Pyrotinib Treatment in Patients With HER2-positive Metastatic Breast Cancer and Brain Metastasis: Exploratory Final Analysis of Real-World, Multicenter Data

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ABSTRACT

Purpose: Patients with HER2-positive ($HER2^+$) metastatic breast cancer (MBC) have poor prognoses. Pyrotinib has shown promising antitumor activity in MBC to improve progression-free survival (PFS). However, findings based on real-world data to analyze whether pyrotinib affects overall survival (OS) remain scarce.

Experimental Design: This real-world study is an exploratory analysis of brain metastasis (BM) and the final update of our preceding study of 168 patients with $HER2^+$ MBC. PFS, OS, tumor mutation burden (TMB), clinical benefit rate (CBR), and overall response rate (ORR) were analyzed.

Results: Pyrotinib treatment led to a median PFS time of 8.00 months and a median OS of 19.07 months in the 168 participants. High TMB was associated with poor OS ($P = 0.0072$) and PFS ($P = 0.0028$). In the 39 patients with BM, the median PFS and OS were 8.67 and 13.93 months, respectively. The surgery/radiation (S/R) group of patients with BM had prolonged survival (PFS: 9.97 vs. 7.73 months $P = 0.19$; OS: 20.67 vs. 12.43 months $P = 0.021$) compared with the no surgery/no radiation group (NS/NR). The CBR was 58.6% (S/R) vs. 41.4% (NS/NR), while the ORR was 24.1% (S/R) vs. 31.0% (NS/NR).

Conclusions: Pyrotinib shows promise as a novel pan-HER2 tyrosine kinase inhibitor (TKI) for the treatment of BM and should be evaluated further. Surgical or radiotherapy in combination with pyrotinib was found to statistically improve OS in our cohort. TMB could be an exploratory biomarker for predicting PFS and OS, but its clinical application still needs further verification.

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Translational Relevance

Patients with HER2-positive ($HER2^+$) metastatic breast cancer (MBC) have a poor prognosis and tend to develop brain metastases (BMs). Pyrotinib, a pan-HER2 receptor tyrosine kinase inhibitor (TKI), has encouraging antitumor effects on progression-free survival (PFS) in patients with $HER2^+$ MBC. However, overall survival (OS) data from $HER2^+$ patients with BM remain scarce. In this multicenter real-world study, pyrotinib shows promise as a novel pan-HER2 TKI for the treatment of BM. Surgical or radiotherapy in combination with pyrotinib was found to improve OS in our BM cohort statistically. Our results could be a crucial complement to current clinical trials. This work also demonstrates tumor mutation burden (TMB) could be an exploratory biomarker for predicting PFS and OS in patients treated with pyrotinib, which warrants further prospective clinical studies.

Introduction

Human epidermal growth factor receptor 2 (HER2) (also referred to as ERBB2) is amplified and/or overexpressed in approximately 15% to 20% of primary breast cancers (1). Patients with HER2-positive ($HER2^+$) breast cancer have a poor prognosis and tend to develop metastatic disease in the central nervous system (CNS), and these patients have an especially high risk of brain metastasis (BM; refs. 2, 3). CNS metastases have been reported in 15%–25% of patients with breast cancer (4), and BM occurs in 30%–55% of patients with metastatic breast cancer (MBC; ref. 5), highlighting the need for safe and effective BM-targeted treatments. Moreover, the survival of these patients is generally poor, and the median overall survival (OS) after the initial diagnosis of CNS metastases is 13.0 months (6). Although anti-HER2 therapeutic options have steadily enhanced the effect of systemic therapy on patients with $HER2^+$ MBC, there is an increased awareness that clinical strategies targeting BM are needed for this subtype of breast cancer (7).

The novel oral pan-ErbB receptor tyrosine kinase inhibitor (TKI) pyrotinib has the advantages of stability, safety, and good tolerance, with encouraging antitumor effects observed in patients with $HER2^+$ MBC (8–14). In August 2018, pyrotinib received its first global conditional approval in China for use in combination with capecitabine for the treatment of patients with $HER2^+$ MBC who had previously received anthracycline or taxane chemotherapy (9). In a phase II study, pyrotinib plus capecitabine yielded a significantly higher overall response rate (ORR; 78.5% vs. 57.1%, $P = 0.01$) and longer progression-free survival (PFS; 18.1 months vs. 7.0 months, $P < 0.001$) than lapatinib plus capecitabine (12). The PHENIX study showed that pyrotinib combined with capecitabine increased the median PFS by 7.0 months (11.1 months vs. 4.1 months, $P < 0.001$) and increased the ORR (68.6% vs. 16.0%, $P < 0.001$) compared with capecitabine monotherapy in women with $HER2^+$ MBC. Patients with BM also achieved a longer PFS (6.9 months vs. 4.2 months, $P = 0.011$; ref. 13). The PHOEBE study revealed that pyrotinib combined with capecitabine increased the median PFS by 5.7 months (12.5 months vs. 6.8 months, $P < 0.0001$) compared with lapatinib plus capecitabine in patients with $HER2^+$ MBC who had been previously treated with trastuzumab and taxane and/or anthracycline (15). Although the promising effects on PFS have demonstrated the significant therapeutic value of pyrotinib, OS data from $HER2^+$ patients with BM are still lacking (14, 16, 17).

In our previous multicenter real-world study, pyrotinib was found to be effective against $HER2^+$ MBC with tolerable side effects. A total of 168 patients were enrolled, and the median PFS time was 8.07 months [95% confidence interval (CI), 7.041–9.099 months] in the entire cohort (17). For patients with BM ($n = 39$, 23.21%) at baseline the median PFS time was 8.80 months (17). After ongoing follow-up for 1 more year, 99 patients reached OS events. Here, we report the final analysis results from all cohorts, with a focus on the results from patients with baseline BM.

Materials and Methods

Data collection and study design

This real-world study is an exploratory analysis of BM and the final update of our preceding study (17), with an extended follow-up through December 2020. Between June 2018 and August 2019, 168 patients with $HER2^+$ MBC treated with pyrotinib were enrolled in our real-world analysis. The enrollment inclusion criteria were as follows: (i) confirmed pathologic diagnosis of $HER2^+$ MBC (IHC category 3+ or positive results of FISH; ref. 18); (ii) a measurable lesion as defined by the revised Response Evaluation Criteria in Solid Tumors 1.1 (ref. 19; RECIST 1.1); and (iii) adequate hematologic, hepatic, and renal functions. The enrollment exclusion criteria were as follows: (i) discontinued pyrotinib treatment; (ii) pyrotinib medication use as neoadjuvant therapy; (iii) severe adverse side effects could not be controlled by dose reductions according to drug instructions; and (iv) loss to follow-up for other unknown reasons.

Most enrolled patients received 400 mg pyrotinib at baseline, and 15 patients (15/168, 8.9%) had a dose reduction to 320-mg pyrotinib once daily in a 21-day meditation cycle because of grade 3 and 4 diarrhea. Informed consent was obtained for clinical information collection and follow-up examination from all patients. Clinical follow-up was scheduled every 2 to 3 weeks during treatment. Imaging follow-up was scheduled every one to two drug cycles (21 days per cycle), according to the standard clinical guidelines. The study was reviewed and approved by the Research Ethics Committee of the Second Xiangya Hospital of Central South University and conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Assessment of tumor mutation burden

Twenty-eight out of 168 patients consented to receive next-generation sequencing (NGS) assays [OncoMD/OncoMD-Plus (http://www.geneplus.org.cn), comprising a customized panel of 1,021 genes] using peripheral blood samples collected before pyrotinib treatment. Circulating tumor DNA (ctDNA) sequencing was used to calculate genetic mutation and tumor mutation burden (TMB). The TMB was calculated as the number of somatic nonsynonymous single-nucleotide variants (SNVs) and small insertions/deletions per megabase in the coding region [mutations per megabase (mut/Mb) with ≥ 0.005 for ctDNA] and by integrated mutation profiling testing of actionable cancer targets within the same gene panel in the samples from the Geneplus database (20–22). The categorization of TMB-high and TMB-low was explained in the preceding article (17).

Statistical analysis

Pearson χ^2 test or Fisher exact test was used to compare categorical variables in different groups of patients. Progression-free survival (PFS) and overall survival (OS) were estimated using Kaplan-Meier curves. OS and PFS were defined as the time from the beginning of treatment with pyrotinib until disease progression (PFS) or death from any cause (OS). Log-rank tests and survival curve plotting were carried

Table 1. Demographic characteristics of patients with and without brain metastasis.

	Non-BM No. (%)	BM No. (%)		*P*
Age				
<50	63 (48.8)	19 (48.7)	0.487	1.000
≥50	66 (51.2)	20 (51.3)	0.513	
ECOG scale				
0–1	120 (93.0)	35 (89.7)	0.897	0.742
≥2	9 (7.0)	4 (10.3)	0.103	
Menopausal status				
Postmenopausal	53 (41.1)	22 (56.4)	0.564	0.133
Premenopausal	76 (58.9)	17 (43.6)	0.436	
HR status				
Positive	69 (53.5)	23 (59.0)	0.590	0.675
Negative	60 (46.5)	16 (41.0)	0.410	
Treatment stage				
First-line	7 (5.4)	2 (5.1)	0.051	0.064
Second-line	56 (43.4)	9 (23.1)	0.231	
Third- or higher-line	66 (51.2)	28 (71.8)	0.718	
Treatment type				
Pyrotinib + capecitabine	89 (69.0)	25 (64.1)	0.641	0.953
Pyrotinib + abraxane	14 (10.9)	5 (12.8)	0.128	
Pyrotinib + trastuzumab	9 (7.0)	3 (7.7)	0.077	
Pyrotinib + others	17 (13.2)	6 (15.4)	0.154	
Prior trastuzumab treatment				
No prior trastuzumab	34 (26.4)	7 (17.9)	0.179	0.507
Previously used trastuzumab	95 (73.6)	32 (82.1)	0.821	
Total	129	39		

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

out using the survival and survminer R packages. Median survival time and 95% confidence intervals (CIs) were calculated, and all reported *P* values (significant cutoff as < 0.05) were two-sided. All statistical calculations and visualizations were executed by R (version 4.0.3, https://www.r-project.org/).

Results

Baseline characteristics

Among the 168 participants, 39 patients were diagnosed with BM, while 129 did not have BM before being treated with pyrotinib. The baseline characteristics of the patients in the study and the *P* values for comparisons between the group of patients with BM and the group of patients without BM are presented in **Table 1**. The 39 enrolled patients with BM were divided into two groups according to whether they received local therapy (surgery/radiation). The surgery/radiation (S/R) group included 43.59% of patients (17/39) who were treated with surgery (4/39) or radiotherapy (15/39) combined with pyrotinib; the no surgery/no radiation group (NS/NR) consisted of 56.41% (22/39) patients with BM. In the other 129 patients who had BM involvement, nine patients were found to have developed BM during pyrotinib treatment. The baseline characteristics of patients with BM are presented in Supplementary Table S1. There was no statistical difference in the baseline data between groups.

Treatment outcomes of all patients

The median follow-up time was 18.50 months. The numbers of PFS and OS events were 141 (83.9%) and 99 (58.9%), respectively. The median PFS time in the entire cohort was 8.00 (7.3–10.53) months (**Fig. 1A**). The OS time was 19.07 (15.13–22.87) months (**Fig. 1B**). The log-rank test results indicated that there was no significant difference between the patients with BM and those without in terms of PFS (8.67; 95% CI, 6.43–11.87 vs. 8.00; 95% CI, 7.07–12.2, $P = 0.16$; **Fig. 1C**), while a significant difference was detected for OS (13.93; 95% CI, 10.53–20.67 months vs. 20.93; 95% CI, 17.13–25.13 months, $P = 0.01$; **Fig. 1D**).

The OS of patients who received pyrotinib as second-line treatment was 19.63 months (95% CI, 13.13–NA), and that of patients who received third or higher line treatment was 18.67 months (95% CI, 14.97–24.47; $P = 0.94$; Supplementary Fig. S1A). The OS of patients with hormone receptor (HR) positivity was 22.33 months (95% CI, 19.53–NA), while that of patients with HR negativity was 14.52 months (95% CI, 12.57–20.67; $P = 0.0075$; Supplementary Fig. S1B).

Treatment outcomes of patients with BM

A total of 39 patients with BM were included in the survival analysis. Seventeen patients (43.59%, 17/39) underwent surgery/radiation, and 22 patients (56.41%, 22/39) did not. The S/R group achieved a longer PFS trend (9.97 months) than the NS/NR group (7.73 months; $P = 0.19$; **Fig. 2A**). In addition, S/R group had significantly improved OS compared with the NS/NR group (20.67 months vs. 12.43 months, $P = 0.021$; **Fig. 2B**).

Focusing on the CNS response to pyrotinib, we evaluated the rates of stable disease (SD), partial response (PR), complete response (CR), and progressive disease (PD) between the S/R and NS/NR groups. Some patients with brain metastasis in the NS/NR group reached the OS endpoint quickly and without enough MRI data to classify their response status to pyrotinib, so they were excluded from analysis. The CBR was 58.6% (S/R) versus 41.4% (NS/NR), while the ORR was 24.1% (S/R) versus 31.0% (NS/NR).

In patients with BM, the median PFS and OS were not affected by the number of prior lines of therapy ($P = 0.73$, $P = 0.053$), HR status ($P = 0.95$, $P = 0.62$), liver metastasis ($P = 0.11$, $P = 0.51$), lung metastasis ($P = 0.75$, $P = 0.13$), or bone metastasis ($P = 0.82$, $P = 0.33$). These survival curves are shown in Supplementary Fig. S1C–S1L.

High TMB is associated with poor PFS and OS

We compared the survival analyses between the TMB-high group and TMB-low group. There was a significant difference in PFS observed in the 28-patient cohort according to the log-rank test [13.44 (7.37–22.9) months vs. TMB-high 4.9 (4.33–NA) months, $P = 0.0072$; **Fig. 3A**].

OS also showed a significant difference in the survival analysis (TMB-low, 22.87; 95% CI, 17.47–NA months vs. 8.2; 95% CI, 4.83–NA months; $P = 0.0028$; **Fig. 3B**). These results reveal that high TMB may be a prognostic marker for poor PFS and OS in patients treated with pyrotinib. In the patients with BM, high TMB was negatively associated with PFS ($P = 0.075$) and OS ($P = 0.032$). Kaplan-Meier curves are shown in **Fig. 3C** and **D**. Nonetheless, further verification of these findings is needed in the future due to the small sample size of this study and its nature.

Discussion

Over the past decades, substantial improvements in the diagnosis and treatment of patients with breast cancer have been achieved, but brain metastasis is still associated with poor outcomes, and these patients lack a sufficiently effective treatment (6, 23, 24). Pyrotinib, as a novel anti-HER2 TKI, shown a potential effectiveness in patients with BM (13). This is the first study to report the OS data in pyrotinib-based

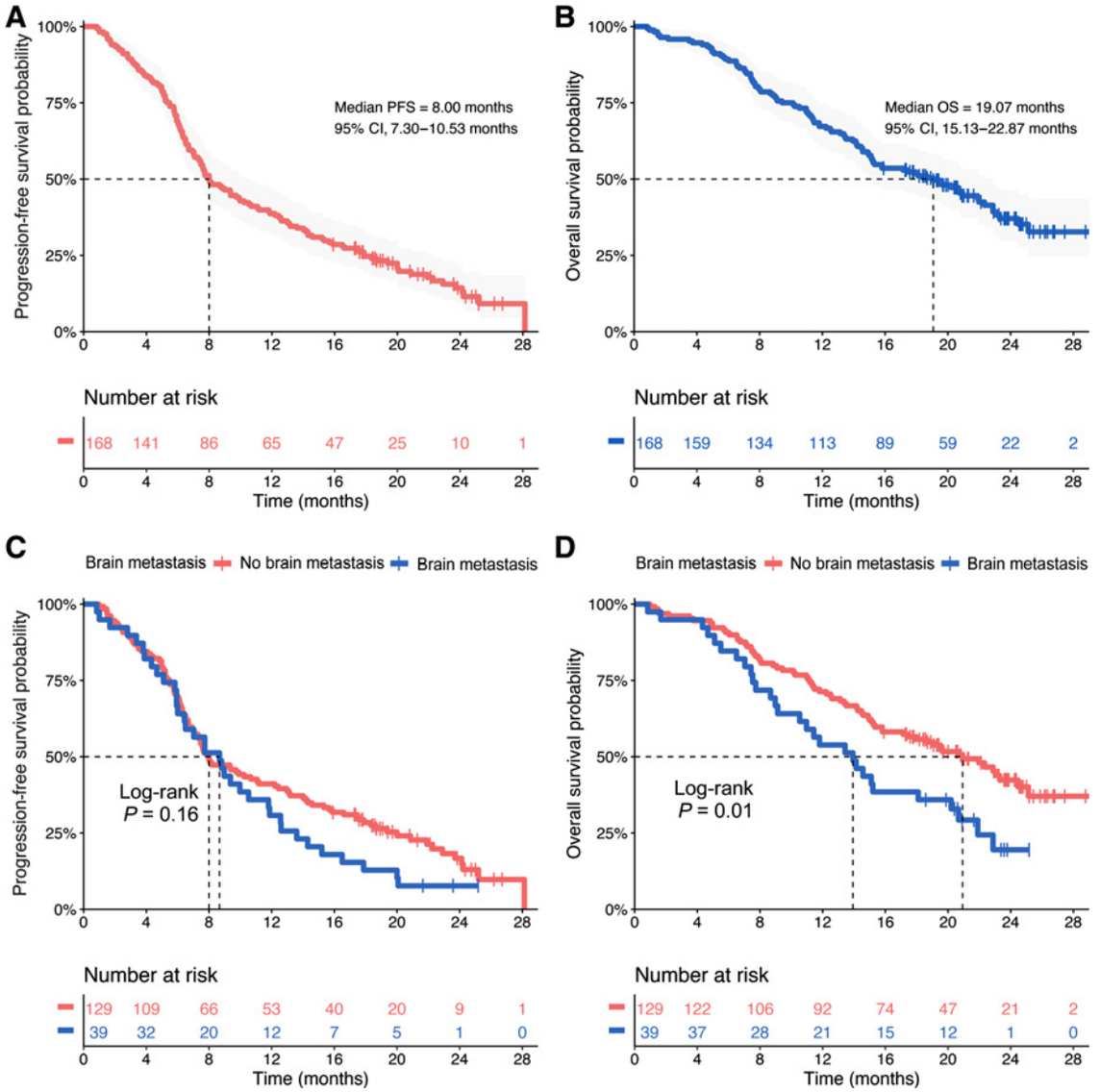

Figure 1.

PFS and OS in patients with and without BM at baseline. **A,** PFS of all patients. **B,** OS of all patients. **C,** PFS of patients with and without BM at baseline. **D,** OS of patients with and without BM at baseline.

treatment, especially in a BM cohort, which could be a crucial complement to current clinical trials.

With the development of HER2-targeted drugs, patients with BM have more options for treatment. The results based on the EMILIA (25) trial confirmed the advantage of T-DM1 for the PFS and OS of HER2$^+$ MBC, but the KAMILLA study indicated that T-DM1 can only bring about a median PFS of approximately 5.5 months for patients with BM (26). Compared with monoclonal antibodies, the physical features of small-molecule TKIs play an important role in allowing them to cross the blood-brain barrier (BBB), thereby improving drug concentrations in the brain (27, 28). This suggests that TKIs could be a rational therapeutic approach to treat CNS metastases (28). Neratinib (29) or lapatinib combined with capecitabine both achieved a median PFS of 5.5 months in the BM group (7). In the HER2CLIMB study, the median PFS was 9.9 months, and the median OS was 18.1 months with tucatinib in combination with trastuzumab and capecitabine compared to placebo plus trastuzumab and capecitabine (PFS, 4.2 months; OS, 12.0 months) among patients with BM (30). However, pyrotinib plus capecitabine reached a median PFS of 6.9 months (13). Meanwhile an 8.67-month PFS and 13.93-month OS were reported in our study, and the number of patients with brain metastases, local treatment of the brain, or third- or higher-line treatment accounted for a higher proportion in this study than in the PHENIX study. Although selection bias may exist in our cohorts, the results of this study provide evidence to support additional clinical trials and indicate that pyrotinib can elicit a good therapeutic effect in HER2$^+$ patients with BM.

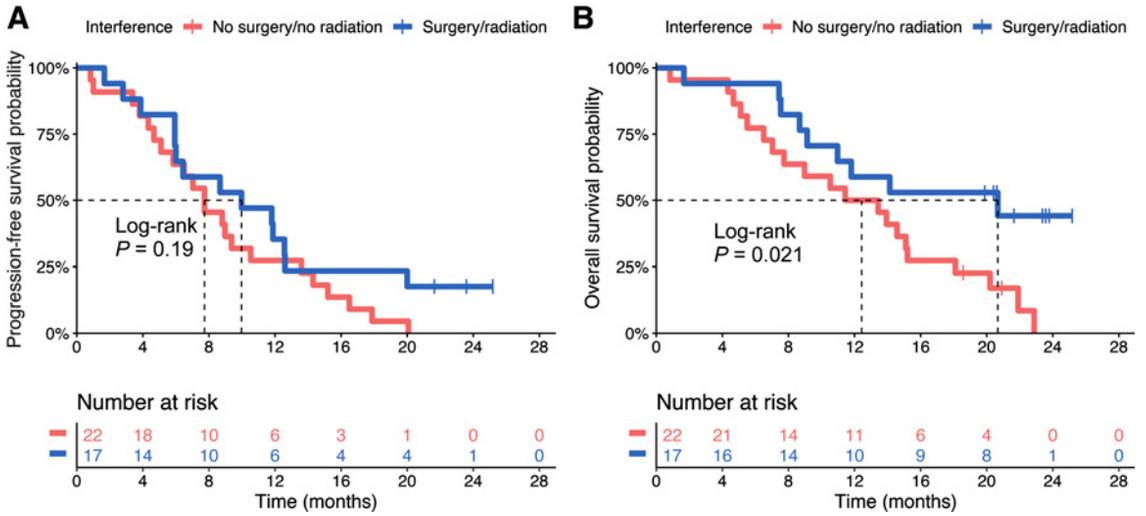

Figure 2.

Survival curve in patients with BM at baseline. **A,** PFS curves of patients with BM (surgery/radiation group and no surgery/no radiation group). **B,** Overall survival curves of patients with BM (surgery/radiation group and no surgery/no radiation group).

Our study showed that 6.98% (9/129) of patients with HER2$^+$ MBC without BM developed brain metastases while receiving pyrotinib treatment. In the KAMILLA study, 9.6% (154/1604) of patients without baseline BM developed brain lesions during T-DM1 treatment (26). The NEfERT-T trial (31) reported that 8.3% of BM recurrence occurred in the neratinib + paclitaxel group. The incidence of CNS metastases as the first site of relapse was 3% (8/251) for lapatinib + capecitabine, as shown in the CEREBEL trial (32). Thus, pyrotinib may have an advantage in preventing brain metastases, and further high-quality trials are expected.

The current systemic treatment of BM is not very effective (33) because monoclonal antibody treatments cannot be transported well across the BBB. Other HER2-targeting agents, including lapatinib (34), afatinib (35), neratinib (29) and tucatinib (30), have also been reported to have synergistic effects with radiotherapy. Several studies have implicated neurosurgery (34) or radiation (35) in changing the permeability of the blood-brain barrier. These agents in combination with neurosurgery and/or radiotherapy could partially improve survival outcomes in patients with oligometastasis (36). Similarly, our study showed that there was a statistically significant OS benefit in the S/R group of patients with BM treated with pyrotinib compared with the NS/NR group with BM. Thus, it suggested a combination therapy option for HER2$^+$ patients with BM, but the specific role and underlying mechanism of pyrotinib in sensitizing HER2+ BC to radiation/surgery needs to be further elucidated in a prospective trial (37). It is undeniable that selection bias may exist among the patients who underwent local interventions.

TMB is an emerging biomarker of the immunotherapeutic response of solid tumors (38) and is defined as the total number of somatic mutations in a defined region of a tumor genome, but the precise definition varies with the sequenced region size and localization and the nature of the mutations included (39). A phase I study of 18 patients with HER2$^+$ MBC suggested that *PIK3CA* and *TP53* mutations in ctDNA ($P = 0.013$) rather than in tumor tissues ($P = 0.474$) may predict the efficacy of monotherapy with pyrotinib (10). However, no single genetic alteration was found to show any predictive value for pyrotinib in combination with capecitabine (11). Research conducted by Conlon revealed that BRCA2 mutations were correlated with responses to neratinib and tucatinib (40). A study of 21 patients with ER$^+$HER2$^+$ breast cancer treated with neoadjuvant letrozole and lapatinib showed that *TP53* and *PIK3CA* were associated with unfavorable clinical outcomes (41). Another study indicated that low *PTEN* and *PIK3CA* mutations were associated with resistance to neoadjuvant treatment with lapatinib and trastuzumab (42). We also explored some of the most frequently mutated genes (*TP53, ERBB2*, and *PIK3CA*) in our cohort, but we did not observe any significant results between these genes and the treatment outcomes of pyrotinib in combination with chemotherapy (data not shown). We considered that whether single-gene tumor mutations may not be good prognostic markers, so we chose TMB to serve as a biomarker to predict PFS and OS. In our previous study, we presented that high TMB was associated with poor PFS under pyrotinib-based treatment (17). In this final analysis of our exploratory study, high TMB was considered a prognostic marker for poor OS in patients with HER2+ MBC. To our knowledge, this result provides the first evidence of the association between ctDNA TMB and PFS and OS in patients receiving pyrotinib therapy. TMB might predict and monitor therapeutic response as an exploratory biomarker in the clinical treatment of MBC. Nevertheless, this finding is based on a small subset of patients. There is an urgent need for prospective biomarker-driven trials to identify targeted drugs for HER2$^+$ MBC.

Several limitations exist in the current study. First, this was a retrospective analysis, not a prospective study, so selection bias was present. Second, the sample size of biomarker analysis was also relatively small. Third, few patients received pertuzumab or T-DM1 prior to pyrotinib because its approval in China almost occurred after our enrollment deadline. Further high-quality clinical trials should be conducted to verify our findings.

In conclusion, pyrotinib shows promise as a novel pan-HER2 TKI for the treatment of BM and should be evaluated further. Surgical or radiotherapy in combination with pyrotinib was found to statistically

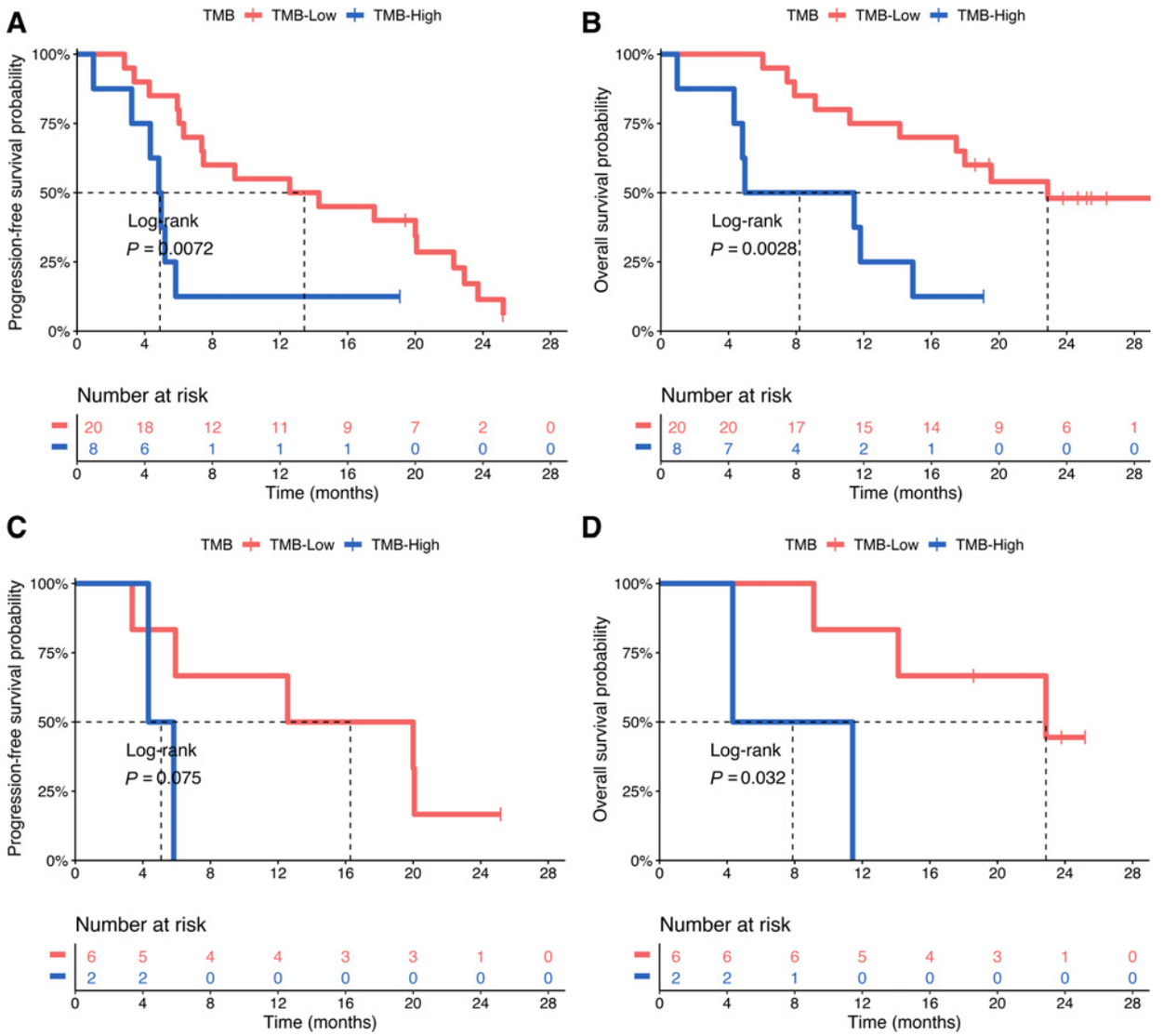

Figure 3.

Kaplan–Meier curves for PFS and OS according to tumor mutation burden (TMB) status. **A,** Kaplan–Meier curves for PFS according to the TMB status of all patients. **B,** Kaplan–Meier curves for OS according to the TMB status of all patients. **C,** Kaplan–Meier curves for PFS according to the TMB status of patients with BM. **D,** Kaplan–Meier curves for OS according to the TMB status of patients with BM.

improve OS in our cohort. TMB could be an exploratory biomarker for predicting PFS and OS, but its clinical application still needs further verification.

Authors' Disclosures

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Authors' Contributions

M. Anwar: Data curation, investigation, methodology, writing–original draft. **Q. Chen:** Software, formal analysis, supervision, visualization, methodology, writing–review and editing. **D. Ouyang:** Investigation, methodology, writing–review and editing. **S. Wang:** Conceptualization, resources, supervision, project administration. **N. Xie:** Resources, data curation. **Q. Ouyang:** Resources, funding acquisition. **P. Fan:** Resources. **L. Qian:** Resources, data curation. **G. Chen:** Resources, data curation. **E. Zhou:** Resources, data curation. **L. Guo:** Resources, data curation. **X. Gu:** Resources, data curation. **B. Ding:** Resources, data curation. **X. Yang:** Resources, data curation. **L. Liu:** Resources, data curation. **C. Deng:** Resources, data curation. **Z. Xiao:** Resources, data curation. **J. Li:** Resources, data curation. **Y. Wang:** Resources, data curation. **S. Zeng:** Resources, data curation. **J. Hu:** Resources, data curation. **W. Zhou:** Resources, data curation. **B. Qiu:** Resources, data curation. **Z. Wang:** Resources, data curation. **J. Weng:** Resources, data curation. **M. Liu:** Resources, data curation. **Y. Li:** Resources, data curation. **T. Tang:** Resources, data curation. **J. Wang:** Resources, data curation. **H. Zhang:** Resources, data curation. **B. Dai:** Resources, data curation. **W. Tang:** Resources, data curation. **T. Wu:** Resources, data curation. **M. Xiao:** Resources, data curation. **X. Li:** Resources, data curation. **H. Liu:** Resources, data curation. **L. Li:** Resources, data curation. **W. Yi:** Conceptualization, resources, supervision, funding acquisition, project administration, writing–review and editing.

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