Breast Cancer: Targets and Therapy

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ORIGINAL RESEARCH

Real-World Outcome and Prognostic Factors Among HER2-Positive Metastatic Breast Cancer Patients Receiving Pyrotinib-Based Therapy: A Multicenter Retrospective Analysis

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Purpose: To explore the efficacy, safety, and potential factors influencing efficacy and outcome of pyrotinib-based therapy in human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer (MBC) in complex clinical practice.

Methods: Real-world data for HER2-positive MBC patients treated with pyrotinib-based regimens from 6 hospitals in Northern Anhui, China, from September 2018 to February 2022, were retrospectively collected, and clinicopathological features, efficacy, prognosis, and safety were analyzed. Potential influencing factors including baseline serum vascular endothelial growth factor-A (VEGF-A) for evaluating pyrotinib's treatment response and outcome were also explored.

Results: A total of 169 patients with HER2-positive MBC were enrolled. The objective response rate (ORR), disease control rate (DCR), and median progression-free survival (mPFS) of the overall cohort were 65.1%, 87.6%, and 12.4 months, respectively. Pyrotinib is highly beneficial as different treatment lines and appears to be a feasible strategy both in combination with chemotherapeutic drugs and alone. The mPFS values were 16.5 months, 12.4 months, and 9.3 months in the first, second, and third-or-higher lines of anti-HER2 therapy, respectively (P=0.027). The most common adverse event (AE) was diarrhea (88.2%), and patients with < grade 3 diarrhea achieved a longer mPFS than patients with ≥ grade 3 diarrhea (13.3 months vs 6.9 months, P=0.007). Among the patients with available baseline VEGF-A data, the ORR was 43.5% in patients with a high level of VEGF-A, compared to 81.5% in patients with a low level of VEGF-A (P=0.005). Moreover, patients in the VEGF-A-high group exhibited a shorter mPFS time than those in the VEGF-A-low group (7.8 months vs 19.1 months, P=0.004). Further analysis demonstrated AE of diarrhea and VEGF-A at baseline to be independent prognostic factors for PFS.

Conclusion: Pyrotinib-based regimens showed promising efficacy, with manageable tolerance, and AE occurrence of severe diarrhea and baseline level of serum VEGF-A are helpful in predicting the treatment outcome of pyrotinib in HER2-positive MBC.

Keywords: breast cancer, HER2, pyrotinib, efficacy, prognostic factor, vascular endothelial growth factor A

Introduction

Breast cancer (BC) is the most common malignancy in women.[1] Human epidermal growth factor receptor 2 (HER2)-positive BC, which accounts for approximately 20%, is highly invasive and has poor prognosis compared to other

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subtypes.[2] The advent of trastuzumab has dramatically changed the clinical outcomes for HER2-positive BC, but drug resistance inevitably occurs. Studies have shown that sustained anti-HER2 therapy after the failure of first-line trastuzumab therapy can bring survival benefits to patients.[3,4] Due to the high price and limited availability of antibody–drug conjugates (ADCs) such as T-DM1 and T-DXd, pyrotinib is favored for second-line anti-HER2 therapy in China. Pyrotinib, an oral irreversible pan-ErbB receptor small-molecule tyrosine kinase inhibitor (TKI) originally developed in China, exhibits excellent performance as rescue therapy for HER2-positive metastatic breast cancer (MBC). For example, the PHENIX study showed that pyrotinib combined with capecitabine significantly prolonged median progression-free survival (mPFS) compared with capecitabine monotherapy (11.1 months vs 4.1 months) in HER2-positive MBC patients with trastuzumab and taxane-based therapy failure.[5] According to the PHOEBE study,[6] pyrotinib combined with capecitabine significantly improved mPFS compared to lapatinib combined with capecitabine (12.5 months vs 5.6 months) in HER2-positive MBC patients who previously received trastuzumab, taxanes, and/or anthracycline. In clinical practice, the application strategies for pyrotinib are diverse, and in addition to capecitabine, pyrotinib often combined with other regimens. Therefore, this real-world study aimed to examine the efficacy and safety data of pyrotinib in the treatment of HER2-positive MBC patients in China to supplement the results of clinical trials and better guide clinical medication. Furthermore, given the lack of effective biomarkers for anti-HER2 therapy efficacy, potential factors influencing pyrotinib's effectiveness in HER2-positive MBC were also explored.

Materials and Methods

General Information

This was a retrospective multicenter real-world study that collected clinical data for HER2-positive MBC patients receiving pyrotinib-based therapy from 6 hospitals in northern Anhui Province, China, from September 2018 to February 2022. Inclusion criteria were as follows: age ≥ 18 years; female with pathologically confirmed BC; imaging- and clinical-confirmed postoperative recurrent or metastatic BC or BC at primary clinical stage IV; positive HER2 status defined as immunohistochemistry (IHC) category 3+ or IHC category 2+ and confirmed by fluorescent in situ hybridization with HER2 gene amplification in primary or metastatic lesions; at least one measurable target lesion evaluable by CT/MRI based on Response Evaluation Criteria in Solid Tumors guidelines version 1.1 (RECIST 1.1); no contraindication of administration of pyrotinib or systemic chemotherapy. Exclusion criteria were as follows: history of other malignancies (excluding cured cervical carcinoma in situ, basal cell carcinoma of the skin, and squamous cell carcinoma of the skin); secondary advanced BC; previously treated with pyrotinib or enrolled in any clinical trial of pyrotinib; incomplete and untraceable clinical data. Resistance to trastuzumab was defined as relapse during or within 12 months after adjuvant trastuzumab or progression at first radiological reassessment within 3 months of trastuzumab treatment for metastatic disease. Moreover, we distinguished anti-HER2 therapy from systematic therapy in this study, for anti-HER2 therapy and systemic therapy are not always synchronized. For particular condition, patients who relapsed within 12 months but exceed 6 months after adjuvant trastuzumab were considered to be resistant to trastuzumab, and the subsequent treatment is considered as second-line anti-HER2 therapy but first-line systemic therapy.

Treatment Regimens

All patients were treated with pyrotinib-based regimens according to routine clinical practice. Dose modification of pyrotinib was allowed according to the label of pyrotinib, and decided by each study center based on the patient's physical condition and drug tolerance.

Efficacy and Safety Assessments

Tumor response was evaluated based on RECIST 1.1, including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The objective response rate (ORR) and disease control rate (DCR) were also calculated: ORR= (CR+PR)/(CR+PR+SD+PD); DCR = (CR+PR+SD)/(CR+PR+SD+PD). PFS was defined as the time from the beginning of treatment with pyrotinib to the occurrence of PD or death from any cause, and overall survival

(OS) was defined as the time from the beginning of treatment with pyrotinib to death from any cause. Adverse events (AEs) were graded with reference to Common Terminology Criteria for Adverse Events, version 5.0.

Observation Indicators

A total of 50 patients for whom serum vascular endothelial growth factor-A (VEGF-A) was measured before the first treatment with pyrotinib were collected. VEGF-A detection was performed using a Weigao JR-1 Chemiluminescent Immunoassay Analyzer and Vascular Endothelial Growth Factor Assay Kit (chemiluminescence) (Shandong Weigao Group Medical Polymer Co., Ltd., Weihai, China) according to the manufacturer's instructions. The reference range of serum VEGF-A was 0–160 pg/mL.

Follow-Up

Inpatient, outpatient, and telephone follow-ups were used and included AEs, PFS, and OS until the patients were lost to follow-up or died. The deadline for follow-up was April 30, 2022, with a median time of 16.2 months.

Statistical Methods

The Pearson $\chi 2$ test was applied for analysis of differences in categorical variables in different groups. The optimal cutoff values of continuous variables related to short-term tumor response were determined by receiver operating characteristic (ROC) curves, and outcome-based cutoff optimization was performed using X-tile software version 3.6.1 (Yale University School of Medicine, New Haven, CT, USA). PFS and OS were calculated by the Kaplan–Meier method, and Log rank tests were used for survival comparisons. Univariate Cox regression analysis was employed to initially screen out candidate prognostic variables related to PFS and OS, and variables with $P < 0.05$ were included in multivariate analysis. The statistical analysis was performed using SPSS statistical software version 25.0. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

A total of 169 female patients with HER2-positive MBC were enrolled. Their baseline characteristics are presented in Table 1.

Treatment Administration

Most of the patients received pyrotinib-based combination regimens, and capecitabine was most commonly used for combination in 125 (74.0%) patients. Pyrotinib combined with other drugs included chemotherapy (except capecitabine), trastuzumab, and endocrine therapy, accounting for 16 (9.5%), 13 (7.7%), and 4 (2.4%), respectively. In addition, 11 (6.5%) patients received pyrotinib monotherapy. A total of 32 patients experienced brain metastases; 23 (71.9%) of them were treated with local radiotherapy and/or surgery. Regarding the initial dose of pyrotinib, 154 (91.1%) patients were given with 400 mg, 12 (7.1%) patients with 320 mg, and 3 (1.8%) patients with 240 mg. During pyrotinib treatment, dose reduction due to AEs occurred in 22 (13.0%) patients. In addition, 27 (16.0%) patients experienced treatment interruption during the use of pyrotinib.

Efficacy Analyses

All patients were evaluable for efficacy, with a CR of 11 (6.5%), PR of 99 (58.6%), SD of 38 (22.5%), PD of 21 (12.4%), ORR of 65.1%, and DCR of 87.6% in the overall cohort. Specific short-term efficacy information is provided in Table 2.

By the end of follow-up, 124 (73.4%) patients had PFS data while the OS data were not mature. mPFS was 12.4 months (95% *CI*, 10.74–14.06 months) in all patients (Figure 1A), and 12.4 months (95% *CI*, 10.70–14.10 months) in patients receiving pyrotinib and capecitabine combination therapy as second-line anti-HER2 therapy (Figure 1B). When receiving first, second, and third-or-higher lines of anti-HER2 therapy, mPFS of the overall cohort was 16.5 months (95% *CI*, 8.40–24.61 months), 12.4 months (95% *CI*, 10.44–14.37 months), and 9.3 months (95% *CI*, 6.16–12.44 months), respectively (Figure 1C).

Table 1 Baseline Characteristics

Characteristics	n (%)
Age (years)	
< 65	157 (92.9)
≥ 65	12 (7.1)
ECOG performance status	
0~1	151 (89.3)
2	18 (10.7)
Hormone receptor status	
ER and PR negative	101 (59.8)
ER and/or PR positive	68 (40.2)
Disease extent at diagnosis	
De novo IV stage	21 (12.4)
Recurrent	148 (87.6)
Sites of metastases	
Visceral	131 (77.5)
Liver	58 (34.3)
Lung	87 (51.5)
Brain	32 (18.9)
Nonvisceral	38 (22.5)
Lymph nodes	88 (52.1)
Bone	63 (37.3)
Number of metastases	
< 3	107 (63.3)
≥ 3	62 (36.7)
Previous anti-HER2 treatment	
Yes	158 (93.5)
(Neo) adjuvant setting	47 (27.8)
Metastatic setting	118 (69.8)
No	11 (6.5)
Previous anti-HER2 drugs	
Trastuzumab	158 (93.5)
Pertuzumab	9 (5.3)
Lapatinib	4 (2.4)
T-DM1	1 (0.1)
Resistance to trastuzumab	
Primary resistance	56 (33.1)
Acquired resistance	92 (54.4)
Non-resistance	21 (12.4)
Lines of anti-HER2 therapy	
1	19 (11.2)
2	119 (70.4)
≥ 3	31 (18.3)
Lines of systematic therapy	
1	42 (24.9)
2	73 (43.2)
≥ 3	54 (31.9)

Abbreviations: ECOG, eastern cooperative oncology group; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Meanwhile, when receiving first, second, and third-or-higher lines of systematic therapy, the mPFS of the overall cohort were 19.4 months (95% *CI*, 14.43–24.38 months), 10.4 months (95% *CI*, 6.87–13.93 months), and 9.6 months (95% *CI*, 6.89–12.31 months), respectively (Figure 1D). Patients treated with pyrotinib monotherapy showed a shorter mPFS than patients treated

Table 2 Subgroup Analysis of Short-Term Response

Therapy Regimens		Total, n (%)	Response, n (%)				ORR, n (%)	DCR, n (%)
			CR	PR	SD	PD		
Total		169	11 (6.5)	99 (58.6)	38 (22.5)	21 (12.4)	110 (65.1)	148 (87.6)
Pyrotinib alone		11 (6.5)	0 (0.0)	7 (63.6)	1 (9.1)	3 (27.3)	7 (63.6)	8 (72.7)
First-line	Anti-HER2 therapy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Systematic therapy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Second-line	Anti-HER2 therapy	9 (81.8)	0 (0.0)	6 (66.7)	1 (11.1)	2 (22.2)	6 (66.7)	7 (77.8)
	Systematic therapy	6 (54.5)	0 (0.0)	4 (66.7)	0 (0.0)	2 (33.3)	4 (66.7)	4 (66.7)
Third-or-higher-line	Anti-HER2 therapy	2 (18.2)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	1 (50.0)
	Systematic therapy	5 (45.5)	0 (0.0)	3 (60.0)	1 (20.0)	1 (20.0)	3 (60.0)	4 (80.0)
Pyrotinib + Capecitabine		125 (74.0)	10 (8.0)	71 (56.8)	30 (24.0)	14 (11.2)	81 (64.8)	111 (88.8)
First-line	Anti-HER2 therapy	11 (8.8)	2 (18.2)	8 (72.7)	1 (9.1)	0 (0.0)	10 (90.9)	11 (100)
	Systematic therapy	32 (25.6)	6 (18.8)	22 (68.8)	4 (12.5)	0 (0.0)	28 (87.5)	32 (100)
Second-line	Anti-HER2 therapy	89 (71.2)	8 (9.0)	50 (56.2)	20 (22.5)	11 (12.4)	58 (65.2)	78 (87.6)
	Systematic therapy	56 (44.8)	2 (3.6)	31 (55.4)	15 (26.8)	8 (14.3)	33 (58.9)	48 (85.7)
Third-or-higher-line	Anti-HER2 therapy	25 (20.0)	0 (0.0)	13 (52.0)	9 (36.0)	3 (12.0)	13 (52.0)	22 (88.0)
	Systematic therapy	37 (29.6)	2 (5.4)	18 (48.6)	11 (29.7)	6 (16.2)	20 (54.1)	31 (83.8)
Pyrotinib + chemotherapy (non-capecitabine)		16 (9.5)	0 (0.0)	10 (62.5)	3 (18.8)	3 (18.8)	10 (62.5)	13 (81.3)
First-line	Anti-HER2 therapy	3 (18.8)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	2 (66.7)	3 (100)
	Systematic therapy	3 (18.8)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)	3 (100)	3 (100)
Second-line	Anti-HER2 therapy	10 (62.5)	0 (0.0)	7 (70.0)	0 (0.0)	3 (30.0)	7 (70.0)	7 (70.0)
	Systematic therapy	6 (37.5)	0 (0.0)	3 (50.0)	0 (0.0)	3 (50.0)	3 (50.0)	3 (50.0)
Third-or-higher-line	Anti-HER2 therapy	3 (18.8)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	1 (33.3)	3 (100)
	Systematic therapy	7 (43.8)	0 (0.0)	4 (57.1)	3 (42.9)	0 (0.0)	4 (57.1)	7 (100)
Pyrotinib + Trastuzumab (± chemotherapy)		13 (7.7)	1 (7.7)	8 (61.5)	3 (23.1)	1 (7.7)	9 (69.2)	12 (92.3)
First-line	Anti-HER2 therapy	4 (30.8)	1 (25.0)	3 (75.0)	0 (0.0)	0 (0.0)	4 (100)	4 (100)
	Systematic therapy	5 (38.5)	1 (20.0)	4 (80.0)	0 (0.0)	0 (0.0)	5 (100)	5 (100)
Second-line	anti-HER2 therapy	8 (61.5)	0 (0.0)	5 (62.5)	2 (25.0)	1 (12.5)	5 (62.5)	7 (87.5)
	Systematic therapy	5 (38.5)	0 (0.0)	4 (80.0)	1 (20.0)	0 (0.0)	4 (80.0)	5 (100)
Third-or-higher-line	Anti-HER2 therapy	1 (7.7)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)
	Systematic therapy	3 (23.1)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	2 (66.7)
Pyrotinib + Endocrine therapy		4 (2.4)	0 (0.0)	3 (75.0)	1 (25.0)	0 (0.0)	3 (75.0)	4 (100)
First-line	Anti-HER2 therapy	1 (25.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)
	Systematic therapy	2 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)	2 (100)
Second-line	Anti-HER2 therapy	3 (75.0)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)	3 (100)	3 (100)
	Systematic therapy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Third-or-higher-line	anti-HER2 therapy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Systematic therapy	2 (50.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (100)	2 (100)

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; HER2, human epidermal growth factor receptor 2.

with pyrotinib combination therapy (9.6 months vs 12.4 months, *P*=0.138, Figure 2A), and the mPFS of pyrotinib plus capecitabine versus non-capecitabine was 12.5 months versus 9.3 months (*P*=0.697, Figure 2B). No significant difference in mPFS was observed in pyrotinib combined with or not trastuzumab (10.9 months vs 12.5 months, *P*=0.387, Figure 2C).

Correlation Between Clinicopathological Characteristics and mPFS

Hormone receptor (HR) status showed no significant correlation with mPFS (12.4 months vs 12.0 months, *P*=0.136, Figure 3A). Patients pretreated with pertuzumab had a shorter mPFS than patients pretreated without pertuzumab (7.7 months vs 12.7 months, *P*=0.017, Figure 3B). It is note that the proportion of patients pretreated with pertuzumab is as low as 5.3% due to the late accessibility and economic burden. The mPFS of patients without resistance to trastuzumab was 16.5 months (95% *CI*, 11.99–21.02 months), whereas that of patients with primary and acquired resistance to trastuzumab was 11.6 months (95% *CI*, 9.21–13.99 months) and 11.8 months (95% *CI*, 9.41–14.19 months), respectively. However, no significant difference

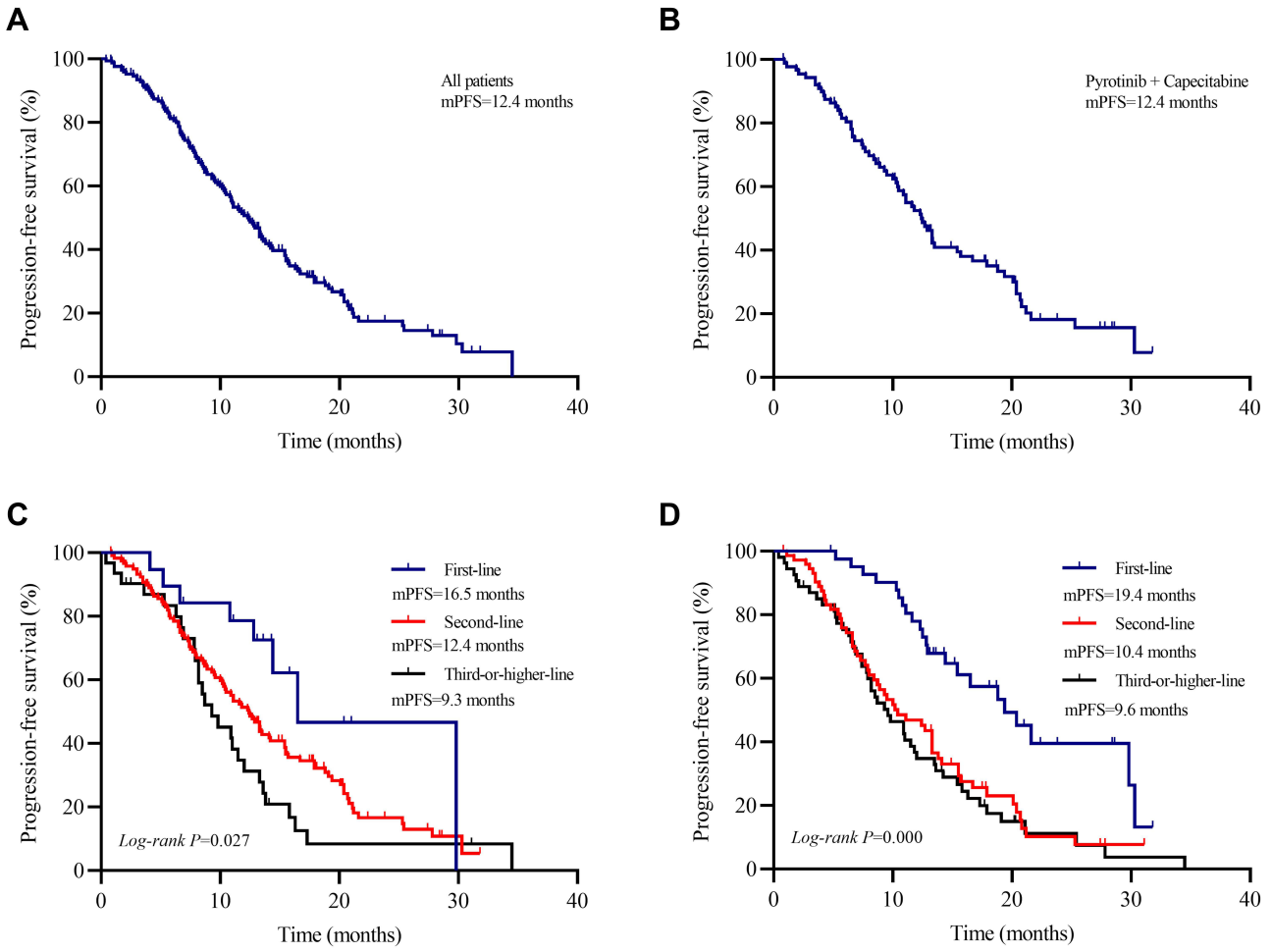

Figure 1 Kaplan–Meier curves of PFS for patients.
Notes: (**A**) Overall cohort. (**B**) Patients treated with pyrotinib combined with capecitabine as second-line anti-HER2 therapy. (**C**) Patients stratified by lines of anti-HER2 therapy. (**D**) Patients stratified by lines of systematic therapy.

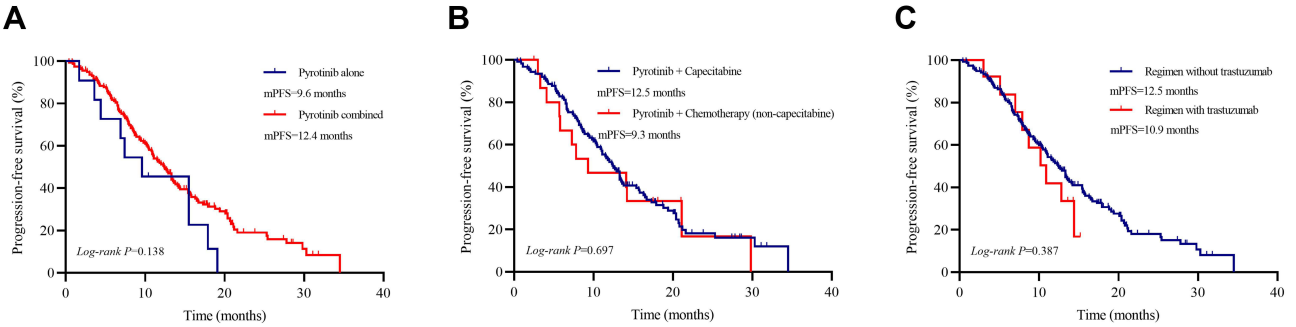

Figure 2 Kaplan–Meier curves of PFS for patients treated with different regimens.
Notes: (**A**) Patients treated with pyrotinib alone or combined regimens. (**B**) Patients treated with pyrotinib combined with capecitabine or other chemotherapeutic drugs (non-capecitabine). (**C**) Patients treated with single (pyrotinib) or dual anti-HER2 (pyrotinib combined with trastuzumab) therapy.

in mPFS was observed among the patients under these three different conditions (P=0.141, Figure 3C). Patients without liver metastases achieved a longer mPFS than patients with liver metastases (12.7 months vs 10.2 months, P=0.026, Figure 3D). In contrast, there was no significant difference in mPFS between patients with and without brain metastases (11.6 months vs 12.8

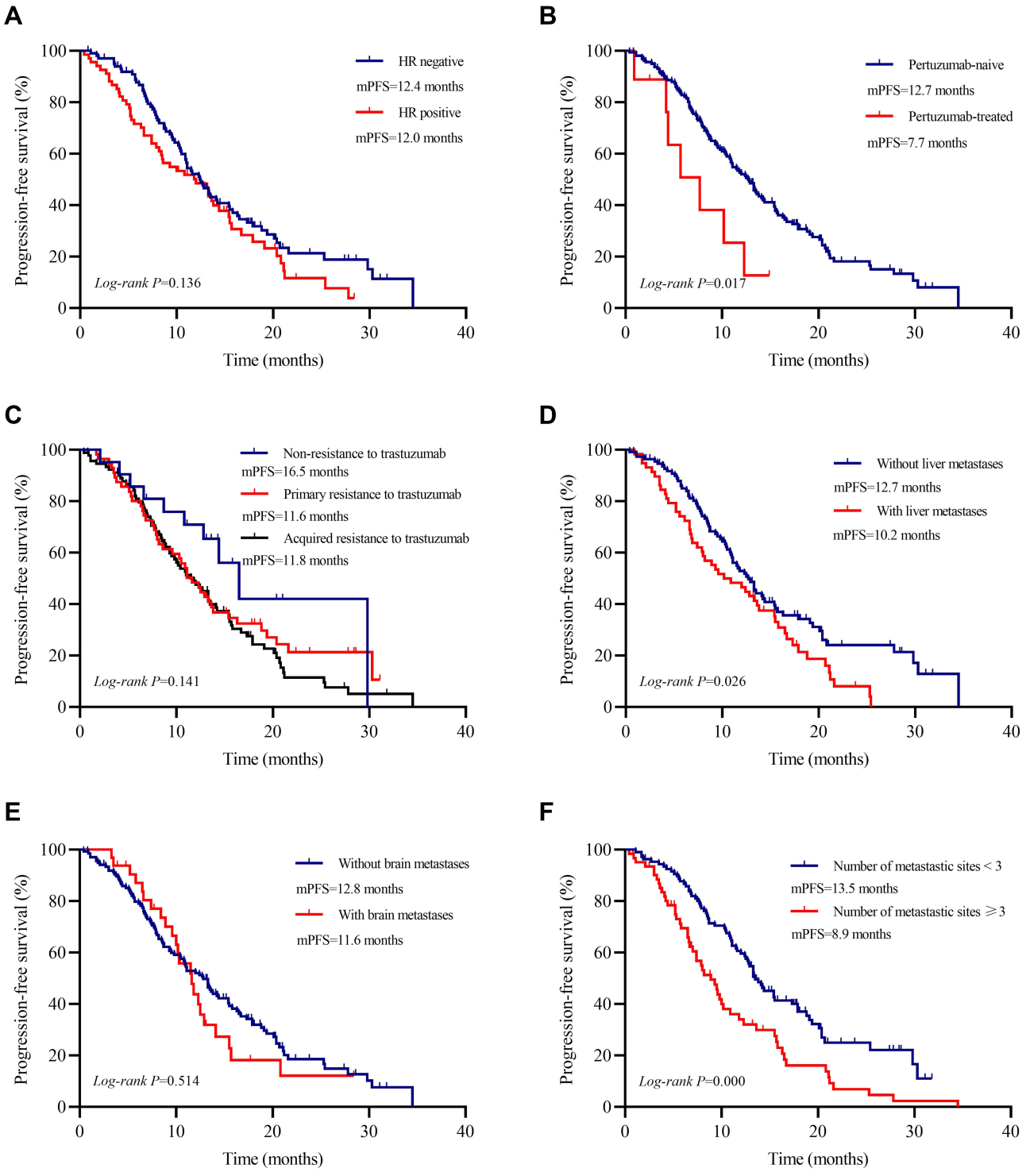

Figure 3 Kaplan–Meier curves of PFS for patients with different characteristics.

Notes: (**A**) Patients stratified by HR status according to pathological characteristics. (**B**) Patients stratified by pertuzumab-treated or pertuzumab-naive according to prior treatment history. (**C**) Patients stratified by sensitivity to trastuzumab according to prior treatment history. (**D**) Patients stratified by liver metastasis according to clinical characteristics. (**E**) Patients stratified by brain metastasis according to clinical characteristics. (**F**) Patients stratified by number of metastatic sites according to clinicopathological characteristics.

Abbreviation: HR, hormone receptor.

months, P=0.514, Figure 3E). In terms of the number of metastases, the mPFS of patients with < 3 metastatic sites was longer than that of patients with ≥ 3 metastatic sites (13.5 months vs 8.9 months, P=0.000, Figure 3F).

Safety

AEs with an incidence of ≥ 20% in this study included diarrhea, hand-foot syndrome, vomiting, anemia, neutropenia, nausea, and hypokalemia. Among them, diarrhea was the most common adverse event (AE), with an incidence of 88.2% for all grades and 16.0% for grades 3~4. Among other gastrointestinal AEs, incidences of vomiting and nausea were 27.2% and 23.1%, and the grades 3~4 rates were 3.6% and 2.4%, respectively. Hand-foot syndrome was the second most common AE, with an incidence of 35.5% for all grades, and 5.3% for grades 3~4. Among hematological AEs, anemia and leukopenia rates were 26.6% and 24.9%, and the grades 3~4 rates were 5.9% and 1.8%, respectively. Specific AEs are presented in Table 3.

Potential Factors Influencing Efficacy and Prognosis

Finally, we explored potential factors influencing either efficacy or treatment outcome for pyrotinib-based therapy. The results showed that, despite no significant difference in ORR (67.6% vs 51.9%, χ^2 =2.478, P=0.115, Figure 4A), patients with < grade 3 diarrhea achieved a longer mPFS than patients with ≥ grade 3 diarrhea (13.3 months vs 6.9 months, P=0.007, Figure 4B).

Detection of serum VEGF-A before pyrotinib administration was available for 50 of 159 patients, and the potential of VEGF-A as a predictive biomarker for the treatment outcome of pyrotinib-based therapy was assessed. Firstly, ROC curves were plotted when taking CR+PR as the effective treatment and SD+PD as the ineffective treatment (Supplementary Figure S1). The area under the curve was 0.739, and the optimal cutoff value of VEGF-A was determined to be 156.16 pg/mL. 23 patients with serum VEGF-A levels ≥156.16 pg/mL were classified into the VEGF-A-high group; 27 patients with serum VEGF-A levels < 156.16 pg/mL were classified into the VEGF-A-low group. When receiving pyrotinib-based therapy, patients in the serum VEGF-A-high group exhibited a lower ORR than patients in the serum VEGF-A-low group (43.5% vs 81.5%, χ^2=7.785, P=0.005, Figure 4C). Subsequently, we compared mPFS associated with these two VEGF-A categories, and the optimal cutoff value was redetermined to be 84.3 pg/mL using X-tile software (Supplementary Figure S2). The mPFS of serum VEGF-A-high levels (≥ 84.3 pg/mL) patients was less than the value of serum VEGF-A-low levels (< 84.3 pg/mL) patients (7.8 months vs 19.1months, P =0.004, Figure 4D).

Table 3 Adverse Events

Adverse Events	All Grade, n (%)	Grade 1~2, n (%)	Grade 3~4, n (%)
Diarrhea	149 (88.2)	122 (72.2)	27 (16.0)
Hand-foot syndrome	60 (35.5)	51 (30.2)	9 (5.3)
Vomiting	46 (27.2)	40 (23.7)	6 (3.6)
Anemia	45 (26.6)	35 (20.7)	10 (5.9)
Leukopenia	42 (24.9)	39 (23.1)	3 (1.8)
Nausea	39 (23.1)	35 (20.7)	4 (2.4)
Hypokalemia	37 (21.9)	26 (15.4)	11 (6.5)
Neutropenia	33 (19.5)	29 (17.2)	4 (2.4)
Creatinine increased	31 (18.3)	30 (17.8)	1 (0.1)
Anorexia	29 (17.2)	25 (14.8)	4 (2.4)
Blood bilirubin increased	24 (14.2)	24 (14.2)	0 (0)
Rash	21 (12.4)	19 (11.2)	2 (1.2)
Aminotransferase increased	18 (10.7)	15 (8.9)	3 (1.8)
Mucositis oral	16 (9.5)	15 (8.9)	1 (0.1)
Hypertriglyceridemia	13 (7.7)	12 (7.1)	1 (0.1)
Thrombocytopenia	10 (5.9)	7 (4.1)	3 (1.8)
Cholesterol high	6 (3.6)	6 (3.6)	0 (0)

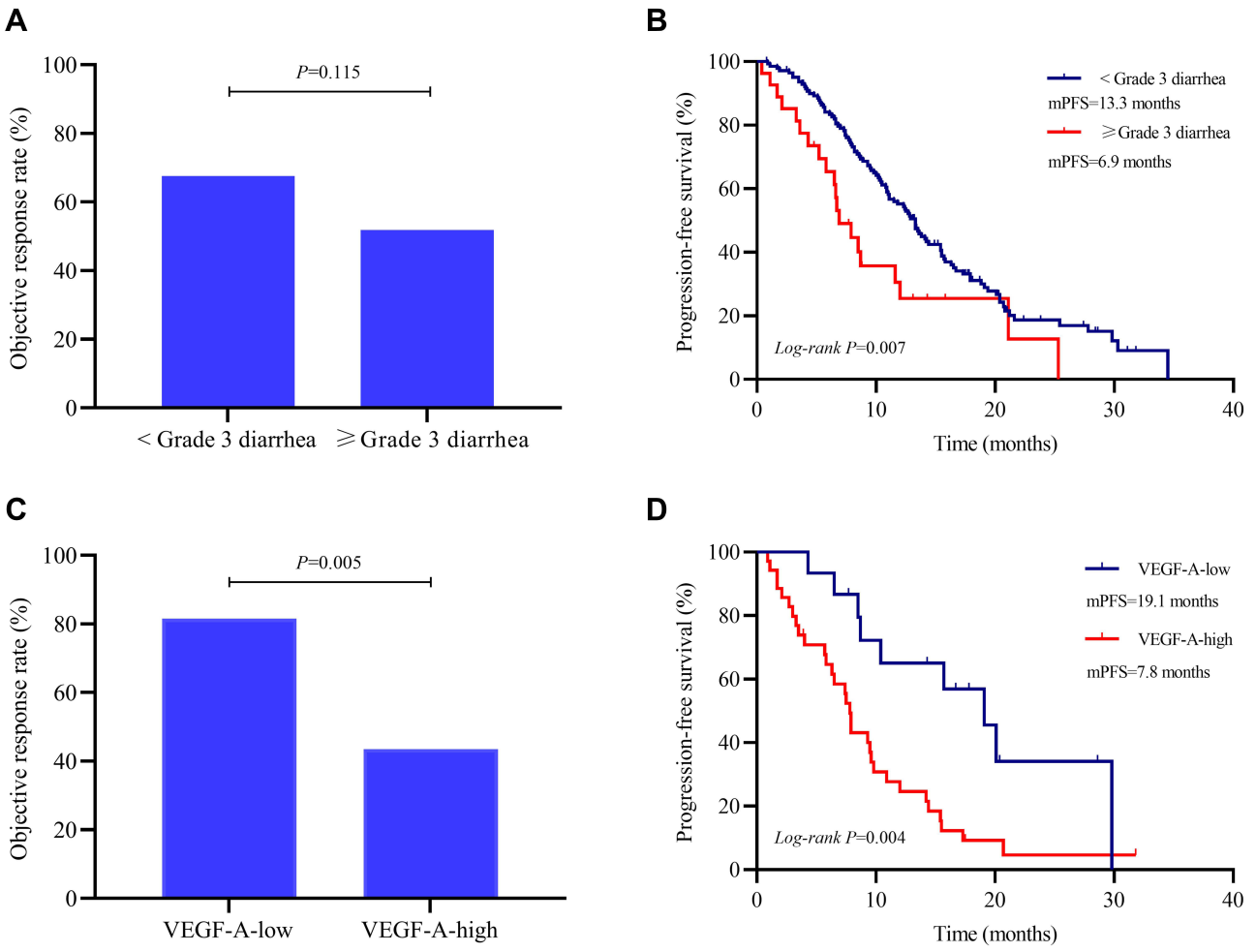

Figure 4 Exploration of potential factors or biomarkers influencing efficacy and outcome of pyrotinib-based therapy.
Notes: (**A**) The objective response rate for patients stratified by diarrhea grade. (**B**) PFS for patients stratified by diarrhea grade. (**C**) The objective response rate for patients stratified by VEGF-A expression level. (**D**) PFS for patients stratified by VEGF-A expression level.
Abbreviation: VEGF-A, vascular endothelial growth factor A.

Based on univariate Cox regression analysis, the number of metastatic sites (P=0.020), line of systematic therapy (both P<0.05), diarrhea severity (P=0.047), and baseline serum VEGF-A level (P =0.006) correlated with PFS (Figure 5A). Multivariate Cox regression analyses also revealed that the line of systematic therapy (both P<0.05), diarrhea severity (P=0.011), and baseline serum VEGF-A level (P=0.022) were independent prognostic factors for patient PFS (Figure 5B).

Discussion

According to our real-world data in the northern area of Anhui Province, China, pyrotinib administration led to an mPFS of 12.4 months, an ORR of 65.2% and a DCR of 87.6% when combined with capecitabine as second-line anti-HER2 therapy for HER2-positive MBC. Patients with this kind of disease receiving T-DM1 as the standard second-line treatment could have an mPFS of 9.6 months as indicated by the results of the EMILIA study.[7] In the Phase III DESTINY-Breast03 study comparing T-DXd with T-DM1 for patients with HER2-positive MBC previously treated with trastuzumab and a taxane, the 12-month PFS rate was 75.8% with T-DXd and 34.1% with T-DM1 (P<0.001), and ORR was 79.7% with T-DXd and 34.2% with T-DM1 (P<0.001).[8] By comparison, the efficacy of pyrotinib was comparable to these ADCs in the treatment setting of second-line from the numerical point of view, however, a head-to-head trial comparing pyrotinib with ADC is lacking to clarify their discrepancy in effectiveness. In the meantime, the data from this

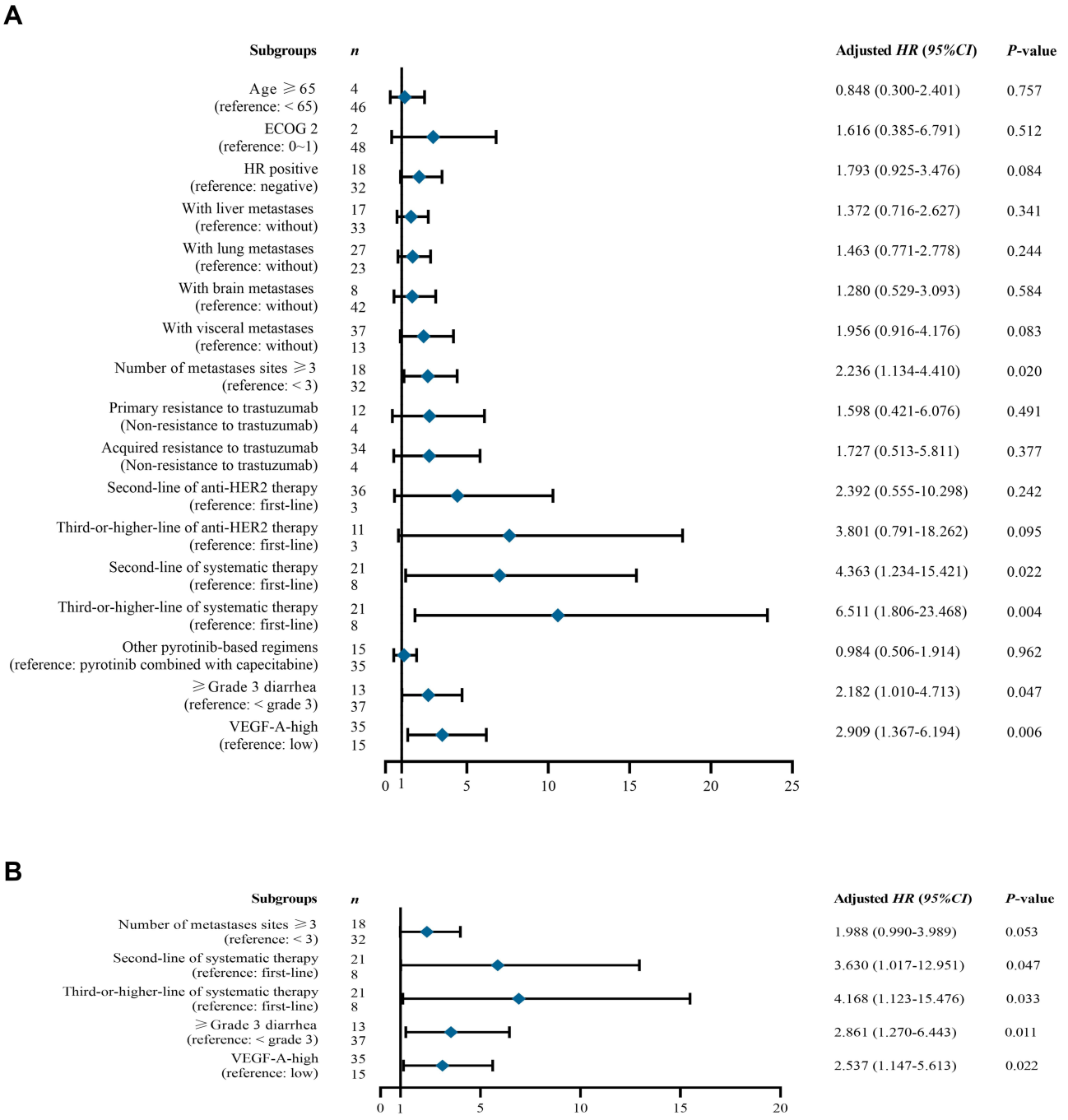

Figure 5 Cox regression analyses of PFS in 50 HER2-positive MBC patients with baseline detection of VEGF-A.
Notes: (**A**) Forest plot of univariate Cox regression analyses. (**B**) Forest plot of multivariate Cox regression analyses.
Abbreviations: ECOG, eastern cooperative oncology group; HR positive, hormone receptor positive; HER2, human epidermal growth factor receptor 2; VEGF-A, vascular endothelial growth factor A; Adjust HR (95% CI), Adjust hazard ratios (95%confidence interval).

real-world study were comparable to the results of the Phase III PHENIX study (ORR of 68.6%, DCR of 91.9%, and mPFS of 11.1 months) and the Phase III PHOEBE study (ORR of 67.2%, DCR of 88.7%, and mPFS of 12.5 months).[5,6] Therefore, our data are representative of the Chinese population and can serve as a complement to clinical trials of pyrotinib.

In the analysis of different regimens, a survival benefit of the classic regimen of pyrotinib combined with capecitabine was not observed when compared to pyrotinib combined with non-capecitabine drugs, and the reason may be related to

the small sample (n=13) receiving the latter therapy. Overall, the best partner for pyrotinib is worthy of further research. Due to the different HER2-targeting domains and synergistic drug interactions, dual anti-HER2 therapy with trastuzumab and TKIs has been proven to provide synergistic antitumor activity and a significant outcome benefit.[9,10] Li et al reported in a real-world study that the mPFS of pyrotinib combined with trastuzumab was significantly longer than that of pyrotinib alone in HER2-positive MBC patients (10.7 months vs 8.8 months, *P*=0.016).[11] Chen et al also analyzed real-world data for pyrotinib but did not find advantages for dual anti-HER2 therapy over pyrotinib monotherapy in a small sample population (n=12).[12] In the present study, 13 patients received the dual-target therapy pyrotinib plus trastuzumab and did not obtain a significant benefit in terms of mPFS compared with single-target pyrotinib therapy (10.9 months vs 12.5 months, *P*=0.387). Therefore, we consider that the best population to receive pyrotinib combined with trastuzumab for HER2-positive MBC still needs further study, especially a large group study, and needs a randomized controlled trial to answer this important question. In addition, based on the benefit of different lines of pyrotinib therapy in this study, more benefit can be achieved with earlier use, which is consistent with the results of the real-world study by Li et al.[11]

Clinicopathological analyses revealed a significant difference in mPFS between patients with and without liver metastases, and this result was consistent with the findings of Goksu et al,[13] who showed liver metastases to be negatively associated with the survival of patients with HER2-positive MBC. The results of studies about larger molecules ADCs such as T-DM1 and T-DXd have indicated clinically relevant intracranial activity in HER2-positive MBC patients with brain metastases.[14,15] Meantime, TKIs which hold smaller molecular sizes also can theoretically penetrate the blood-brain barrier and their clinical benefits for patients with brain metastatic MBC have been demonstrated by a series of clinical trials.[16–18] Thus, we consider ADCs and TKIs are both favorable options for this kind of patient. In this real-world study, pyrotinib-based therapy resulted in an mPFS of 11.6 months in patients with brain metastases, which is highly consistent with that of 11.3 months and 11.1 months reported in the PERMEATE study and by Gao et al for pyrotinib plus capecitabine in patients with brain metastases from HER2-positive MBC,[19,20] respectively. Although the prognosis of patients with brain metastases remains poor, treatment strategies range from local to systemic anti-HER2 therapies. However, there was no difference in mPFS between BC patients with and without brain metastases in this study (11.6 months vs 12.8 months, *P*=0.514). We hypothesize that this might be related to the limited number of patients with brain metastases enrolled in this study (n=32). Another important factor is the fact that up to 71.9% of them received local treatments such as radiotherapy and/or surgery and that these local interventions can contribute to survival improvement, indicating the importance of comprehensive treatment in achieving favorable efficacy for brain metastasis.

Consistent with the results reported in previous clinical trials of pyrotinib, diarrhea was the most common AE with pyrotinib treatment, but the incidence of severe diarrhea was 16.0%, which was lower than the rates of 26.5%-31% from other studies.[5,6,21] This discrepancy may be attributed to improved knowledge and management of the use of pyrotinib in clinical practice, including more routine administration of preventive medication and better management of AEs. The incidence of hand-foot syndrome in this study was lower than that reported in Phase II and phase III clinical trials of pyrotinib,[6,22] and we consider that this may be related to the use of different combination drugs, as capecitabine was not the only drug used in combination. The categories and incidences of other AEs in this study were similar to those reported in previous real-world studies.

Exploration of factors influencing the efficacy and prognosis is very important for the management of HER2-positive MBC. Chen et al and Anwar et al reported that a high tumor mutation burden is associated with poorer PFS in patients treated with pyrotinib-based therapies.[12,23] However, analysis of this biomarker has mainly been focused at the molecular level, and there are limitations of testing, including its high price and inconsistent testing standards; thus, it is difficult to promote its application in clinical practice. AEs have potential in predicting survival with certain targeted drugs. For example, it has been reported that rash is able to predict the survival benefit of cetuximab for metastatic colorectal cancer;[24] diarrhea has a valuable role in assessing the efficacy of sorafenib in patients with advanced hepatocellular carcinoma.[25] Hence, the association between diarrhea and pyrotinib effectiveness or patient outcome was explored in this study, and a negative correlation between diarrhea and pyrotinib efficacy was found. We speculate that the occurrence of severe diarrhea may affect the quality of life and reduce a patient's tolerance to treatment and drug dose, resulting in impaired benefit from pyrotinib. Moreover, a unique relationship between severe diarrhea and pyrotinib efficacy may exist. Wen et al developed a population pharmacokinetic model for pyrotinib administration in patients with HER2-

positive MBC and found that concomitant use of montmorillonite for the treatment of diarrhea reduced the bioavailability of pyrotinib by 50.3%.[26] Therefore, the role of diarrhea, especially in the case of anti-diarrheal drug intervention, as an influencing factor of pyrotinib effectiveness needs to be further assessed.

Most importantly, the remarkable finding of this study is that serum VEGF-A levels are promising for predicting both the tumor response and treatment outcome of pyrotinib-based therapy. Cox regression analysis revealed baseline serum VEGF-A to be an independent prognostic indicator of PFS. During tumor growth, microenvironmental hypoxia may occur, and molecular pathways such as PI3K/Akt/HIF-1α signaling may be activated, leading to an increase in VEGF-A expression and secretion and ultimately tumor angiogenesis.[27] Thus, patients with a high tumor burden may be more likely to have high serum levels of VEGF-A, rendering them more likely to develop resistance to routine treatment, including pyrotinib, due to remodeling of the tumor microenvironment. The value of baseline serum VEGF-A in predicting the efficacy and survival outcome of first-line chemotherapy in patients with advanced small-cell lung cancer has already been demonstrated by our previous study,[28] and the findings support our hypothesis. Moreover, Zhu et al proposed combined detection of multiple indicators, including VEGF and HER2, as diagnostic and prognostic markers for early BC.[29] These together provided the reason why VEGF-A was chosen as a biomarker in this study. Considering that serum VEGF-A measurement is a hematological test with the advantages of being noninvasive, convenient, and repeatable, it is a promising biomarker for optimizing the management of HER2-positive MBC patients in the future. More generally, we believe that the combination of anti-VEGF and anti-HER2 therapy holds promise. A previous study found that inhibiting VEGF-dependent angiogenesis can lead to partial reversal of trastuzumab resistance in HER2-positive ovarian cancer cells,[30] therapy combined with anti-HER2 and anti-VEGF has also been applied in patients with HER2-positive MBC who had previously received an average of approximately 3 lines of treatment, providing some benefit, with an mPFS of 24.7 weeks and a CBR of 30.8%.[31] Therefore, anti-angiogenesis plus anti-HER2 treatment strategies should be given more attention and warrant continued investigation in anti-HER2 therapy.

Conclusion

In conclusion, this real-world study offers a complement to clinical trials of pyrotinib by validating its efficacy and safety in the treatment of HER2-positive MBC. Pyrotinib is highly beneficial for different treatment lines and appears to be a feasible strategy both in combination with chemotherapeutic drugs and alone. Severe diarrhea AEs and the level of serum VEGF-A at baseline are potential prognostic factors for HER2-positive MBC patients receiving pyrotinib-based therapy, and these findings will be of importance for the management of patients with this disease.

Data Sharing Statement

The data supporting the results in the manuscript can be obtained from the corresponding author based on reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the independent ethics committee for each of the six participating medical institutions including the First Affiliated Hospital of Bengbu Medical College, Suzhou Municipal Hospital, the First Affiliated Hospital of Anhui Medical University, the Third People's Hospital of Bengbu, the Second Affiliated Hospital of Bengbu Medical College, and the Fifth People's Hospital of Fuyang. Given that this study was conducted relying on retrospective data collected as part of routine clinical practice, those ethical committees waived the consent, and individual informed consent was not obtained. In this retrospective study, no patient identifiers were used and data were anonymized.

Consent for Publication

All authors approved the version submitted for publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest.

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