disease, an ORR of 50.0% and a clinical benefit rate (CBR) of 61.1% were impressive. In previous monotherapy studies

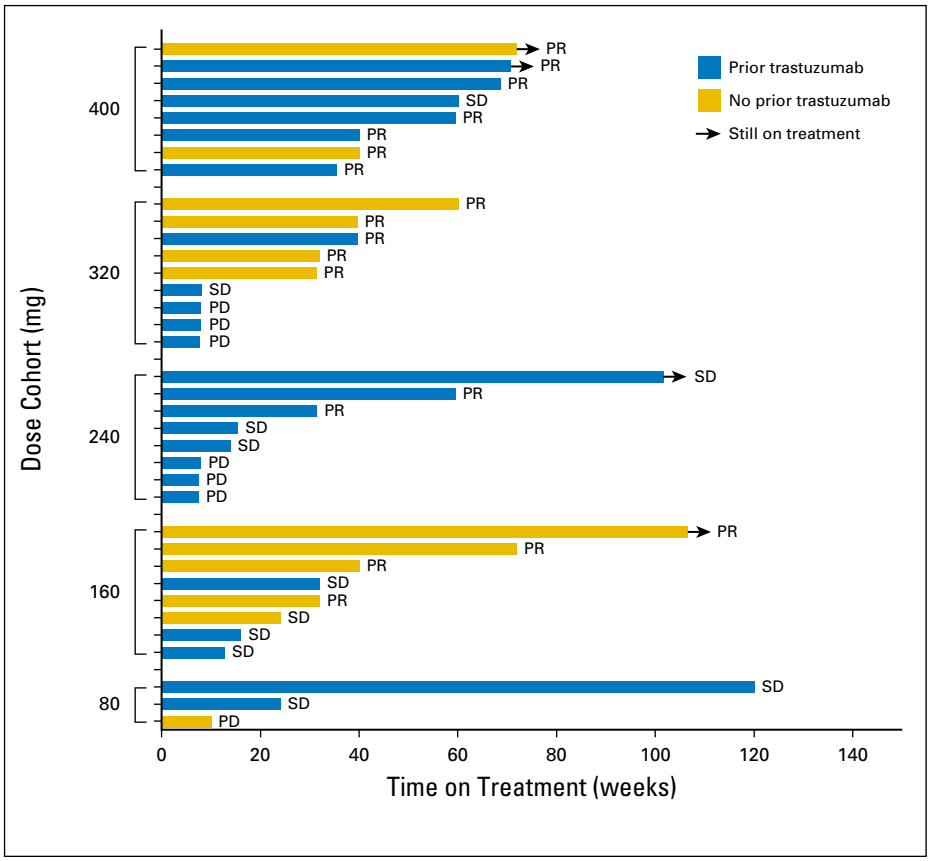

Fig 2. Time on treatment for patients in the 80- to 400-mg dose cohorts. All data were cut off on December 31, 2015. PD, progressive disease; PR, partial response; SD, stable disease.

Table 4. PK Parameters of Pyrotinib in Chinese Patients With MBC

		Parameter (CV%)													
		C_{max} (ng/mL)		t_{max} (h)		$t_{1/2}$ (h)		AUC (ng × h/mL)*		CL/F (L/h/kg)		V_z/F (L/kg)		*R*	
Dose (mg)	No.	Mean	%	Median	Range	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
Study day 1															
80	3	38.8	10.8	3.00	2.00-4.00	9.43	22.3	415	5.0	—		—		—	
160	8	80.8	27.7	4.50	2.00-6.00	11.7	16.7	926	25.7	—		—		—	
240	8	98.7	69.4	5.00	3.00-6.00	16.5	41.3	1,320	73.7	—		—		—	
320	9	143	38.0	4.00	3.00-12.00	13.0	26.2	1,970	36.4	—		—		—	
400	8	147	57.2	4.00	1.00-6.00	12.2	34.1	1,860	67.3	—		—		—	
Study day 28															
80	3	43.0	36.0	2.00	2.00-3.00	11.4	31.7	549	25.0	149	25.8	2,570	39.9	1.32	28.3
160	8	102	46.1	3.50	1.00-4.00	14.1	39.8	1,260	46.1	141	48.8	3,110	60.3	1.35	28.0
240	8	156	91.0	4.00	3.00-5.00	15.0	39.9	2,080	110.1	136	46.5	2,960	60.1	1.57	28.4
320	9	175	32.8	4.00	0.00-6.00	15.9	37.5	2,660	29	125	29.9	3,040	45.9	1.35	38.6
400	8	170	54.4	3.00	2.00-4.00	12.9	18.5	2,270	59.3	213	66.8	3,820	55.7	1.22	35.9

Abbreviations: AUC, area under the concentration-time curve; C_{max}, peak concentration; CL/F, apparent oral dose clearance; CV%, percent coefficient of variation; MBC, metastatic breast cancer; *R*, accumulation ratio (quotient of $AUC_{0\text{-}24\ hours}$ on day 28 to $AUC_{0\text{-}24\ hours}$ on study day 1); $t_{1/2}$, terminal phase elimination half-life; t_{max}, time to peak concentration; V_z/F, apparent volume of distribution.
*Reported as AUC from time zero extrapolated to 24 hours ($AUC_{0\text{-}24\ hours}$) for study days 1 and 28.

of other small molecular anti-HER1/HER2 TKIs,[20-23] for example, in a phase I trial of neratinib in 25 patients with breast cancer, the ORR was 32% and the CBR was 36%,[20] and lapatinib demonstrated a response of only 4.3% as monotherapy in patients with MBC.[24] Although the ORR and CBR were numerically higher in our study, we could not make direct comparison on the basis of the small sample sizes and different cohorts of these studies. In the 400-mg dose group (n = 8) in our study, the ORR and CBR were as high as 87.5% and 100.0%, respectively. The phase II study is ongoing to prove the efficacy of pyrotinib at the daily dose of 400 mg.

In our study, 83.3% (10 of 12) of patients with no prior trastuzumab exposure experienced PR to pyrotinib, which was significantly higher than in patients with prior trastuzumab exposure (eight of 24 [33.3%]; $P = .001$). Trastuzumab-naive patients made up most of the population that responded to pyrotinib. The ORR of pyrotinib in patients previously treated with trastuzumab in both adjuvant and metastatic settings was numerically lower than that in patients with trastuzumab exposure in either an adjuvant or metastatic setting. However, because of the small sample size of our study, further phase II and III pyrotinib trials are required to evaluate the effects of prior trastuzumab exposure on the efficacy of pyrotinib.

In an exploratory biomarker analysis, significant discordance was observed between the mutational analysis of ctDNA and archival tumor tissue. Previous studies had reported controversial results regarding the relationship between *PIK3CA* mutations and responses to anti-HER2 treatment. In the NeoALTTO (Lapatinib With Trastuzumab for HER2-Positive Early Breast Cancer) trial, activating mutations in *PIK3CA* significantly predicted poorer response to neoadjuvant anti-HER2 therapy ($P = .012$).[25] However, in National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 (A Randomized Trial Comparing Doxorubicin and Cyclophosphamide Followed by Paclitaxel, With or Without Trastuzumab as Adjuvant Therapy in Node-Positive, HER2-Overexpressing Breast Cancer), when archived tissue blocks were used in a retrospective biomarker analysis, *PIK3CA* mutations were demonstrated to have no predictive value in adjuvant trastuzumab treatment.[26] Several meta-analyses also indicated no significant correlation between *PIK3CA* mutation status and the clinical benefit of anti-HER2 therapy in both early-stage and metastatic settings.[13,14] This inconsistency was probably the result of the heterogeneity between specimens and tumors as well as intratumor heterogeneity.[15,16] To the best of our knowledge, our study is the first to correlate the mutation status of *TP53* and *PIK3CA* in ctDNA with the benefit of anti-HER2 therapy. Mutational analysis of ctDNA could potentially overcome this inconsistency caused by intratumor heterogeneity and could become a candidate predictive biomarker for anti-HER2 therapy. Because of the small sample size in this study, further investigation with a larger sample size is required. Mutational analysis of plasma samples was more convenient and practical compared with repeated biopsies of metastatic sites.

In our previous study of dynamic ctDNA monitoring,[27] we evaluated the changes between pre- and post-treatment ctDNA sequencing results, and demonstrated that variations of post-treatment ctDNA profiling were in good concordance with CT scans in response assessments. An association was observed in baseline ctDNA between mutations in *TP53* and *PIK3CA* and primary resistance to trastuzumab (n = 6). In our present study, we analyzed the baseline mutation status of both ctDNA and archived tumor tissue, and compared the predictive and prognostic value between baseline ctDNA and archived tissue for all patients with ctDNA (n = 18). These two studies proposed two different aspects of potential ctDNA applications in future clinical practice: dynamic ctDNA monitoring might contribute to more accurate response assessments; baseline ctDNA profiling might serve as predictive as well as prognostic biomarker of anti-HER2 treatment.

In conclusion, pyrotinib is well-tolerated at a dose of 400 mg once per day, and its encouraging antitumor activity in patients with HER2-positive breast cancer warrants further evaluation in HER2-positive MBC phase II trials with a dose of 400 mg once per day. In addition, our preliminary results suggest that the mutation

status of ctDNA is a potential candidate biomarker for anti-HER2 therapy and merits further evaluation in future studies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

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Collection and assembly of data: All authors
Data analysis and interpretation: Fei Ma, Qiao Li, Binghe Xu
Manuscript writing: All authors
Final approval of manuscript: All authors
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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Phase I Study and Biomarker Analysis of Pyrotinib, a Novel Irreversible Pan-ErbB Receptor Tyrosine Kinase Inhibitor, in Patients with Human Epidermal Growth Factor Receptor 2–Positive Metastatic Breast Cancer

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Appendix

Biomarker Analyses

All biomarker analyses were prospectively planned. Informed consents for collection of blood and archival tumor samples were obtained from 18 and 19 patients, respectively. Both blood and tumor samples were collected before treatment was initiated. Plasma was separated at room temperature by centrifugation once at 3,000 $\times$ *g* for 10 minutes. Both plasma and peripheral blood cells were retained and stored at -80°C until circulating tumor DNA (ctDNA) and genomic DNA (gDNA) were extracted. gDNA from the formalin-fixed paraffin-embedded tumor samples was extracted by using QIAamp DNA FFPE Tumor Tissue Kit (Qiagen, Valencia, CA). ctDNA and gDNA from plasma samples were extracted by using the QIAamp Circulating Nucleic Acid Kit (Qiagen) and QIAamp DNA Blood Mini Kit (Qiagen), respectively, according to the manufacturer's instructions. ctDNA was sequenced to detect somatic alterations, whereas gDNA was adopted as the normal control.

Next-generation sequencing was performed on ctDNA and gDNA of baseline plasma and tumor samples. A total of 368 genes were selected from four sources: (1) known oncogenes and tumor suppressor genes, (2) genes that are targets of agents approved by the US Food and Drug Administration or have been assessed in clinical trials, (3) genes implicated in major cancer-related signaling pathways, and (4) genes identified in the findings of The Cancer Genome Atlas network, which covers 12 cancer types. Sequencing libraries were prepared from ctDNA by using KAPA DNA Library Preparation Kits (Kapa Biosystems, Wilmington, MA), and gDNA sequencing libraries were prepared by using the protocols recommended by the Illumina TruSeq DNA Library Preparation Kit (Illumina, San Diego, CA). DNA sequencing was performed on a HiSeq2500 sequencing system (Illumina) with 2 $\times$ 101 bp paired-end reads. The reads were aligned to the human genome build GRCh37 by using a Burrows-Wheeler aligner. Somatic single nucleotide variant was generated by using MuTect software.

All variants identified by the bioinformatics pipeline were manually reviewed by an experienced bioinformatics director to assess the quality of base calls, the mapping quality of the reads, and the overall read depth at the site. Variations meeting any of the following criteria were filtered: low base quality (Phred score $<$ 13) in all reads supporting the variation, mutant reads all in the plus or minus strands, all the reads with mutant alleles that did not meet mapping confidently (quality score $\geq$ 30), reads supported at variant position $<$ 3, and variants detected near the start or end of sequencing reads.

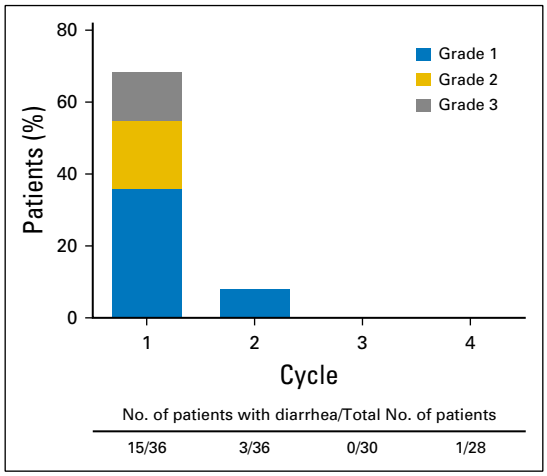

Fig A1. Reported diarrheal events over time for patients in all of the dose cohorts. For patients with multiple toxicity grades in a given cycle, the maximum grade was reported.

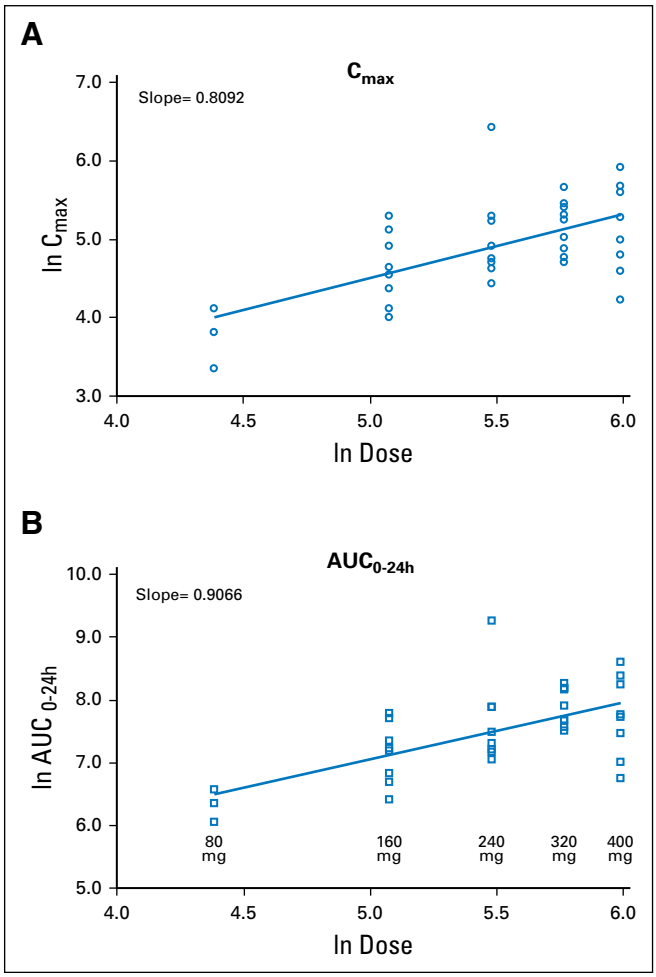

Fig A2. Individual and mean (standard deviation) plasma pyrotinib exposure versus dose on study day 28. (A) Peak concentration (C_{max}) versus dose and (B) area under the concentration-time curve from time zero to 24 hours after the dose ($AUC_{0\text{-}24\ hours}$) versus dose. Patients with human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer received oral doses of pyrotinib once per day.

Table A1. Summary of Dose-Escalation Cohorts and DLT Rates

	Pyrotinib Dose Cohorts (mg)									
	80 (n = 3)	160 (n = 8)	240 (n = 8)	320 (n = 9)		400 (n = 8)		480 (n = 2)		
Parameter				No.	%	No.	%	No.	%	
No. of patients with DLT	0	0	0	1		2		2		
No. of DLTs	0	0	0	1		2		2		
DLT rate	0	0	0		11.1		25.0		100.0	

NOTE. Grade 3 diarrhea was the only reported DLT in our study.
Abbreviation: DLT, dose-limiting toxicity.

Table A2. Summary and Management of Diarrhea AEs

	Pyrotinib Dose Cohorts (mg)											
	80 (n = 3)	160 (n = 8)	240 (n = 8)		320 (n = 9)		400 (n = 8)		480 (n = 2)		Total (n = 38)	
Parameter	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Incidence												
All grades	0	0	4	50.0	4	44.4	7	87.5	2	100.0	17	44.7
Grade 3 to 4	0	0	0		1	11.1	2	25.0	2	100.0	5	13.2
Median time to onset, days (range)	N/A	N/A	3.0 (2.0-14.0)		8.0 (2.0-24.0)		13.5 (2.0-54.0)		3.5 (1.0-55.0)		7.5 (2.0-55.0)	
Median duration of an event, days (range)	N/A	N/A	4.0 (1.0-15.0)		4.0 (1.0-13.0)		4.0 (1.0-66.0)		2.0 (1.0-55.0)		4.0 (1.0-66.0)	
Diarrhea management												
Received dose reduction	0	0	0		0		0		2	100.0	2	5.3
Received dose delay	0	0	0		0		1	12.5	1	50.0	2	5.3
Received antidiarrheal medication	0	0	0		1	11.1	2	25.0	2	100.0	5	13.2
Discontinued treatment because of diarrhea	0	0	0		0		0		2	100.0	2	5.3

Abbreviations: AE, adverse event; N/A, not applicable.

Table A3. Management of Diarrhea After First Cycle of Pyrotinib Treatment

Grade of diarrhea	Management
1 or 2	Continue pyrotinib and adjust diet.
≥ 3	Discontinue pyrotinib. Start loperamide at 4 mg followed by 2 mg after each episode of diarrhea (up to 16 mg/day). Administer isotonic solution (1 to 1.5 L/day) plus intravenous fluids if necessary. If diarrhea resolves to grade ≤ 1, continue with the same dose of pyrotinib. If dose discontinuation is required for a second time, dose reduction is required after diarrhea resolves to grade ≤ 1.

NOTE. Obtain thorough GI history and rule out infection.

Table A4. Comparison of Mutation Status of PIK3CA and TP53 Genes in Tumor Tissue and ctDNA

	No. of patients		
PIK3CA + TP53	Sensitive	Insensitive	Fisher's Exact Test *P*
Tumor tissue			
Wild-type	0	2	.474
Mutation	9	8	
ctDNA			
Wild-type	6	4	.013
Mutation	0	8	

NOTE. Sensitive patients were defined as patients who achieved a best response of complete or partial response; insensitive patients were defined as patients with a best response of stable or progressive disease according to RECIST. Mutational status of PIK3CA and TP53 was significantly related to response to pyrotinib ($P = .013$).
Abbreviation: ctDNA, circulating tumor DNA.

Table A5. Summary of 12 Patients With Mutational Data for ctDNA and Archival Tumor Tissues

	Mutation Status of PIK3CA and TP53		
Patient ID	Tumor Tissue	ctDNA	Best Response (weeks)
204	Mutated*	Wild-type	PR
205	Wild-type	Wild-type	SD < 24
206	Mutated†	Mutated‡	SD < 24
208	Mutated‡	Mutated‡	SD ≥ 24
304	Mutated*	Wild-type	PR
305	Mutated‡	Mutated*	SD < 24
306	Wild-type	Wild-type	PD
308	Mutated*	Wild-type	SD ≥ 24
403	Mutated*	Mutated*	PD
405	Mutated†	Mutated†	PD
407	Mutated†	Wild-type	PR
408	Mutated‡	Wild-type	PR

NOTE. Different mutational status was observed in five (204, 304, 308, 407 and 408) of these 12 patients. These 5 patients had PIK3CA and TP53 wild-type in circulating tumor DNA (ctDNA) tests and had PIK3CA and/or TP53 mutations in archival tumor tissue analysis. Meanwhile, these five patients experienced the best response of partial response (PR) or stable disease (SD) ≥ 24 weeks, which is consistent with the mutation results of ctDNA.
Abbreviations: ID, identification; PD, progressive disease.
*Mutations of TP53.
†Mutations of PIK3CA.
‡Mutations of both PIK3CA and TP53.