# Effect of HER2 expression status on the prognosis of patients with HR$^+$/HER2$^-$ advanced breast cancer undergoing advanced first-line endocrine therapy

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**Abstract.** The present study aimed to retrospectively assess the effects of human epidermal growth factor receptor 2 (HER2) expression on the diagnosis of patients with hormone receptor (HR)$^+$/HER2$^-$ late-stage breast cancer undergoing advanced first-line endocrine-based treatment. A total of 72 late-stage breast tumor cases from June 2017 to June 2019 were selected from the Department of Surgical Oncology, Shaanxi Provincial People's Hospital (Xi'an, China) and included in the present study. The expression of estrogen receptor, progesterone receptor and HER2 was detected by immunohistochemistry. The subjects were divided into two groups: the HER2-negative (0) cohort (n=31) and the HER2 low expression cohort (n=41). The age, BMI, Karnofsky Performance Status (KPS) score, tumor size, lymph node metastasis, pathological type, Ki-67 expression and menopausal status of the patients were obtained through the electronic medical record system of Shaanxi Provincial People's Hospital. Progression-free survival (PFS) and overall survival (OS) were evaluated for all patients. The median PFS and OS of the HER2(0) cohort were longer than those of the HER2 low expression cohort (all P<0.05). It was shown that age (hazard ratio, 6.000 and 5.465), KPS score (hazard ratio, 4.000 and 3.865), lymph node metastasis (hazard ratio, 3.143; 2.983) and HER2 status (hazard ratio, 3.167 and 2.996) were independent influencing factors of the prognosis of patients with HR$^+$/HER2$^-$ advanced breast cancer (ABC) (all P<0.05). Three models (model 1, no parameters adjusted; model 2, BMI, tumor size, pathological type, Ki-67 and menopausal status adjusted; and model 3, age, KPS functional status score and lymph node metastasis adjusted based on model 2) were established within the HER2(0) cohort as the reference for statistical analysis using the multivariate Cox's regression test. In models 2 and 3, the risk of poor prognosis of ABC within the HER2 low expression cohort was significantly higher compared with that in the HER2(0) cohort (hazard ratio, 3.558 and 4.477; 95% CI, 1.349-9.996 and 1.933-11.586; P=0.003 and P<0.001). The HER2 expression status of patients with HR$^+$/HER2$^-$ ABC receiving advanced first-line endocrine therapy may affect PFS and OS.

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## Introduction

Breast cancer (BC) is a prevalent malignancy in Chinese female patients. A survey in 2020 showed that the incidence rate was 36.1 cases per 100,000 individuals, and the mortality rate was 8.8 cases per 100,000 individuals (1). Although diagnosis and treatment technology have improved in recent years, no curative options exist for advanced BC (ABC), driving the predominance of mortality incidences in such cases (2). Endocrine therapy, including tamoxifen, aromatase inhibitors and flurvist, is the first choice for patients with hormone receptor (HR)$^+$/human epidermal growth factor receptor-2 (HER2)$^-$ advanced, postmenopausal ABC (3,4). Emerging research on drugs used to overcome endocrine resistance, including CDK4/CDK6 and mTOR inhibitors, continuously optimize this treatment method (5,6). HER2 is a transmembrane receptor on the cell surface, amplified in 20-30% of patients with invasive BC, and it is associated with a poor prognosis. The HER2 status of patients with BC has notable guiding value for developing treatment plans (7-9). A number of studies refer to patients with HER2$^-$, HER2 1$^+$ and HER2 2$^+$ FISH as patients with low HER2 expression (10,11). In clinical studies, the influence by different HER2 expression-profiles upon survival of patients with HR$^-$/HER2$^+$ ABC undergoing endocrine-based treatment(s) is still unclear, thus creating the basis for the current study. HER2-low expression has been shown to affect the overall survival (OS) period and lead to a shorter survival time. The present study therefore investigated the effects of low HER2 expression on survival in patients with HR$^+$/HER2$^-$ ABC, in order to help develop treatments.

## Materials and methods

*Research materials*. The cases of 72 patients with ABC who underwent treatment within the medical center of The Department of Surgical Oncology, Shaanxi Provincial People's Hospital (Xi'an, China) between June 2017 and June 2019 were include in this study. Inclusion criteria were as follows: i) Female patients diagnosed with ABC; ii) patients who did not receive any BC-related treatment before admission; iii) pathological manifestations were estrogen receptor $(ER)^+$ and/or progesterone receptor $(PR)^+$ and $HER2^-$; iv) endocrine therapy was the first-line treatment; and v) the general clinical data and follow-up data were complete. Exclusion criteria: i) Patients with bilateral BC; ii) presence of other malignant tumors; iii) patients whose treatment was interrupted or withdrawn due to severe toxic side effects during treatment; iv) immune system disease or mental illness; and v) lack of follow-up. This is a retrospective study, reviewed and approved by the Ethics Committee of Shaanxi Provincial People's Hospital.

*Detection of ER, PR and HER2*. ER and PR were detected by immunohistochemistry, and a ratio of nuclear staining ≥2% was described as positive. $ER^+$ and $PR^+$ together were used to indicate $HR^+$. HER2 was evaluated using the immunohistochemical scoring system (12) suggested by the American Society of Clinical Oncology and the Society of American Pathologists: i) HER2(0), $HER2^+$ or $HER2^{++}$ was negative, and ii) HER2 $1^+$ or $2^{++}$ and FISH was negative was considered low expression of HER2. Subjects were divided into two cohorts: A HER2-negative (0) cohort (n=31) and a HER2 low expression cohort (n=41). Immunohistochemistry was used to detect the expression of ER, PR and HER2. Paraffin-embedded wax blocks were made into 4 $\mu$m serial sections. Bake at 65˚C for 2 h, dewaxed with xylene to water. The sections were stained with 3% hydrogen peroxide for 10 min to exclude endogenous peroxidase. The sections were washed with tap water and distilled water, and antigen repair was performed. Briefly, 10% neutral formalin fixation, dewaxing and hydration were performed, then the sections were washed with PBS, incubated with 3% $H_2O_2$ at room temperature for 10 min, and then washed again with phosphate buffer. The sections were immersed in 0.01 mol/l citrate buffer, heated to 92-96˚C in microwave oven for 10 min, cooled to room temperature for 10-30 min, and rinsed with PBS. Next, 2% sheep serum (Shanghai Yuanye Biotechnology Co., Ltd.) was added at room temperature for 20 min, excess liquid was removed, mouse anti-human ER, PR and HER2 antibodies (BD Biosciences) were added at 37˚C for 1-2 h, and then tissues were rinsed with PBS buffer. The 2% biotinylated anti-mouse antibody (cat. no. RMB98601; OriGene Technologies, Inc.) was added dropwise followed by incubation at 37˚C for 30 min; the sections were then rinsed with PBS and stained with 3,3'-diaminobenzidine. Re-staining was performed using hematoxylin staining for 5-10 min, followed by washing twice with distilled water for 5 min each time. Sectioned were soaked in 75, 85 and 95 ethanol at room temperature for 5 min, and then dimethylbenzene was added at room temperature for 5 min. The results of the staining were obtained from the electronic medical record system of Shaanxi Provincial People's Hospital before the study.

*Data gathering*. The clinicopathological characteristics of the patients with BC were obtained through the electronic patient medical history platform. These included information on age, BMI, Karnofsky Performance Status (KPS) functional status score (13), tumor size, lymph node metastasis, pathological type, Ki-67 and menopausal status; menopause was defined as the decline of ovarian function and cessation of menstruation.

*Patient follow-up*. Follow-up was completed through outpatient review, inpatient examination or by telephone, with a follow-up interval of 3 months. The follow-up endpoint was July 2022, and both PFS and OS of all patients were analyzed.

*Statistical analysis*. Data were assessed using SPSS (v.21.0; IBM Corp.). The descriptive statistics that satisfied the normal distribution are presented as the mean ± SEM, and the two cohorts were compared using the independent samples t-test. Count datasets were denoted to be n (%), and either $\chi^2$ or Fisher's exact test was used for comparison. A rank sum test was applied to compare ranked data. Kaplan-Meier OS and PFS curves were compared by logrank. GraphPad software (version 8.0; Dotmatics) was used for plotting, and the baseline HER2 status was used as a categorical variable. Multivariate Cox's regression test was used to assess the relationship between HER2 status and OS, DFS and prognosis. Model 1, no parameters were adjusted; model 2, adjusted BMI, tumor size, pathological type, Ki-67 and menopausal status; Model 3, age, KPS functional status score and lymph node metastasis were adjusted based on model 2. $P<0.05$ indicated statistical significance for any denoted variation(s).

## Results

*Comparative analysis of clinicopathological data of cases with different HER2 status*. No significant difference was found in the age, BMI, KPS functional status score, tumor size, lymph node metastasis, pathological type, CDK4/6 inhibitor use, metastatic site, Ki-67 and menopausal status among patients with ABC at different HER2 status at baseline ($P>0.05$; Table I).

*Comparison of survival analysis between two cohorts*. The average follow-up time of the 72 patients with ABC who received endocrine treatment was 35 months (range, 23-58 months). Median OS for the HER2(0) cohort was 49 months (range, 43-58 months), while median OS for the HER2 low expression cohort was 41 months (range, 35-50 months) ($P<0.05$; Fig. 1). The median PFS for the HER2(0) cohort was 20 months (range, 13-38 months) months, whereas median PFS for the HER2 low expression cohort was 15 months (range, 9-28 months; $P<0.05$; Fig. 2).

*Multivariate Cox regression test to assess influencing factors for the prognosis of patients with $HR^+/HER2^-$ ABC*. Age, KPS functional status score, lymph node metastasis and HER2 status were assessed by Cox's regression test. It was revealed that age (hazard ratio, 6.000, 5.465), KPS functional status score (hazard ratio, 4.000, 3.865), lymph node metastasis (hazard ratio, 3.143, 2.983) and HER2 status (hazard ratio, 3.167, 2.996) were independent risk factors for the prognosis of patients with $HR^+/HER2^-$ (all $P<0.05$; Table II).

Table I. Comparative analysis for clinicopathological data of clinical cases in the HER2(0) cohort and in the HER2 low expression cohort.

| Clinicopathological characteristics | HER2(0) (n=31) | HER2 low expression (n=41) | $t/\chi^2$ | P-value |
|---|---|---|---|---|
| Age, years | 64.53±8.34 | 65.86±7.67 | 0.702 | 0.485 |
| BMI, kg/m$^2$ | 23.86±2.20 | 23.61±2.14 | 0.485 | 0.629 |
| KPS functional status score, Points | 75.63±13.56 | 70.76±12.18 | 1.6 | 0.114 |
| Tumor dimension, cm | 6.13±1.36 | 6.31±1.40 | 0.547 | 0.586 |
| Lymph node metastasis, n (%) | 21 (67.74) | 28 (69.29) | 0.002 | 0.96 |
| Pathological type, n (%) | | | | |
| Invasive ductal carcinoma | 20 (64.52) | 30 (73.17) | - | 0.665[a] |
| Invasive lobular carcinoma | 7 (22.58) | 6 (14.63) | | |
| Others | 4 (12.90) | 5 (12.20) | | |
| Ki-67, n (%) | | | | |
| ≤30 | 13 (41.94) | 19 (46.34) | 0.139 | 0.71 |
| >30 | 18 (58.06) | 22 (53.66) | | |
| Menopause, n (%) | 22 (70.96) | 35 (85.37) | 2.219 | 0.136 |
| CDK4/6 inhibitor use | 6 (19.35) | 10 (24.39) | 0.259 | 0.611 |
| Metastatic sites, n (%) | | | | |
| No metastatic | 24 (77.42) | 30 (73.17) | | 0.663[a] |
| Visceral metastasis | 7 (22.58) | 10 (24.39) | | |
| Brain metastasis | 0 | 1 (2.44) | | |

[a]Calculated using Fisher's exact test. HER2, human epidermal growth factor receptor 2; KPS, Karnofsky Performance Status.

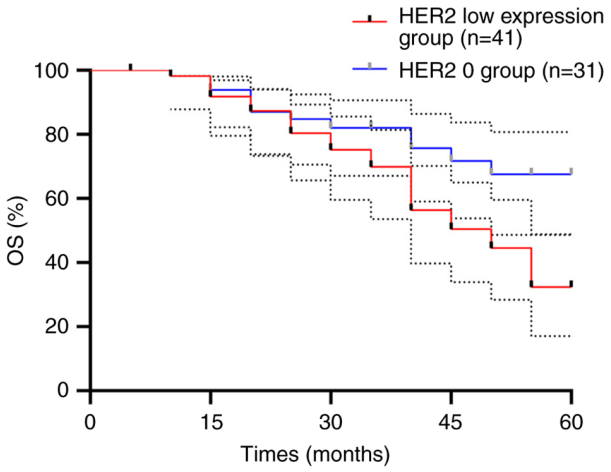


Figure 1. PFS comparative analysis across all cohorts.

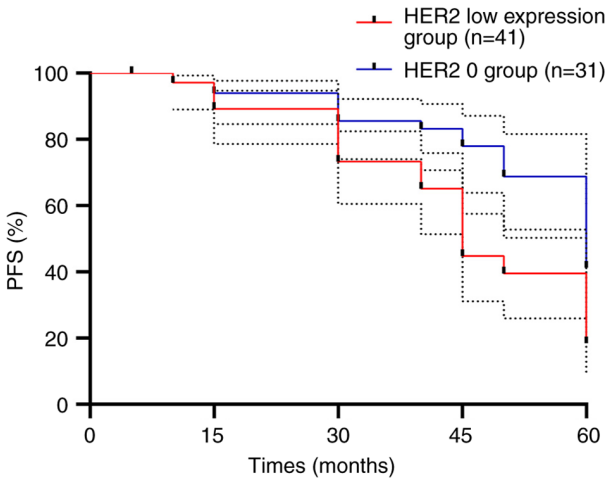


Figure 2. OS comparative analysis across all cohorts.

*Effect of HER2 baseline status on the prognosis of patients with HR$^+$/HER2$^-$ ABC under different models*. Three models were established for multivariate Cox's regression test with the HER2(0) cohort as reference. In model 2 and 3 (model 1, no parameters adjusted; model 2, BMI, tumor size, pathological type, Ki-67 and menopausal status adjusted; and model 3, age, KPS functional status score and lymph node metastasis adjusted based on model 2), the risk of poor prognosis within the HER2 low expression cohort was significantly higher compared with the HER2(0) cohort (hazard ratio, 1.985,2.133 and 3.558; 95% CI, 0.689-5.748,0.803-6.889 and 1.349-9.996; P=0.210,0.166 and 0.003; Table III).

## Discussion

HER2 forms heterodimers with other members of the epidermal growth factor family to activate the MAPK, Janus kinase, PI3K and other signaling pathways, and promote the occurrence and development of various tumors (14); It is one of the main mechanisms of tumor cell proliferation, migration, invasion and survival (2,15-17). Recent studies have shown that 15-20% of BC cases harbor HER2 upregulation (18,19). Harbeck (20) and Huai *et al* (21) showed that low expression of HER2 in BC cases with lymph node metastases was associated with a poor prognosis. Furthermore, in

Table II. Multivariate COX regression analysis for analyzing the influencing factors of prognosis of patients with $HR^+/HER2^-$ advanced breast cancer.

| | PFS | | | OS | | |
|---|---|---|---|---|---|---|
| Variable | Hazard ratio | 95% CI | P-value | Hazard ratio | 95% CI | P-value |
| Age, years | 6.000 | 2.105-17.11 | <0.001 | 5.465 | 1.986-15.676 | <0.001 |
| KPS functional status score | 4.000 | 1.693-23.093 | <0.001 | 3.865 | 1.670-21.346 | <0.001 |
| Lymph node metastasis | 3.143 | 1.518-6.508 | <0.001 | 2.983 | 1.339-6.236 | <0.001 |
| HER2 status | 3.167 | 1.446-6.936 | <0.001 | 2.996 | 1.362-6.660 | <0.001 |

HER2, human epidermal growth factor receptor 2; HR, hormone receptor; KPS, Karnofsky Performance Status; OS, overall survival; PFS, progression-free survival.

Table III. Effect of HER2 baseline status on the prognosis of patients with $HR^+/HER2^-$ advanced breast cancer under different models.

| | HER2 low expression group | | |
|---|---|---|---|
| Model | Hazard ratio | 95% CI | P-value |
| Model 1[a] | 1.985 | 0.689-5.748 | 0.210 |
| Model 2[b] | 2.133 | 0.803-6.889 | 0.166 |
| Model 3[c] | 3.558 | 1.349-9.996 | 0.003 |

[a]Model 1, no parameters were adjusted. [b]Model 2, BMI, tumor size, pathological type, Ki-67 and menopausal status were adjusted. [c]Model 3, age, KPS functional status score and lymph node metastasis were adjusted based on model 2. HER2, human epidermal growth factor receptor 2; HR, hormone receptor; KPS, Karnofsky Performance Status.

cases of early BC, reduced expression in HER2 is markedly associated with the prognosis of patients having previously received surgical treatment, rendering this a separate parameter in predicting poor prognosis within clinical cases (22). In a study by Luque *et al* (23), patients with HER2 $2^+$ ABC were characterized by larger tumor volume, higher lymph node involvement, more severe clinical stage and higher Ki-67 expression at the first diagnosis. Yu *et al* (24) reported that, compared with the negative patient cohort, Ki-67 was markedly increased within the low-expression cohort; the case population having T3 stage was markedly elevated, the lymph node metastasis rate was markedly increased, the axillary lymph node clearance rate notably increased and the rate of recurrence or metastasis was markedly higher within three years after the operation.

The present study is one of the first to compare medical statistics of ABC cases with differing HER2 status at baseline. It was shown that no major variation existed within baseline age, BMI, KPS functional status score, tumor size, lymph node metastasis, pathological type, Ki-67 and menopausal status among patients with ABC with different HER2 status, indicating that HER2 had no marked influence on the first-diagnosis status in such patients. In the further survival analysis, median PFS and OS for the HER2(0) cohort were enhanced compared with those of the HER2 low expression cohort, indicating that HER2 status had a particular impact on the short-term prognosis of patients. Endocrine therapy has been widely recognized in recent years (25) and its scope of application in treating different types of patients with BC is also expanding. Neoadjuvant endocrine therapy, instead of neoadjuvant chemotherapy, is an efficient clinical healing strategy for patients with $ER^+$ BC, which can reduce the tumor stage, to receive breast-conserving operation for BC and reduce the postoperative adjuvant chemotherapy (26). However, there are still some controversies about the optimal duration of endocrine therapy and the optimal individualized treatment plan. The results of the present study show that HER2 status has a particular impact on the short-term prognosis of patients with ABC undergoing first-line endocrine-based treatment and has value in guiding the formulation of treatment plans. In this study, multivariate Cox's regression test under different adjustment models was further carried out, and the results showed that HER2 has an independent effect on OS and PFS of patients and was not restricted by other clinical factors. Results were consistent with previous findings in patients with other types of BC. Recently, researchers have found that the mutual regulation of ER/HER2 within aromatase inhibitor-resistant BC can upregulate the expression of the oncogene transcription factor c-Myc (27). At the same time, c-Myc has a role in regulating glutamine metabolism related to endocrine resistance (28), which may be associated with the difference in diagnosis of patients with different HER2 status in the current study. However, this hypothesis needs further *in vitro* studies to confirm.

In conclusion, the results from the present study indicated that HER2 expression status may affect PFS and OS in patients with $HR^+/HER2^-$ advanced BC treated with advanced first-line endocrine therapy. However, this investigation remained at single-center level and had transitory follow-up, thus requiring to a larger cohort size to further improve and supplement the study conclusions.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

KW and QD confirm the authenticity of all the raw data. KW, QD, JY developed the methodology used, carried out the investigation and data curation, and prepared the original draft. XZ and KW wrote, reviewed and edited the manuscript. XZ and YL conceived the idea for the research study, and reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

## Ethics approval and consent to participate

All the subjects provided their informed consent for the investigation, and this study was reviewed and approved by the Ethics Committee of the Shaanxi Provincial People's Hospital (Xi'an, China).

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Qiu Y, Yang L, Liu H and Luo X: Cancer stem cell-targeted therapeutic approaches for overcoming trastuzumab resistance in HER2-positive breast cancer. Stem Cells 39: 1125-1136, 2021.
3. Horisawa N, Adachi Y, Takatsuka D, Nozawa K, Endo Y, Ozaki Y, Sugino K, Kataoka A, Kotani H, Yoshimura A, *et al*: The frequency of low HER2 expression in breast cancer and a comparison of prognosis between patients with HER2-low and HER2-negative breast cancer by HR status. Breast Cancer 29: 234-241, 2022.
4. Tung NM, Zakalik D and Somerfield MR; Hereditary Breast Cancer Guideline Expert Panel: Adjuvant PARP inhibitors in patients with high-risk early-stage HER2-Negative breast cancer and germline BRCA Mutations: ASCO Hereditary breast cancer guideline rapid recommendation update. J Clin Oncol 39: 2959-2961, 2021.
5. Tarantino P, Gandini S, Nicolò E, Trillo P, Giugliano F, Zagami P, Vivanet G, Bellerba F, Trapani D, Marra A, *et al*: Evolution of low HER2 expression between early and advanced-stage breast cancer. Eur J Cancer 163: 35-43, 2022.
6. Jackisch C, Cortazar P, Geyer CE Jr, Gianni L, Gligorov J, Machackova Z, Perez EA, Schneeweiss A, Tolaney SM, Untch M, *et al*: Risk-based decision-making in the treatment of HER2-positive early breast cancer: Recommendations based on the current state of knowledge. Cancer Treat Rev 99: 102229, 2021.
7. Zimmer AS, Van Swearingen AED and Anders CK: HER2-positive breast cancer brain metastasis: A new and exciting landscape. Cancer Rep (Hoboken) 5: e1274, 2022.
8. Haji F and Hurvitz SA: Can women with HER2-Positive metastatic breast cancer Be Cured?. Clin Breast Cancer 21: 526-531, 2021.
9. Martínez-Sáez O and Prat A: Current and future management of HER2-Positive metastatic breast cancer. JCO Oncol Pract 17: 594-604, 2021.
10. Modi S: Trastuzumab deruxtecan in previously treated HER2-positive metastatic breast cancer: Plain language summary of the DESTINY-Breast01 study. Future Oncol 17: 3415-3423, 2021.
11. Schettini F and Prat A: Dissecting the biological heterogeneity of HER2-positive breast cancer. Breast 59: 339-350, 2021.
12. O'Grady A, Allen D, Happerfield L, Johnson N, Provenzano E, Pinder SE, Tee L, Gu M and Kay EW: An immunohistochemical and fluorescence in situ hybridization-based comparison between the Oracle HER2 Bond Immunohistochemical System, Dako HercepTest, and Vysis PathVysion HER2 FISH using both commercially validated and modified ASCO/CAP and United Kingdom HER2 IHC scoring guidelines. Appl Immunohistochem Mol Morphol 18: 489-493, 2010.
13. Ishida Y, Kamibeppu K, Sato A, Inoue M, Hayakawa A, Shiobara M, Yabe H, Koike K, Adachi S, Yamashita T, *et al*: Karnofsky performance status and visual analogue scale scores are simple indicators for quality of life in long-term AYA survivors who received allogeneic hematopoietic stem cells transplantation in childhood. Int J Hematol 116: 787-797, 2022.
14. Ling Y, Liang G, Lin Q, Fang X, Luo Q, Cen Y, Mehrpour M, Hamai A, Liu Z, Shi Y, *et al*: circCDYL2 promotes trastuzumab resistance via sustaining HER2 downstream signaling in breast cancer. Mol Cancer 21: 8, 2022.
15. Chen J, Colosimo M and Lim E: The management of HER2-positive early breast cancer: Current and future therapies. Asia Pac J Clin Oncol 17 (Suppl 6): S3-S12, 2021.
16. Le Du F, Diéras V and Curigliano G: The role of tyrosine kinase inhibitors in the treatment of HER2+ metastatic breast cancer. Eur J Cancer 154: 175-189, 2021.
17. Lloyd MR, Spring LM, Bardia A and Wander SA: Mechanisms of Resistance to CDK4/6 blockade in advanced hormone Receptor-positive, HER2-negative breast cancer and emerging therapeutic opportunities. Clin Cancer Res 28: 821-830, 2022.
18. Antolín S, García-Caballero L, Reboredo C, Molina A, Mosquera J, Vázquez-Boquete Á, Gallego R, Santiago MP, Concha Á, Pérez E, *et al*: Is there a correlation between HER2 gene amplification level and response to neoadjuvant treatment with trastuzumab and chemotherapy in HER2-positive breast cancer?. Virchows Arch 479: 853-857, 2021.
19. Md Pauzi SH, Masir N, Yahaya A, Mohammed F, Tizen Laim NMS, Mustangin M, Aizudin AN, Talib A, Teoh KH, Karim N, *et al*: HER2 testing by immunohistochemistry in breast cancer: A multicenter proficiency ring study. Indian J Pathol Microbiol 64: 677-682, 2021.
20. Harbeck N: Neoadjuvant and adjuvant treatment of patients with HER2-positive early breast cancer. Breast 62 (Suppl 1): S12-S16, 2022.
21. Huai J, Cao M, Jiang Y, Yang X, Zhu Y, Si Y, Xu M, Shen C, Han T and Lian X: Evaluation of liquid biopsy in patients with HER2-Positive breast cancer. Biomed Res Int 2021: 6388492, 2021.
22. Peckys DB, Gaa D and de Jonge N: Quantification of EGFR-HER2 Heterodimers in HER2-Overexpressing breast cancer cells using liquid-phase electron microscopy. Cells 10: 3244, 2021.
23. Luque M, Sanz-Álvarez M, Santamaría A, Zazo S, Cristóbal I, de la Fuente L, Mínguez P, Eroles P, Rovira A, Albanell J, *et al*: Targeted therapy modulates the secretome of cancer-associated fibroblasts to induce resistance in HER2-Positive breast cancer. Int J Mol Sci 22: 13297, 2021.
24. Yu H, Qiu F, Gu X and Bian XB: Clinical characteristics and prognosis of patients with triple-negative breast cancer with low HER2 expression. J China Med Univ 51: 721-724, 2022 (In Chinese).

25. Barrio AV, Montagna G, Mamtani A, Sevilimedu V, Edelweiss M, Capko D, Cody HS III, El-Tamer M, Gemignani ML, Heerdt A, *et al*: Nodal recurrence in patients with node-positive breast cancer treated with sentinel node biopsy alone after neoadjuvant Chemotherapy-A Rare Event. JAMA Oncol 7: 1851-1855, 2021.
26. Dowsett M, Kilburn L, Rimawi MF, Osborne CK, Pogue-Geile K, Liu Y, Jacobs SA, Finnigan M, Puhalla S, Dodson A, *et al*: Biomarkers of response and resistance to palbociclib plus letrozole in patients with ER+/HER2- breast cancer. Clin Cancer Res 28: 163-174, 2022.
27. Botty van den Bruele A, Ferraro E, Sevilimedu V, Hogan MP, Javed-Tayyab S, Le T, Fornier MN, Morrow M and Sacchini V: Does preoperative MRI accurately stratify early-stage HER2+breast cancer patients to upfront surgery vs neoadjuvant chemotherapy?. Breast Cancer Res Treat 189: 307-315, 2021.
28. Hart V, Silipo M, Satam S, Gautrey H, Kirby J and Tyson-Capper A: HER2-PI9 and HER2-I12: Two novel and functionally active splice variants of the oncogene HER2 in breast cancer. J Cancer Res Clin Oncol 147: 2893-2912, 2021.