

Plasma IFN- γ may predict pyrotinib efficacy in patients with HER2-positive advanced breast cancer

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Abstract. Pyrotinib has been extensively utilized in the treatment of patients with human epidermal growth factor receptor 2 (HER2)-positive advanced breast cancer (ABC), yet few reliable markers for predicting pyrotinib efficacy in these patients have been identified. Therefore, the present study aimed to explore the predictive clinical factors for treating patients with HER2-positive ABC with pyrotinib. To this aim, a prospective observational study was conducted on the prognostic potential of serum cytokines in a cohort of 58 patients with HER2-positive cancer receiving pyrotinib treatment, enrolled from January 2020 to December 2022. Patients were treated with oral pyrotinib (400 mg/day, 30 min after eating) combined with capecitabine (1,000 mg/m² twice daily on days 1-14 of a 21-day cycle) or vinorelbine soft capsules (40 mg/day, 3 times weekly). Peripheral blood was collected from these patients before and 4 weeks after the initial administration of pyrotinib. Plasma cytokine levels (IL-6, IL-8, IL-10, IL-17 and IFN- γ) were measured via multiple microsphere flow cytometry luminescence. Univariate analysis revealed that patients who received pyrotinib as second-line therapy had an improved progression-free survival (PFS) time compared with those who received it as third- or later-line therapy [median PFS (mPFS), 17.0 vs. 9.5 months; $P=0.016$]. Furthermore, patients with increased plasma IL-8 levels had a poorer PFS time than those with decreased levels (mPFS, 9.4 vs. 14.0 months; $P=0.021$). Patients with increased plasma IFN- γ levels had a longer PFS time than those with decreased levels (mPFS, 13.0 vs. 11.0 months; $P=0.010$). Multivariate Cox regression analysis revealed that pyrotinib application beyond third-line therapy and decreased plasma IFN- γ levels

were independent risk factors for PFS ($P=0.025$ and $P=0.033$, respectively). In conclusion, early application of pyrotinib may improve the PFS time of patients with HER2-positive ABC who are resistant to trastuzumab; however, whether these findings translate into an improved overall survival time requires further investigation. Additionally, plasma IFN- γ has potential prognostic value in patients with HER2-positive ABC treated with pyrotinib.

Introduction

Patients overexpressing human epidermal growth factor receptor 2 (HER2) account for 15-20% of all breast cancer (BC) cases and have a poor prognosis (1,2). Pyrotinib is an irreversible small molecule tyrosine kinase inhibitor (TKI) drug that targets HER1, HER2 and HER4 and has robust antitumor effects in patients with HER2-positive BC (3). It has been shown that, compared with lapatinib plus capecitabine, pyrotinib plus capecitabine significantly improves the progression-free survival (PFS) of patients with HER2-positive metastatic BC after trastuzumab and chemotherapy (4).

Dying cancer cells exert both immuno-activating and immunosuppressive effects, depending on the intracellular signals, extracellular context and systemic crosstalk (5). Immune cells communicate with the microenvironment via cytokines, which are mainly produced by macrophages, T cells, B cells and cancer cells. These cytokines can promote or inhibit malignant tumor progression through the complex cytokine network between tumor cells and the tumor microenvironment (TME) (6,7). Cytokines from the TME can be released into the circulation and detected, making them potentially useful as biomarkers to predict disease outcomes and guide therapeutic decisions (8). In BC, cytokines serve a crucial role in regulating immunity (9). The levels of cytokines such as TNF, colony stimulating factor 1, IL-6 and endogenous IFN in the circulation of patients with BC are significant markers of tumor progression (10). These cytokines also form an autocrine, pro-inflammatory feed-forward loop that facilitates the accumulation of drug-resistant BC cells (11).

The aim of the present study was to explore the relationship between plasma cytokines and the efficacy of pyrotinib. For this purpose, plasma cytokines such as IL-6, IL-8, IL-10, IL-17 and IFN- γ were measured before and 4 weeks after pyrotinib treatment.

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Key words: human epidermal growth factor receptor 2-positive breast cancer, pyrotinib, cytokine, interferon- γ , clinical efficacy, adverse events

Materials and methods

Study design. A total of 58 patients with HER2-positive advanced BC (ABC) treated with pyrotinib at Tangshan People's Hospital (Tangshan, China) were enrolled in the present single-center prospective study from January 2020 to December 2022. Since only patients who were already scheduled to receive pyrotinib-containing treatments were included in the present study, patient randomisation was not applicable. The study protocol was approved by the Ethics Committee of Tangshan People's Hospital (approval no. RMY-LLKS-2019-058; Tangshan, China). The key inclusion criteria were as follows: i) Histopathological confirmation of invasive ductal carcinoma with clear immunohistochemistry; ii) HER2 3+ or 2+ detected by fluorescence *in situ* hybridization (FISH); iii) Eastern Cooperative Oncology Group (ECOG) score of 1-3; and iv) patients expected to survive ≥ 3 months. According to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) for efficacy evaluation, at least one measurable target lesion was detected, and previous trastuzumab-based targeted therapy had failed. The key exclusion criteria were as follows: i) Had other malignant tumors; ii) were under the age of 18; and iii) were unwilling to provide informed consent. Written informed consent was obtained from all participants prior to study enrollment.

Treatment and follow-up. All patients received pyrotinib (400 mg, orally, once daily) combined with capecitabine (1,000 mg/m², orally, twice daily on days 1-14) or vinorelbine soft capsules (40 mg, orally, 3 times weekly), which was for patients who had previously taken capecitabine in a 3-week cycle. Upon the development of grade ≥ 3 adverse events, symptomatic treatment was administered and pyrotinib was suspended or reduced to 320 mg daily. In the present study, treatment was continued until disease progression (PD), unacceptable toxicity or withdrawal of consent. Treatment responses were determined according to the RECIST 1.1 guidelines. Adverse events were assessed according to the Common Terminology Criteria for Adverse Events Version 4.0 and graded as 0-4. Follow-up was performed upon clinic visit, hospital admission, and telephone contact until December 2023.

Sample and data collection. In total, 2 ml peripheral blood (PB) samples were collected from patients before and 4 weeks after the initial pyrotinib treatment using EDTA anticoagulant tubes. The plasma samples were centrifuged at 1,000 $\times$ g for 10 min for cytokine detection. The plasma cytokine levels, including IL-6, IL-8, IL-10, IL-17 and IFN- γ , were measured using a multi-microsphere immunofluorescence assay according to the manufacturer's instructions (plasma cytokine test kit; cat. no. 20202400205; Qingdao Ruisikaier Biotechnology Co., Ltd.) and a Navios flow cytometer analyser with Kaluza 1.2 software (Beckman Coulter, Inc.).

Statistical analysis. Statistical analyses were performed using SPSS 26.0 software (IBM Corp.). For descriptive analysis, qualitative variables are expressed as absolute values and percentages, and quantitative variables are expressed as the mean and range. Kaplan-Meier curves were used for survival

analysis. Based on the results of the univariate logistic regression analysis ($P < 0.10$), candidate variables were selected for inclusion in the multivariate Cox regression analysis, which was performed to assess the prognostic variables. The relationship between non-normally distributed variables was analysed by Spearman's rank correlation analysis. The predictive accuracy of the nomograms was evaluated using receiver operating characteristic (ROC) curves. $P < 0.05$ was considered to indicate a statistically significant defined difference.

Results

Clinical data. A total of 58 patients were included in the present study. The median follow-up time was 33.25 months (range, 14.0-49.0 months) and the follow-up rate was 98.28%. The median age of the included patients was 53 years. The detailed clinical data of the 58 patients are shown in Table I. The findings on the IL-6, IL-8, IL-10 and IL-17 plasma levels before and 4 weeks after pyrotinib treatment are shown in Table II.

Short-term efficacy. The short-term efficacy evaluation according to the RECIST 1.1 guidelines revealed that 7 patients had PD (12.1%), 28 had stable disease (48.3%), 22 had partial remission (37.9%), and 1 had complete remission (1.7%).

Log-rank univariate analysis. On December 2023 data cut-off date, the median PFS (mPFS) time of the included patients was 11.0 months, the 2-year survival rate was 66%, the 3-year survival rate was 52% and the median overall survival (OS) was not reached. The results revealed that patients with a HER2 3+ status had an improved PFS time compared with those with a HER2 2+ status (FISH-positive) (mPFS, 13.0 vs. 9.5 months; $P = 0.053$; Fig. 1A). Patients whose HER2 expression status changed after recurrence exhibited a trend towards a poorer PFS time compared with those with stabilized HER2 expression (mPFS, 9.5 vs. 13.0 months; $P = 0.072$). Patients who received pyrotinib as the second-line therapy had a longer PFS time than patients who received pyrotinib as the third- or later-line therapy (mPFS, 17.0 vs. 9.5 months; $P = 0.016$; Fig. 1B). Patients who had primary resistance to trastuzumab also had a poorer PFS time than those who had secondary resistance (mPFS, 9.5 vs. 13.0; $P = 0.057$). Patients whose plasma IL-8 levels increased after 4 weeks of pyrotinib treatment had a poorer PFS time than those whose plasma IL-8 levels decreased (mPFS, 9.4 vs. 14.0 months; $P = 0.021$; Fig. 1C). Patients with increased plasma IFN- γ levels had an improved PFS time compared with patients with decreased plasma IFN- γ levels (mPFS, 13.0 vs. 11.0 months; $P = 0.013$; Fig. 1D). The results of the univariate analysis of PFS with various clinical parameters in patients with pyrotinib-treated ABC are included in Table III.

Multivariate Cox regression analysis. Multivariate Cox regression analysis revealed that the number of cycles of pyrotinib treatment after recurrence and elevated plasma IFN- γ levels after treatment were independent predictive factors for PFS (Table IV and Fig. 2).

Table I. Baseline characteristics of human epidermal growth factor receptor 2-positive patients with advanced breast cancer treated with pyrotinib.

Clinical characteristics (n=58)	n (%)
Eastern Cooperative Oncology Group performance status	
0	7 (12.10)
1	46 (79.3)
2	5 (8.60)
Menopause	
No	38 (65.50)
Yes	20 (34.50)
Oestrogen ⁺	
≥20%	19 (32.76)
<20%	39 (67.24)
Location of metastasis	
Lung	39 (67.24)
Liver	14 (24.14)
Brain	7 (12.07)
Bone	18 (31.03)
Serous effusion	3 (5.17)
Lymph node	29 (50.0)
Multiple organs metastasis	
Yes	46 (79.31)
No	12 (20.69)
Chemotherapy cycles	
2 lines	21 (36.21)
≥3 lines	37 (63.79)
Combined	
Capecitabine	35 (60.30)
Vinorelbine	23 (39.7)

Table II. Plasma cytokine levels in patients with advanced breast cancer treated with pyrotinib.

Cytokines	Pretreatment, median (range)	Post-treatment, median (range)
IL-6, pg/ml	7.95 (1.23-56.83)	8.81 (1.33-71.72)
IL-8, pg/ml	7.41 (0.37-32.06)	8.03 (0.1-65.05)
IL-10, pg/ml	1.98 (0.71-4.62)	1.68 (0.18-3.63)
IL-17, pg/ml	4.64 (0.53-19.23)	2.87 (0.1-32.46)
Interferon-γ	6.28 (0.29-27.09)	6.65 (0.5-25.99)
IL, interleukin.		

Spearman's rank correlation analysis. Spearman's rank correlation analysis demonstrated that there was no correlation between the levels of plasma IFN-γ and other cytokines ($P>0.05$). There was a correlation between IL-17 and IL-8 after treatment, with a correlation coefficient of 0.431 ($P<0.001$).

There was a correlation between IL-17 and IL-10, with a correlation coefficient of 0.666 ($P<0.001$) but there was no correlation between IL-8 and IL-10, with a correlation coefficient of 0.212 ($P=0.110$) (Table V).

ROC curve analysis. ROC curve analysis demonstrated that the change in plasma IFN-γ level had an area under the curve of 0.728 (95% confidence interval, 0.544-0.912) for predicting the PFS of patients with HER-2 positive ABC treated with pyrotinib (Fig. 3).

Adverse reactions. There were no deaths associated with adverse events in any of the included patients. The most commonly adverse events were diarrhea (91.38%), bone marrow suppression (34.48%), hand-foot syndrome (32.76%), nausea (21.69%), fatigue (5.17%) and oral mucositis (6.9%) (Table VI). Most adverse events were well tolerated in all patients and were alleviated after diet modification, symptomatic treatment or by adjusting the dose of pyrotinib.

Discussion

BC is currently the most common malignant tumor and HER2-positive BC accounts for 15-20% of all BC cases (1,2). Trastuzumab is the most commonly used anti-HER2 monoclonal antibody, but ~50% of patients show resistance (12,13). Numerous patients with HER2-positive BC do not respond to initial therapy with trastuzumab, and a vast majority of these patients develop resistance to this monoclonal antibody within 1 year (14). A randomized phase II trial demonstrated that, pyrotinib (an irreversible anti-HER2 TKI) plus capecitabine demonstrated a significantly improved objective response rate (78.5 vs. 57.1%) and PFS time (18.1 vs. 7.0 months) than lapatinib plus capecitabine in patients with HER2-positive metastatic BC (15). The phase III PHOEBE clinical study also identified that the mPFS time was significantly longer in patients treated with pyrotinib plus capecitabine (12.5 months) compared with those treated with lapatinib plus capecitabine (6.8 months) who had been previously treated with trastuzumab and taxanes (4). The present study enrolled 58 patients, including 7 patients with brain metastasis. The mPFS time of these patients was 11.0 months, the 2-year survival rate was 66%, the 3-year survival rate was 52% and the median OS had not yet been reached. Notably, in the present study, all patients exhibited primary or secondary resistance to trastuzumab and some patients had previously received heavily treatment of them even had been heavily treated. A previous study demonstrated that the mPFS time of patients with HER2-positive ABC treated with pyrotinib was 14.1 months and that there were no significant differences in PFS or OS among patients receiving pyrotinib as first-, second-, and third or later-line therapy (16). In the present study, patients who received pyrotinib beyond the third-line a had poorer PFS time than those who received pyrotinib at the second-line (mPFS, 9.5 vs. 17.0 months; $P=0.016$). Multivariate analysis also revealed that the application of pyrotinib beyond third-line therapy was an independent risk factor for PFS. A previous study reported that, for patients with HER2-positive metastatic BC receiving first-, second- and third or later-line therapy with pyrotinib, the mPFS time was 16.5, 12.4 and 9.3 months, respectively (17).

Table III. Univariate analysis of the prognosis of patients with advanced breast cancer treated with pyrotinib.

Clinical parameters	Total (n=58)	Median progression-free survival (months)	χ^2	P-value
Age, years				
≤53	32	13.0	1.801	0.180
>53	26	9.0		
ECOG				
1	7	7.0	0.882	0.643
2	46	12.0		
3	5	6.5		
Menopause				
Yes	38	11.0	1.023	0.880
No	20	11.0		
Oestrogen status				
<20%	48	11.0	0.041	0.839
≥20%	10	8.0		
Brain metastasis				
Yes	7	13.0	0.316	0.574
No	51	11.0		
HER2 status when relapse				
3 ⁺	43	13.0	3.744	0.053
2 ⁺ (FISH ⁺)	15	9.5		
HER2 status changed after recurrence				
Yes	13	9.5	3.231	0.072
No	45	13.0		
Therapy cycles				
2 lines	21	17.0	5.766	0.016
≥3 lines	37	9.5		
Resistance to trastuzumab				
Primary	20	9.5	3.637	0.057
Secondary	36	13.0		
Combined drug				
Capecitabine	35	13.0	1.176	0.278
Vinorelbine	23	9.0		
Lapatinib application				
Yes	9	9.0	1.457	0.227
No	49	12		
IL-6 level				
Increase	28	11.0	3.296	0.069
Decrease	30	17.0		
IL-8 level				
Increase	29	9.4	5.332	0.021
Decrease	29	13.0		
IL-10 level				
Increase	30	13.0	1.346	0.246
Decrease	28	10.7		
IL-17 level				
Increase	29	11.0	0.031	0.861
Decrease	29	13.0		
Interferon- γ level				
Increase	29	14.0	6.701	0.013
Decrease	29	9.50		

HER2, human epidermal growth factor receptor 2; IL, interleukin.

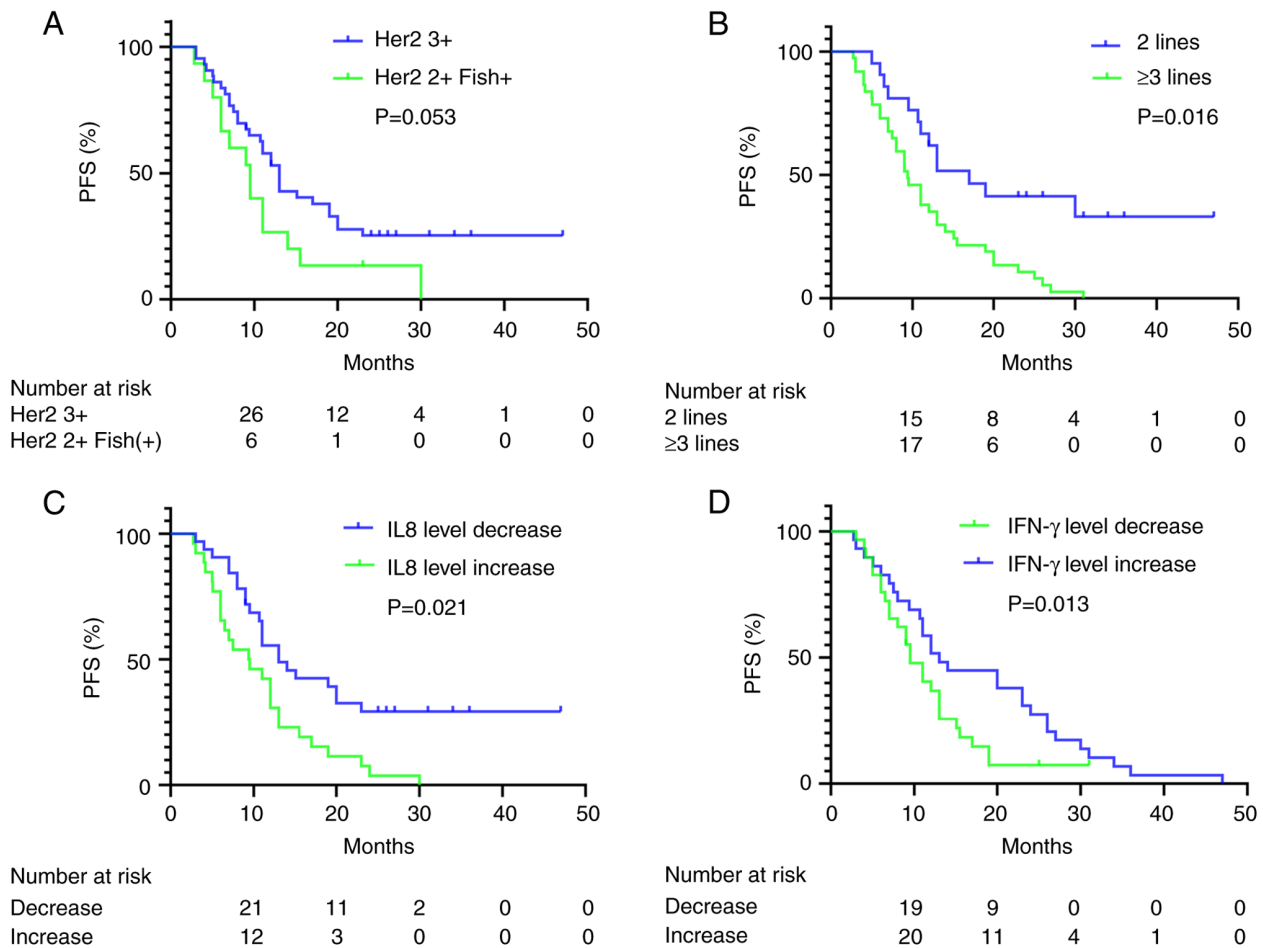


Figure 1. Kaplan-Meier survival curves of clinical factors and the PFS of patients with HER2-positive ABC treated with pyrotinib. (A) Patients with a HER2 status 3+ status who relapse have an improved PFS time compared with those with a HER2 2+ status (fluorescence *in situ* hybridization-positive) (median, 13.0 vs. 9.5 months; P=0.053). (B) Patients receiving pyrotinib as second-line therapy have an improved PFS time compared with patients receiving third-line therapy or latter (median, 17.0 vs. 9.5 months; P=0.015). (C) Survival data of patients with decreased plasma IL-8 levels at 4 weeks after the initiation of pyrotinib compared with patients with increased plasma IL-8 levels (median, 13.0 vs. 9.4 months; P=0.021). (D) Patients with increased plasma IFN-γ levels have an improved PFS time compared with those with decreased IFN-γ levels (median, 14.0 vs. 9.5 months; P=0.013). ABC, advanced breast cancer; PFS, progression-free survival; HER2, human epidermal growth factor receptor 2; IL, interleukin; IFN, interferon.

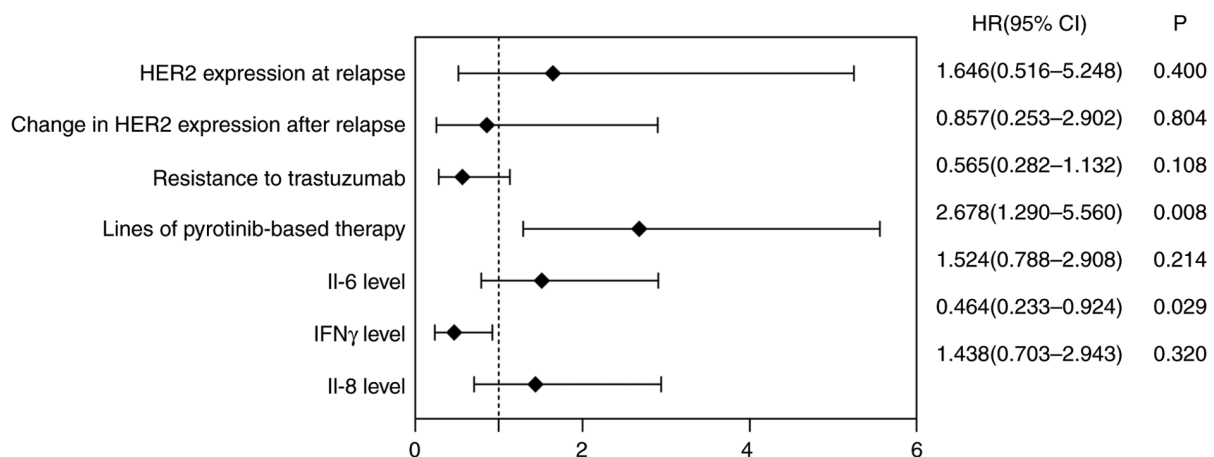


Figure 2. Early application of pyrotinib after recurrence and increased plasma IFN-γ levels post-treatment of pyrotinib are independently correlated with a prolonged progression-free survival time. IFN, interferon; HER2, human epidermal growth factor receptor 2; IL, interleukin; HR, hazard ratio; CI, confidence interval.

Another study suggested that patients who received second-line pyrotinib had a significantly longer PFS time than those who

received third-line pyrotinib (18). Therefore, early application of pyrotinib results in a longer mPFS time, however, whether

Table IV. Multivariable analysis of the prognosis of patients with advanced breast cancer treated with pyrotinib.

Clinical parameters	B	SE	Wald	df	P-value	Exp(B)
HER2 expression at relapse	0.498	0.592	0.710	1	0.400	1.646 (0.516-5.248)
Change in HER2 expression after relapse	-0.155	0.623	0.062	1	0.804	0.857 (0.253-2.902)
Resistance to trastuzumab	-0.571	0.355	2.590	1	0.108	0.565 (0.282-1.132)
Number of cycles of pyrotinib-based therapy	0.985	0.373	6.989	1	0.008	2.678 (1.290-5.560)
IL-6 level increase	0.414	0.333	1.547	1	0.214	1.514 (0.788-2.908)
IL-8 level increase	0.363	0.365	0.989	1	0.320	1.438 (0.703-2.943)
Interferon- γ level increase	-0.768	0.352	4.769	1	0.029	0.464 (0.233-0.924)

HER2, human epidermal growth factor receptor 2; IL, interleukin.

Table V. Correlations between IFN- γ and IL-6, IL-8, IL-10, IL-17 in advanced breast cancer patients after pyrotinib therapy.

Cytokines		IL-6	IL-8	IL-10	IL-17	IFN- γ
IL-6	Coefficient	1.000	0.242	0.194	0.158	-0.113
	P-value	-	0.068	0.144	0.235	0.399
IL-8	Coefficient	0.242	1.000	0.212	0.431	0.068
	P-value	0.068	-	0.110	0.001	0.610
IL-10	Coefficient	0.194	0.212	1.000	0.666	0.104
	P-value	0.144	0.110	-	0.000	0.435
IL-17	Coefficient	0.158	0.431	0.666	1.000	-0.124
	P-value	0.235	0.001	0.000	-	0.355
IFN- γ	Coefficient	-0.113	0.068	0.104	-0.124	1.000
	P-value	0.399	0.610	0.435	0.355	-

IL, interleukin.

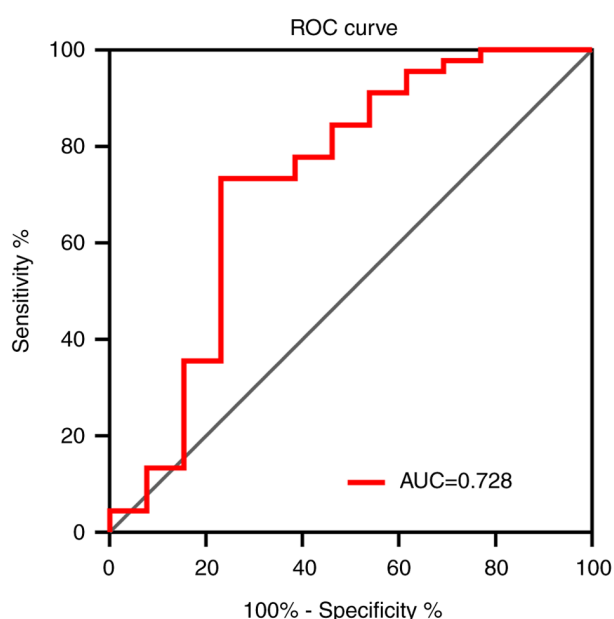


Figure 3. ROC curve analysis demonstrated that the change in plasma interferon- γ levels had an AUC of 0.728 (95% confidence interval, 0.544-0.912) for predicting the progression-free survival of patients with human epidermal growth factor receptor 2-positive advanced breast cancer receiving pyrotinib treatment. ROC, receiver operating characteristic; AUC, area under the curve.

Table VI. Incidence of the main adverse effects related to pyrotinib.

Adverse effects	All patients (%)	III-IV patients (%)
Hematology		
Neutropenia	20 (34.48%)	2 (3.45%)
Thrombocytopenia	6 (10.34%)	0 (0%)
Non-haematology		
Diarrhea	53 (91.38%)	11 (18.96%)
Hand-foot syndrome	19 (32.76%)	2 (3.45%)
Nausea	12 (21.69%)	0 (0%)
Fatigue	3 (5.17%)	0 (0%)
Oral mucositis	4 (6.90%)	0 (0%)

early application of pyrotinib can improve OS requires further follow-up observation.

In total, ~15.7% of patients with BC experience a change in HER2 status during tumor progression (17). In the present study, 13 patients experienced HER2 status alterations during tumor progression and had a worse PFS time than patients

without HER2 alterations (mPFS, 9.5 vs. 13.0 months; $P=0.072$). Additionally, patients with a HER2 3⁺ status had a longer PFS time than patients with a HER2 2⁺ status (mPFS; 13.0 vs. 9.5 months; $P=0.053$). Patients who had primary resistance to trastuzumab also had a poorer PFS time than those who had secondary resistance, when treated with pyrotinib (mPFS; 9.5 vs. 13.0 months; $P=0.057$). However, according to the results of the multivariate Cox regression analysis in the present study, there was no statistically significant difference. These results require verification in further studies with larger sample sizes and extended the follow-up periods to evaluate the long-term efficacy of pyrotinib and the survival benefits for patients.

Pyrotinib resistance is inevitable in patients with advanced HER2⁺ ABC, thus identifying a biomarker that predicts treatment efficacy is crucial. The multicentre phase II Panphila study evaluated pyrotinib plus trastuzumab, docetaxel and carboplatin as neoadjuvant therapies for early BC and found a significant association between the pathological complete response and a greater baseline infiltration of stromal (s)-CD20⁺, s-CD8⁺ and s-CD4⁺ cells (19). Cytokines produced by T-helper cells play a crucial role in regulating cellular responses between tumors and immune system (20). Cytokines are also derived from stromal, parenchymal and immune cells that reside in the tumor tissue, under the activation of cancer-related inflammation or by treatments for cancer (21). Certain cytokines may not be beneficial for cancer treatment and can cause immunosuppression, promote tumor progression and induce drug resistance (22).

HER2-positive BC is accompanied by a significant increase in PB IL-6 and IL-8 levels (23), particularly in the presence of trastuzumab-resistant HER2-positive BC cells (24). The synthesis and secretion of the IL-8 chemokine family are enhanced, which are involved in HER2-positive BC metastasis and endocrine resistance. The circulating levels of the IL-8 and GRO cytokines may represent new biomarkers for monitoring the responses of patients with BC to endocrine therapy and HER2-targeted therapy (25). In the present study, elevated IL-6 and IL-8 levels indicated a poor prognosis, but the differences were not statistically significant. IFN- γ can cause significant surface loss of HER2, diminished growth and induced tumor senescence (26). IFN- γ -secreting T cells have been observed to enhance tumor infiltration after treatment with doxorubicin and/or lapatinib in a HER2-positive BC animal model (27). The mechanism of pyrotinib, an irreversible small molecule TKI of HER1, HER2 and HER4, is similar to that of lapatinib. At present, few studies investigating biomarkers associated with the efficacy of pyrotinib have been conducted. In the present study, the plasma levels of the IL-6, IL-8, IL-10, IL-17 and IFN- γ cytokines were measured before and 4 weeks after pyrotinib treatment. Univariate analysis revealed that increased expression levels of IL-6 and IL-8 in the PB after treatment were risk factors for PFS. However, the prolonged mPFS time was associated with increased IFN- γ expression. Multivariate analysis revealed that increased PB IFN- γ levels are an independent influencing factor for mPFS time in patients with HER2-positive ABC tumors treated with pyrotinib. ROC curve analysis also demonstrated that serum IFN- γ levels have value in predicting the efficacy of pyrotinib in the treatment of HER-2 positive ABC. However,

the underlying biological mechanisms were not thoroughly investigated in the present study. The next step is to investigate the mechanisms of IFN- γ in pyrotinib treatment using *in vitro* experiments or animal models.

The main adverse effect observed in the present study was diarrhea, but this symptom can be managed through targeted symptomatic treatment and diet adjustment. Overall, the side effects included diarrhea (91.38%), bone marrow suppression (34.48%), hand-foot syndrome (32.76%), nausea (21.69%), fatigue (5.17%) and oral mucositis (6.9%), which are similar to a previous study (16) and demonstrate that the treatment is tolerable.

While the present study provides valuable insights, its findings must be interpreted considering its sample size limitations. The relatively small cohort ($n=58$) may restrict the statistical power to detect subtle effects. Reduced power increases the risk of Type II errors, wherein true associations may remain undetected due to insufficient sensitivity. Despite these limitations, the study's rigorous methodology and novel findings offer a meaningful foundation for future research. The small size of the present study means that a larger randomised study will be required to define the predictive role of PB IFN- γ levels in HER2-positive ABC treated with pyrotinib.

In summary, pyrotinib is well-tolerated by patients with HER2-positive ABC and is more efficacious with early application. Further follow-up and larger patient cohorts are required to determine whether these promising findings translate into improvements in the OS of patients. Furthermore, PB IFN- γ levels can predict the effect of pyrotinib in patients with HER2-positive ABC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JJ contributed to the design of the study, performed the data curation and analyses, performed the literature search and wrote the first draft of the manuscript. JW contributed to the design of the study, performed the literature search, and edited the manuscript. XY and JL contributed to study design, data curation, data analysis, and manuscript editing. JJ and JW confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was performed in line with the principles of the Declaration of Helsinki, and in accordance with the

Australian Code for the Responsible Conduct of Research and the National Statement on Ethical Conduct in Human Research. Approval was granted by the Ethics Committee of Tangshan People's Hospital (Tangshan, China; approval no. RMY-LLKS-2019-058). Written informed consent was obtained from all participants prior to study enrollment.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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