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Unveiling the mysteries of HER2-low expression in breast cancer: pathological response, prognosis, and expression level alterations

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Abstract

Background The novel anti-HER2 antibody drug conjugates (ADCs) can effectively improve the long-term survival of patients with HER2-low expression breast cancer. However, pathological responses to neoadjuvant therapy (NAT) within HER2-low expression breast cancer, the relationship between pathological response and prognosis and the transformation of HER2 status are all now poorly understood.

Methods The patients with HER2-0 and HER2-low expression breast cancer receiving NAT at Harbin Medical University Cancer Hospital between Jan. 2014 and Nov. 2018 were retrospectively explored. HER2 low expression refers to the IHC 1 + or 2 + and FISH negative. The Kappa test was utilized for analyzing the consistency rate of HER2 expression. To evaluate disease-free survival (DFS) and overall survival (OS), this research employed both the Kaplan-Meier analysis and the Cox regression.

Results In this study, 178 patients with HER2-0 and 344 patients with HER2-low expression breast cancer were included. In comparison with the HER2-0 group, it is shown that patients in the HER2-low group have more possibility to be younger compared to those 50 years old ($P < 0.014$), have more premenopausal patients ($P < 0.001$), a higher proportion of hormone receptor (HR) positive patients ($P < 0.001$), and less proportion of stage III V patients ($P < 0.034$). When NAT was finished, the pCR rate became 23.6% in the HER2-0 group while 22.1% in the HER2-low group, and there was also a higher pCR rate in HR- patients in comparison with that in HR + patients ($P < 0.01$). Considering HER2 expression inconsistency, the overall HER2 inconsistency rate was 30.4% (Kappa = 0.431, $P < 0.01$). Among patients initially diagnosed as HER2-0, 34% ($N = 61$) were re-diagnosed as HER2-low after NAT. After stratification by HR expression status, HR+/HER2-0 patients transformed to HER2-low after NAT in 37%, and 32% of HR- patients changed from HER2-0 to HER2-low. In this survival analysis, there were both better DFS rates ($P = 0.009$) and OS rates ($P = 0.026$) in the HR-/HER2-low patients in comparison with the HR-/HER2-0 patients, while the HER2-0 and HER2-low patients in the HR + group had no significant survival difference. Additionally, for non-pCR patients, there was better DFS ($P = 0.029$) and OS ($P = 0.038$) in the HER2-low group in comparison with that of the HER2-0 group, while no significant survival difference exists between pCR patients.

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Conclusion After HR stratification, there are unique clinical characteristics and prognostic outcomes in HER2-low expression breast cancer, which indicates the potential to become a specific molecular subtype of breast cancer. The significant instability of HER2-low expression status between primary tumor and residual invasive disease suggests that multiple detections of HER2 status should be emphasized in NAT strategies.

Keywords Breast cancer, HER2, Neoadjuvant therapy, Prognosis, HER2-low

Background

Breast cancer is the world's most common cancer type among women [1]. As a transmembrane protein encoded by the ERBB2 gene, the human epidermal growth factor receptor 2 (HER2) is lowly expressed in nearly 45-55% of breast cancers and overexpressed in nearly 15% of breast cancers [2, 3]. As suggested by guidelines of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP), HER2-positive lesions can be identified by using immunohistochemistry (IHC) and in situ hybridization (ISH) [4]. In the IHC scoring, it can be seen that HER2 expression is categorized between 1+and 3+. At present, clinicians usually pay much attention to tumors with HER2 overexpression, which are classified as IHC 3+or IHC 2+with positive ISH [4]. However, there are still HER2-low expression tumors in approximately 50% of breast cancer patients, in which around 20% involve triple-negative breast cancer and nearly 80% are hormone receptor (HR) positive diseases [3]. However, patients with HER2-low expression now are not adequately diagnosed and treated. In recent days, an expert consensus statement was issued by European Society for Medical Oncology (ESMO) to analyze HER2-low expression breast cancer management [5]. The novel ADC drug fam-trastuzumab deruxtecan-nxki (T-DXd) is reported to be utilized to treat HER2-low expression breast cancer, which can greatly improve the progression-free survival in HER2-low patients [6, 7].

Taking into account the patients that have early or locally advanced breast cancer, neoadjuvant therapy (NAT) contributes much to diagnosis and treatment decisions by effectively determining the patient's drug sensitivity and providing more precise treatment decisions based on different tumor subtypes [8–10]. Over the past few decades, NAT has shown the ability to assess treatment sensitivity, which downstages the primary tumor and tailors post-neoadjuvant approaches [11]. Some research, before the ADCs era, has shown that a high inconsistency exists in HER2 status from baseline biopsy to residual disease in patients that receive NAT, possibly brought by tumor heterogeneity, changing treatment choices and assessment technique differences [12–14]. However, effective treatment and prognosis data are lacking for patients initially diagnosed as HER2-low or those whose HER2 status changes to HER2-low after NAT. Additionally, it is not clear whether pathological complete response (pCR) after the NAT and the pCR can represent a surrogate prognostic marker for that new subtype.

Thus, the patients that have HER2-0 and HER2-low breast cancer receiving NAT were chosen to evaluate the clinical and pathological characteristics of HER2-low breast cancer patients. For the purpose of treating HER2-low breast cancer patients more accurately, inconsistencies of HER2 low expression from primary breast cancer to matched residual disease were analyzed.

Methods

Study population

There was a retrospective study on early or locally advanced breast cancer patients who received NAT from Jan. 2014 to Nov. 2018. All data were obtained from the medical records of patients at Harbin Medical University Cancer Hospital. Below are the inclusion criteria: (a) female gender; (b) histologically confirmed invasive breast cancer before NAT through core needle biopsy; (c) tumor confirmed as HER2-negative or HER2-low expression before NAT through core needle biopsy; (d) receipt of ≥4 cycles of NAT; and (e) availability of complete the pathological data and the clinical information before and after NAT. Below are the exclusion criteria: (a) male patients; (b) patients with occult breast cancer; (c) patients with inflammatory breast cancer; (d) patients with bilateral breast cancer; and (e) individuals with incomplete basic information or pathological data. This study was conducted in accordance with the Declaration of Helsinki, and approved by the independent Ethical Committees (IEC) of Harbin Medical University Cancer Hospital. Given the anonymized nature of the data, the requirement for informed consent was waived by the IEC of Harbin Medical University Cancer Hospital.

Clinical and pathological interpretation criteria

The physical examination or diagnostic ultrasound determines the clinical tumor and lymph node status prior to NAT. TNM staging of breast cancer was built upon the staging manual of the 8th edition of the American Joint Committee on Cancer (AJCC) [15]. Guided by the preoperative therapy ultrasound, a core biopsy can diagnose breast cancer. According to ASCO/CAP recommendations, pathological and IHC evaluation of the tumor was carried out via standard techniques and antibodies by at least two experienced local pathologists. The positive assessment for estrogen receptor (ER) and progesterone

receptor (PR) on IHC referred to infiltration tumor cells positively stained by IHC, which was not less than 1%. Hormone receptor (HR) positivity is defined as ER and/or PR positivity. After being determined by IHC, HER2 protein expression was classified into IHC 0, IHC 1+, IHC 2+and IHC 3+separately. Additional fluorescence in situ hybridization (FISH) testing was carried out on IHC 2+samples, in which the HER2/CEP17 dual-probe was employed to detect the HER2 gene amplification. In the findings of the IHC and FISH, HER2 status has the following classification: HER2-positive when IHC was 3+or IHC was 2+and FISH was positive; HER2-low when IHC was 1+or IHC was 2+and FISH was negative; and HER2-0 when IHC was 0. The HER2 expression status was reviewed and determined again by two pathologists based on the patient's medical data.

This study referred to RECIST version 1.1 to evaluate efficacy after NAT [16]. pCR refers to the absence of residual invasive disease in primary breast lesions and axillary lymph nodes (ypT0/Tis, ypN0). Patients who have not achieved pCR are defined as non-pCR. The disease-free survival (DFS) and overall survival (OS) served as the primary endpoints for survival analysis. To be specific, DFS refers to the time from breast cancer diagnosis to the earliest local occurrence or contralateral recurrence, distant metastasis, or death from any cause. In addition, OS refers to the time from breast cancer pathological diagnosis to death from any cause.

All the patients received NAT consisting of 4 or more cycles based on anthracycline and/or taxane. The AC regimen included doxorubicin (A) at 60 mg/m^2 and cyclophosphamide (C) at 600 mg/m^2; the AC-T regimen involved A at 60 mg/m^2, C at 600 mg/m^2, and docetaxel (T) at 90 mg/m^2; and the EC-T regimen consisted of epirubicin (E) at 90–100 mg/m^2, C at 600 mg/m^2, and T at 80–100 mg/m^2. Each of these regimens had a 21-day interval between cycles. In addition, the PC regimen consisted of paclitaxel (P) at 80 mg/m^2 and carboplatin (C) at AUC=2, with a 7-day interval between cycles. HR+patients received endocrine therapy based on tamoxifen or aromatase inhibitors.

Statistical analysis

Through employing the IBM SPSS version 25 (SPSS, Inc., Chicago, IL) and GraphPad Prism version 8.0 (GraphPad Software, CA, USA), this research employed the figure construction and statistical analysis, in which the two-tailed p-value<0.05 was thought to be statistically significant. For clinicopathological comparisons, continuous variables are described as mean (±standard deviation, SD) or median (with minimum–maximum). Categorical variables are described as number and percentage for each modality. Percentages were calculated on complete data. Continuous variables were compared between two groups using the Student t test in case of normally distributed variables, and otherwise, using a Wilcoxon test. The Shapiro–Wilk test was used to check the normality of the distribution. Categorical variables were compared between groups using the Chi-square test, or Fisher's exact test as appropriate. The concordance rates of HER2 status from primary breast cancer to residual disease after neoadjuvant treatment were analyzed by using the Kappa test, and Kappa value<0.2, 0.2–0.4, 0.4–0.6 and >0.6 were considered as poor, fair, moderate and good agreement, respectively. The category change of HER2 expression was graphically reported by building Sankey diagrams.Survival curves were generated by using the Kaplan-Meier method and compared by applying the log-rank test. Differences were considered statistically significant when the p value<0.05. Cox hazard proportional models were applied to analyze clinicopathological factors affecting DFS and OS, and the variables related to OS or DFS (p values<0.05) in the univariate analysis were selected for multivariate analysis.

Results

Patient cohort and clinical characteristics

In the HR+subgroup analysis, age and menopausal status between the HER2-0 and HER2-low groups showed no statistically significant difference (Table 1). Like the overall cohort, there was a lower ratio of stage III patients in the HER2-low group (P<0.001). Additionally, there was a higher rate of invasive ductal carcinoma (IDC) at initial diagnosis within the HER2-low group (P=0.005). However, a higher rate of breast conservation exists after treatment in the HER2-0 group (P=0.002). Besides, the HR+subgroup showed no statistically significant difference in NAT cycles, tumor size, neoadjuvant NAT strategy and lymph node status between the two groups.

The HR- subgroup showed statistically significant differences in age, KI67 expression and menopausal status between HER2-0 and HER2-low patients (Table 2). HER2-low patients, in comparison with the HER2-0 group, had more possibilities to be younger compared to those 50 years old (P=0.012) and premenopausal (P=0.002), consistent with the overall cohort. Additionally, there were more HER2-low patients with KI67>15% at initial diagnosis (P=0.042).

HER2 expression status and pCR and clinical characteristics

In the overall cohort, the pCR rate after receiving NAT was 23.6% in the HER2-0 group and 22.1% in the HER2-low group respectively (Fig. 1A; Table 3). The study also found that there was a higher pCR rate in HR- patients than HR+patients (P<0.01) (Fig. 1B). Therefore, stratified analysis based on HR revealed that in the HR+group, the pCR rate was respectively 21.0% in the HER2-0 group and 17.5% in HER2-low group (Fig. 1C; Table 1). By

Table 1 Patient demographics and baseline characteristics (HR+)

Characteristic	HER2		*P*-value
	HER2-0 (*n* = 81)	HER2-low (*n* = 228)	
Mean age			0.438
Mean ± SD	50 ± 11	49 ± 9	
Age			0.130
< 50	39 (48.1%)	132 (57.9%)	
≥ 50	42 (51.9%)	96 (42.1%)	
BMI			0.591
Mean ± SD	24.7 ± 3.4	24.9 ± 3.6	
Menstruation			0.147
Pre-menopausal	43 (53.1%)	142 (62.3%)	
Post-menopausal	38 (46.9%)	86 (37.7%)	
Tumour size			0.415
≤ 2 cm	9 (11.1%)	27 (11.8%)	
2–5 cm	56 (69.1%)	170 (74.6%)	
> 5 cm	16 (19.8%)	31 (13.6%)	
Lymph node			0.158
Negative	40 (49.4%)	92 (40.4%)	
Positive	41 (50.6%)	136 (59.6%)	
Grade			**0.001**
I	15 (18.5%)	23 (10.1%)	
II	36 (44.4%)	154 (67.5%)	
III	30 (37.0%)	51 (22.4%)	
Histology			**0.005**
IDC	66 (81.5%)	211 (92.5%)	
non-IDC	15 (18.5%)	17 (7.5%)	
ER			**0.036**
Positive	69 (85.2%)	212 (93.0%)	
Negative	12 (14.8%)	16 (7.0%)	
PR			**0.002**
Positive	66 (81.5%)	213 (93.4%)	
Negative	15 (18.5%)	15 (6.6%)	
Ki67			0.102
≤ 15%	14 (17.3%)	60 (26.3%)	
> 15%	67 (82.7%)	168 (73.7%)	
NAT strategy			0.300
Anthracycline + Taxane	70 (86.4%)	208 (91.2%)	
Anthracycline	10 (12.3%)	16 (7.0%)	
Taxane	1 (1.2%)	4 (1.8%)	
NAT Cycle			0.949
4–6	49 (60.5%)	137 (60.1%)	
> 6	32 (39.5%)	91 (39.9%)	
Breast Surgery			**0.002**
Mastectomy	61 (75.3%)	204 (89.5%)	
BCS	20 (24.7%)	24 (10.5%)	
pCR			0.492
non-pCR	64 (79.0%)	188 (82.5%)	
pCR	17 (21.0%)	40 (17.5%)	

Abbreviations: *HR* hormone receptor, *IDC* invasive ductal carcinoma, *non-IDC* non-invasive ductal carcinoma, *ER* estrogen receptor, *PR* progesterone receptor, *NAT* neoadjuvant therapy, *pCR* pathologic complete response, *non-pCR* non-pathologic complete response

contrast, the HR- subgroup showed 31.0% in HER2-low patients and the pCR rate was 25.8% in HER2-0 patients respectively (Fig. 1D; Table 2). Further analysis of factors influencing pCR found that the univariate analysis shows that the influencing factors consisted of the grade (OR = 0.51, P = 0.038), BMI (OR = 0.93, P = 0.039), tumor size (OR = 0.50, P = 0.015; OR = 0.41, P = 0.019), presence of IDC (OR = 10.52, P < 0.001), HR status (OR = 0.56,

Table 2 Patient demographics and baseline characteristics (HR-)

Characteristic	HER2		*P*-value
	HER2-0 (*n* = 97)	HER2-low (*n* = 116)	
Mean age			**0.013**
Mean ± SD	52 ± 9	49 ± 9	
Age			**0.012**
< 50	36 (37.1%)	63 (54.3%)	
≥ 50	61 (62.9%)	53 (45.7%)	
BMI			0.882
Mean ± SD	24.2 ± 3.2	24.3 ± 3.1	
Menstruation			**0.002**
Pre-menopausal	38 (39.2%)	70 (60.3%)	
Post-menopausal	59 (60.8%)	46 (39.7%)	
Tumour size			0.072
≤ 2 cm	8 (8.2%)	21 (18.1%)	
2–5 cm	67 (69.1%)	77 (66.4%)	
> 5 cm	22 (22.7%)	18 (15.5%)	
Lymph node			0.823
Positive	55 (56.7%)	64 (55.2%)	
Negative	42 (43.3%)	52 (44.8%)	
Grade			0.521
I	6 (6.2%)	12 (10.3%)	
II	53 (54.6%)	58 (50.0%)	
III	38 (39.2%)	46 (39.7%)	
Histology			0.134
IDC	78 (80.4%)	83 (71.6%)	
non-IDC	19 (19.6%)	33 (28.4%)	
Ki67			**0.042**
≤ 15%	25 (25.8%)	17 (14.7%)	
> 15%	72 (74.2%)	99 (85.3%)	
NAT strategy			0.808
Anthracycline	12 (12.4%)	14 (12.1%)	
Anthracycline + Taxane	83 (85.6%)	101 (87.1%)	
Taxane	2 (2.1%)	1 (0.9%)	
NAT Cycle			0.796
4–6	56 (57.7%)	69 (59.5%)	
> 6	41 (42.3%)	47 (40.5%)	
Breast Surgery			0.275
Mastectomy	84 (86.6%)	94 (81.0%)	
BCS	13 (13.4%)	22 (19.0%)	
pCR			0.398
pCR	25 (25.8%)	36 (31.0%)	
non-pCR	72 (74.2%)	80 (69.0%)	

Abbreviations: *HR* hormone receptor, *IDC* invasive ductal carcinoma, *non-IDC* non-invasive ductal carcinoma, *ER* estrogen receptor, *PR* progesterone receptor, *NAT* neoadjuvant therapy, *pCR* pathologic complete response, *non-pCR* non-pathologic complete response

$P=0.007$) and KI67 status (OR = 3.42, $P<0.001$). By contrast, the multivariate analysis showed that independent factors affecting pCR consisted of the tumor T stage (OR = 0.55, $P=0.046$; OR = 0.68, $P=0.001$), KI67 (OR = 2.82, $P=0.004$), grade (OR = 0.36, $P=0.013$), and presence of IDC (OR = 10.99, $P<0.001$) (Table 4).

Furthermore, this study demonstrated that some patients had inconsistent HER2 expression between pre-NAT core biopsy samples and the HER2 status of residual disease after treatment (Fig. 2). As for HER2, the overall discordance rate was 30.4% (Kappa = 0.431, $P<0.01$). Among patients initially diagnosed as HER2-0, 34% ($N=61$) were diagnosed as HER2-low after treatment, and 5% ($N=9$) were diagnosed as HER2+. In addition, among patients initially diagnosed as HER2-low, 17.4% ($N=60$) were diagnosed as HER2-0 after treatment, and 4.4% ($N=15$) were diagnosed as HER2+. Stratifying by HR expression status, among HR+/HER2-0 patients, 37%

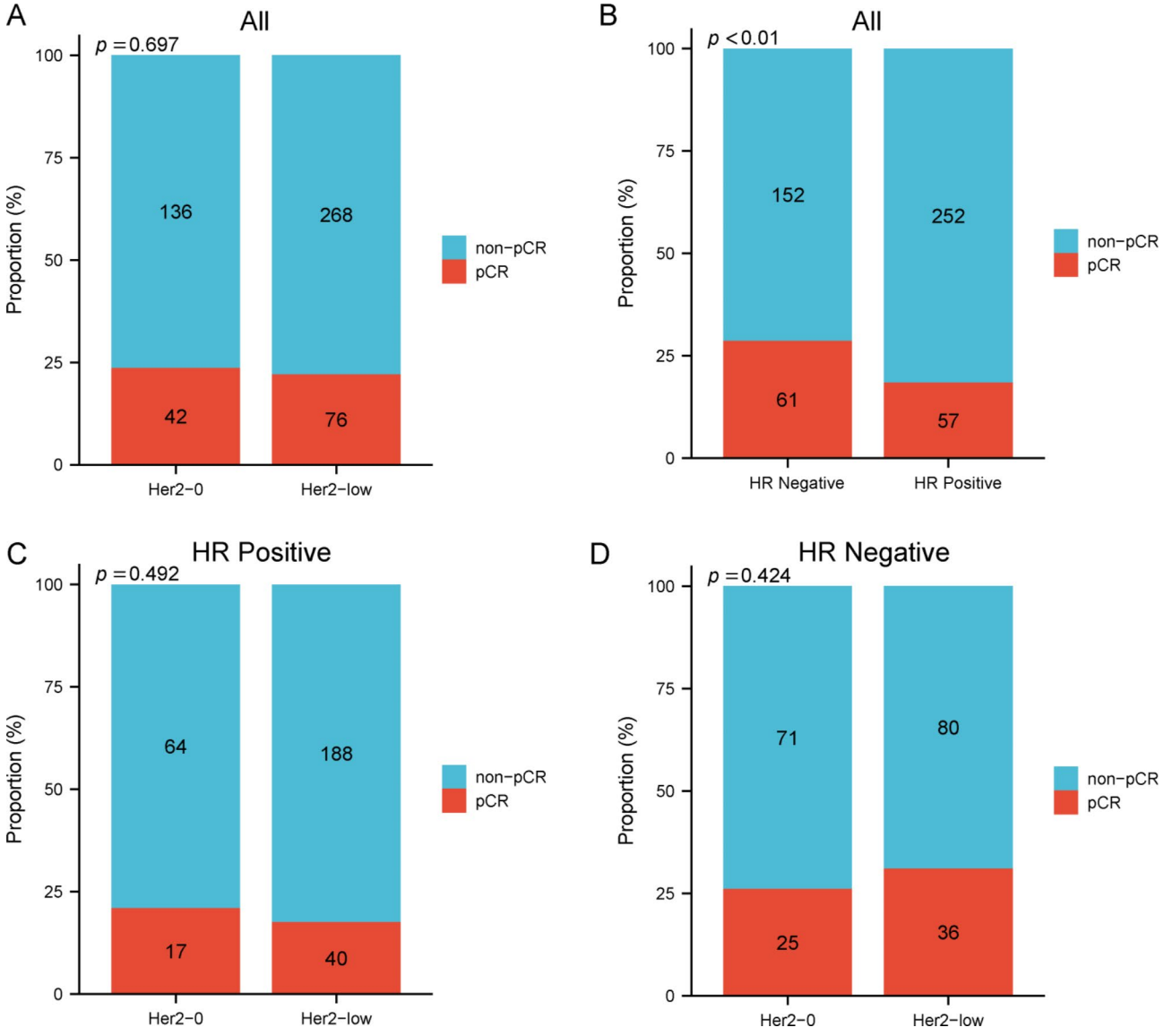


Fig. 1 pCR rate in HR + and in HR– breast cancer according to HER2-low and HER2-0 status. (**A**) Pathological complete response (pCR) rates in patients with HER2-0 and HER2-low breast cancer. (**B**) pCR rates in patients with HR-negative and HR-positive breast cancer. (**C**) pCR and non-pCR rates in patients with HR-positive breast cancer. (**D**) pCR and non-pCR rates in patients with HR-negative breast cancer

transformed to HER2-low after NAT, while 5% transformed to HER2+. HR+/HER2-low patients saw that 18% were transformed to HER2-0 and 4% were transformed to HER2+. Moreover, among HR- patients, 32% transformed from HER2-0 to HER2-low and 5% transformed from HER2-0 to HER2+. Among patients initially diagnosed as HR-/HER2-low, 16.4% transformed to HER2-0 and 5.2% transformed to HER2+.

HER2-low and pCR and survival analysis

DFS and OS were primarily assessed via the survival analysis. The median follow-up time was 44.6 months, in which the follow-up was carried out till January 2024. In the overall cohort, the univariate and multivariate analysis showed independent factors affecting DFS were lymph node status (HR=1.35, P=0.021), HER2 expression status (HR=0.60, P=0.037), achievement of pCR (HR=2.23, p=0.015), and NAT strategy (HR=1.97, P=0.032; HR=3.18, P=0.014) (Table 5). Additionally, in univariate analysis, HR status and NAT cycles showed some correlation with DFS but lacked statistical significance (Table 5). Figure 3A shows that in comparison with patients with HER2-0 expression, there was a better DFS in those with HER2-low expression (P=0.047). When stratifying by HR, in comparison with HR-/HER2-0 patients, there was a better DFS rate in HR-/HER2-low patients (P=0.009). However, no significant DFS

Table 3 Patient demographics and baseline characteristics

Characteristic	HER2		*P*-value
	HER2-0 (*n* = 178)	HER2-low (*n* = 344)	
Mean age			**0.014**
Mean ± SD	51 ± 10	49 ± 9	
Age			**0.002**
< 50	75 (42.1%)	195 (56.7%)	
≥ 50	103 (57.9%)	149 (43.3%)	
BMI			0.368
Mean ± SD	24.4 ± 3.3	24.7 ± 3.4	
Menstruation			**< 0.001**
Pre-menopausal	81 (45.5%)	212 (61.6%)	
Post-menopausal	97 (54.5%)	132 (38.4%)	
Tumour size			0.064
≤ 2 cm	17 (9.6%)	48 (14.0%)	
2–5 cm	123 (69.1%)	247 (71.8%)	
> 5 cm	38 (21.3%)	49 (14.2%)	
Lymph node			0.358
Positive	96 (53.9%)	200 (58.1%)	
Negative	82 (46.1%)	144 (41.9%)	
Grade			**0.034**
I	21 (11.8%)	35 (10.2%)	
II	89 (50.0%)	212 (61.6%)	
III	68 (38.2%)	97 (28.2%)	
Histology			0.178
IDC	144 (80.9%)	294 (85.5%)	
non-IDC	34 (19.1%)	50 (14.5%)	
ER			**< 0.001**
Positive	69 (38.8%)	212 (61.6%)	
Negative	109 (61.2%)	132 (38.4%)	
PR			**< 0.001**
Negative	112 (62.9%)	131 (38.1%)	
Positive	66 (37.1%)	213 (61.9%)	
Ki67			0.902
≤ 15%	39 (21.9%)	77 (22.4%)	
> 15%	139 (78.1%)	267 (77.6%)	
NAT strategy			0.362
Anthracycline + Taxane	153 (86.0%)	309 (89.8%)	
Anthracycline	22 (12.4%)	30 (8.7%)	
Taxane	3 (1.7%)	5 (1.5%)	
NAT Cycle			0.843
4–6	105 (59.0%)	206 (59.9%)	
> 6	73 (41.0%)	138 (40.1%)	
Breast Surgery			0.118
Mastectomy	145 (81.5%)	298 (86.6%)	
BCS	33 (18.5%)	46 (13.4%)	
pCR			0.697
pCR	42 (23.6%)	76 (22.1%)	
non-pCR	136 (76.4%)	268 (77.9%)	

Abbreviations: *IDC* invasive ductal carcinoma, *non-IDC* non-invasive ductal carcinoma, *ER* estrogen receptor, *PR* progesterone receptor, *NAT* neoadjuvant therapy, *pCR* pathologic complete response, *non-pCR* non-pathologic complete response

difference was shown between patients with HER2-0 and HER2-low expression in the HR + group (Fig. 3B-C).

For OS, both univariate and multivariate analysis found that independent factors that affect OS in the overall cohort included the tumor size (T_2 stage) (HR = 3.22, $p = 0.014$), the inclusion of taxane-based drugs in the NAT strategy (HR = 2.50, $P = 0.009$), and achievement of pCR (HR = 2.65, $P = 0.027$) (Table 6). The lymph

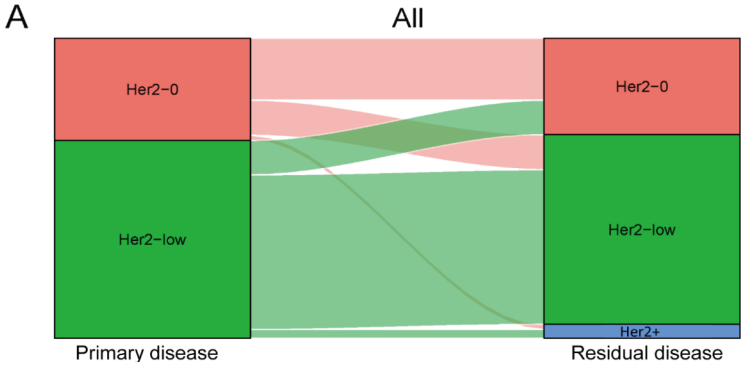
Table 4 Analysis of the effect of the patient characteristics on pCR

		Non-pCR *N* (%)	pCR *N* (%)	Univariable analysis *P*-value	Multivariable analysis OR (95%CI, *P*-value)
Age	< 50	214 (79.3)	56 (20.7)	-	-
	≥ 50	190 (75.4)	62 (24.6)	0.292	-
Menstruation	Pre-menopausal	233 (79.5)	60 (20.5)	-	-
	Post-menopausal	171 (74.7)	58 (25.3)	0.189	-
BMI	Mean (SD)	24.8 (3.4)	24.0 (3.3)	**0.039**	0.94 (0.87–1.01, $p=0.117$)
Tumour size	≤ 2 cm	42 (64.6)	23 (35.4)	-	-
	2–5 cm	291 (78.6)	79 (21.4)	**0.015**	**0.55 (0.29–0.98, $p=0.046$)**
	> 5 cm	71 (81.6)	16 (18.4)	**0.019**	**0.68 (0.21–0.76, $p=0.011$)**
Lymph node	Negative	178 (78.8)	48 (21.2)	-	-
	Positive	226 (76.4)	70 (23.6)	0.514	-
Grade	I	38 (67.9)	18 (32.1)	-	-
	II	242 (80.4)	59 (19.6)	**0.038**	0.55 (0.27–1.19, $p=0.119$)
	III	124 (75.2)	41 (24.8)	0.288	**0.36 (0.16–0.81, $p=0.013$)**
Histology	IDC	374 (85.4)	64 (14.6)	-	-
	non-IDC	30 (35.7)	54 (64.3)	**< 0.001**	**10.99 (6.15–20.27, $p<0.001$)**
HR	Negative	152 (71.4)	61 (28.6)	-	-
	Positive	252 (81.6)	57 (18.4)	**0.007**	0.82 (0.51–1.33, $p=0.410$)
HER2	HER2-0	136 (76.4)	42 (23.6)	-	-
	HER2-low	268 (77.9)	76 (22.1)	0.697	-
Ki67	≤ 15%	105 (90.5)	11 (9.5)	-	-
	> 15%	299 (73.6)	107 (26.4)	**< 0.001**	**2.82 (1.45–5.95, $p=0.004$)**
NAT strategy	Anthracycline + Taxane	360 (77.9)	102 (22.1)	-	-
	Anthracycline	37 (71.2)	15 (28.8)	0.272	-
	Taxane	7 (87.5)	1 (12.5)	0.524	-
NAT Cycle	4–6	243 (78.1)	68 (21.9)	-	-
	> 6	161 (76.3)	50 (23.7)	0.623	-
Breast Surgery	Mastectomy	349 (78.8)	94 (21.2)	-	-
	BCS	55 (69.6)	24 (30.4)	0.115	-

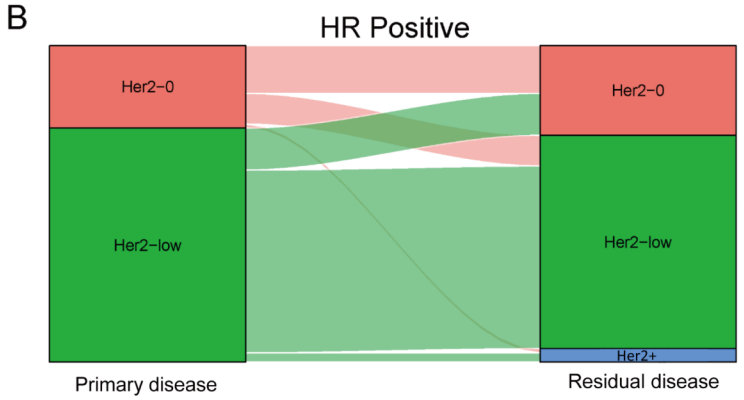
Abbreviations: *HR* hormone receptor, *IDC* invasive ductal carcinoma, *non-IDC* non-invasive ductal carcinoma, *ER* estrogen receptor, *PR* progesterone receptor, *NAT* neoadjuvant therapy, *pCR* pathologic complete response, *non-pCR* non-pathologic complete response, *CI* Confidence interval

node status in the univariate analysis was linked to OS as well ($P=0.038$). Additionally, although not reaching statistical significance, the HER2-low expression status showed correlations with OS in the univariate and multivariate analyses (Table 6). As can be seen in Fig. 3D, although not statistically significant, the survival curves for HER2-0 and HER2-low began to diverge at around 50 months. In comparison with those with HER2-0 expression ($P=0.064$), there was a favorable trend in OS in patients with HER2-low expression. In the further stratification by HR status, there was a better OS rate in HR-/HER2-low patients compared to that in HR-/HER2-0 patients ($P=0.026$). However, no significant differences were seen in OS between patients with HER2-0 and HER2-low expression in the HR+group (Fig. 3E-F).

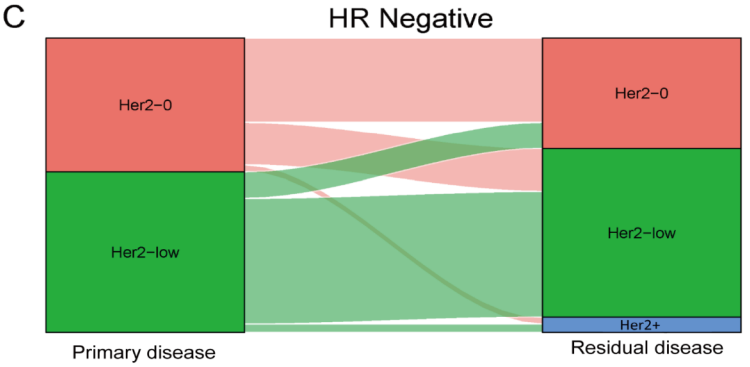
Patients were classified into the pCR and the non-pCR groups for evaluating survival outcomes of HER2-low patients in accordance with the pCR status. Figure 4 shows a better DFS rate ($P=0.027$) and OS rate ($P=0.038$) exits within the pCR group compared to that in the Non-pCR group. In the subgroup analysis in accordance with the achievement of pCR, for patients achieving pCR, no significant difference exists in DFS ($P=0.736$) and OS ($P=0.891$) regardless of the HER2 expression status. However, non-pCR patients saw the HER2-low group had better DFS ($P=0.029$) and OS ($P=0.038$) than the HER2-0 group.



ALL		Residual disease *n* (%)			Kappa	*p* value
		HER2-0	HER2-low	HER2+		
Primary disease *n* (%)	HER2-0	108 (61)	61 (34)	9 (5)	0.431	<0.01
	HER2-low	60 (17.4)	269 (78.2)	15 (4.4)		



HR+		Residual disease *n* (%)			Kappa	*p* value
		HER2-0	HER2-low	HER2+		
Primary disease *n* (%)	HER2-0	47 (58)	30 (37)	4 (5)	0.291	<0.01
	HER2-low	41 (18)	178 (78)	9 (4)		



HR-		Residual disease *n* (%)			Kappa	*p* value
		HER2-0	HER2-low	HER2+		
Primary disease *n* (%)	HER2-0	61 (63)	31 (32)	5 (5)	0.316	<0.01
	HER2-low	19 (16.4)	91 (78.4)	6 (5.2)		

Fig. 2 HER2 category change between primary and residual disease. (**A**) Changes in HER2 categories between primary and residual diseases in the overall patient cohort. (**B**) Changes in HER2 categories between primary and residual diseases in the HR-positive patients. (**C**) Changes in HER2 categories between primary and residual diseases in the HR-negative patients

Table 5 Analysis of the effect of the patient characteristics on DFS

		N (%)	Univariable analysis *P*-value	Multivariable analysis HR (95%CI, *P*-value)
Age	< 50	270 (51.7)	-	-
	≥ 50	252 (48.3)	0.239	-
Menstruation	Pre-menopausal	293 (56.1)	-	-
	Post-menopausal	229 (43.9)	0.101	-
BMI	Mean (SD)	24.6 (3.4)	0.410	-
Tumour size	≤ 2cm	65 (12.5)	-	-
	2-5cm	370 (70.9)	0.754	-
	> 5cm	87 (16.7)	0.185	-
Lymph node	Negative	226 (43.3)	-	-
	Positive	296 (56.7)	**0.035**	**1.35 (1.12–1.85, *p* = 0.021)**
Grade	I	56 (10.7)	-	-
	II	301 (57.7)	0.542	-
	III	165 (31.6)	0.348	-
Histology	IDC	438 (83.9)	-	-
	non-IDC	84 (16.1)	0.671	-
HR	Negative	213 (40.8)	-	-
	Positive	309 (59.2)	0.454	-
HER2	HER2-0	178 (34.1)	-	-
	HER2-low	344 (65.9)	**0.047**	**0.60 (0.37–0.97, *p* = 0.037)**
Ki67	≤ 15%	116 (22.2)	-	-
	> 15%	406 (77.8)	0.910	-
NAT strategy	Anthracycline + Taxane	462 (88.5)	-	-
	Anthracycline	52 (10.0)	**0.034**	**1.97 (1.06–3.28, *p* = 0.032)**
	Taxane	8 (1.5)	**0.048**	**3.18 (1.67–7.10, *p* = 0.014)**
NAT Cycle	4–6	311 (59.6)	-	-
	> 6	211 (40.4)	**0.067**	0.67 (0.41–1.11, *p* = 0.120)
Breast Surgery	Mastectomy	443 (84.9)	-	-
	BCS	79 (15.1)	0.817	-
pCR	pCR	118 (22.6)	-	-
	non-pCR	404 (77.4)	**0.027**	**2.23 (1.17–4.26, *p* = 0.015)**

Abbreviations: *HR* hormone receptor, *IDC* invasive ductal carcinoma, *non-IDC* non-invasive ductal carcinoma, *ER* estrogen receptor, *PR* progesterone receptor, *NAT* neoadjuvant therapy, *pCR* pathologic complete response, *non-pCR* non-pathologic complete response, *CI* Confidence interval, *DFS* disease-free survival

Discussion

In recent years, researchers have proposed that there were unique biological characteristics in HER2-low expression breast cancer, which were distinct from those with HER2-0 expression [17, 18]. HER2-low can represent a new classification to guide clinicians in NAT for breast cancer patients [19, 20]. However, controversies regarding specific biological characteristics of HER2-low expression breast cancer affecting patient prognosis due to a lack of relevant evidence still exist. The research showed in the overall cohort, no statistical difference in OS can be seen in the low-expression status of HER2, while there was higher DFS in HER2-low patients. When clinical characteristics of HER2-0 and HER2-low were compared, it can be seen tumor size, Ki67 expression and lymph node status between both groups showed no significant differences, conforming to results of the prior research [21, 22]. However, this research revealed higher rates of ER and PR positivity existed in HER2-low patients. Previous studies have suggested that based on PAM50 intrinsic subtyping analysis, a higher correlation with luminal-like gene expression can be seen in HER2-low tumors, whereas HER2-0 tumors are more associated with basal-like subtyping [3]. After stratifying patients based on HR status, our study also found HER2-low had an obvious increase in DFS and OS in comparison with HER2-0 in HR- patients. Thus, it is suitably speculated HR status is likely to serve as a primary influencing factor

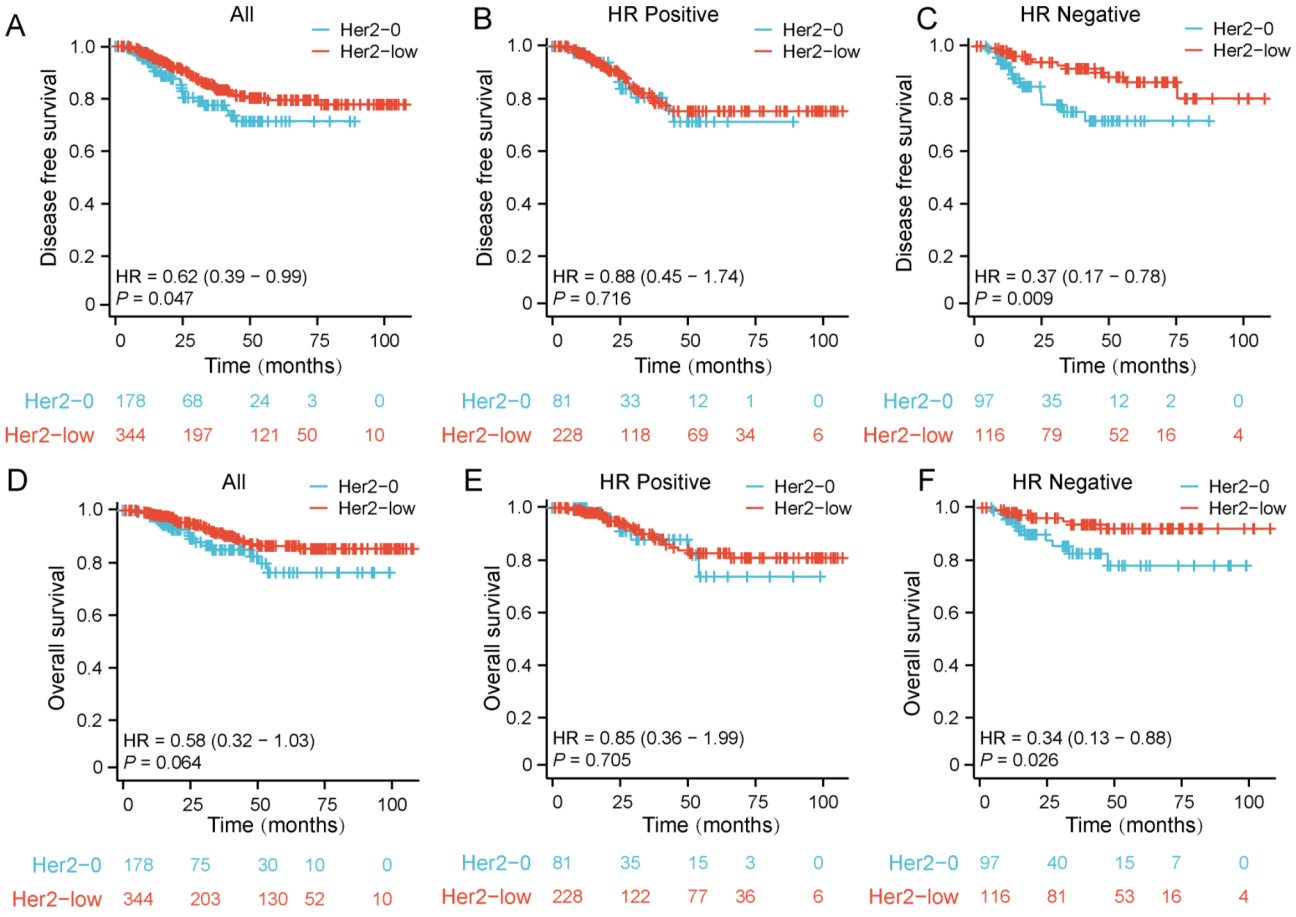


Fig. 3 Kaplan–Meier survival curves in HR + and HR– for DFS and for OS. (**A**) The Kaplan–Meier curve for disease-free survival in the HER2-0 and HER2-low patients. (**B**) The Kaplan–Meier curve for disease-free survival in the HR+/HER2-0 and HR+/HER2-low patients. (**C**) The Kaplan–Meier curve for disease-free survival in the HR-/HER2-0 and HR-/HER2-low patients. (**D**) The Kaplan–Meier curve for overall survival in the HER2-0 and HER2-low patients. (**E**) The Kaplan–Meier curve for overall survival in the HR+/HER2-0 and HR+/HER2-low patients. (**F**) The Kaplan–Meier curve for overall survival in the HR-/HER2-0 and HR-/HER2-low patients

for the survival benefits of HER2-low patients. In addition, HR is likely to serve as a primary molecular marker that affects unique biological characteristics of HER2-low expression breast cancer.

Given evaluations of therapeutic efficacy in NAT, our study found significantly higher DFS and OS existed in patients achieving pCR than non-pCR patients, conforming to findings of prior exploration [23, 24]. Yet, the stratified analysis displayed no significant differences in DFS and OS between HER2-0 and HER2-low patients achieving pCR. The retrospective study that included 446 patients indicated that there was a lower pCR rate in HER2-low expression patients than HER2-0 patients [25]. However, the respective evaluation of the pCR rate in the HR+ /HER2- and triple-negative breast cancer subgroups showed no longer a significant relationship between HER2 expression and pCR. According to Denkert et al., there was a significantly lower pCR rate of the HER2-low expression tumors than the HER2-0 tumors, while in the subgroup analysis, this difference exists significantly not in HR-negative subgroup but just in the HR-positive subgroup [17]. All of those findings indicate in HER2-negative cohort, the HR status instead of the HER2 expression serves as the major determinant of chemotherapy sensitivity and HR positivity is likely to serve as a confounding factor when HER2-low and HER2-0 tumors are compared. In this way, HR status has the possibility to serve as a primary influencing factor for the pathological response to NAT and survival outcomes.

Meanwhile, this research also noticed an interesting phenomenon, that is, in non-pCR patients, HER2-low tumors had better prognoses compared to HER2-0 tumors. That may be because characteristics of HER2-low tumors tend to be luminal-like, while HER2-0 tumors are more similar to basal-like. In addition, it is indicated HER2-low and HER2-0 patients have different outcomes from the perspective of efficacy and prognosis of NAT after refining stratification. For patients initially diagnosed as HER2-negative, it is highly recommended identification of HER2-low expression and HER2-0

Table 6 Analysis of the effect of the patient characteristics on OS

		N (%)	Univariable analysis *P*-value	Multivariable analysis HR (95%CI, *P*-value)
Age	< 50	270 (51.7)	-	-
	≥ 50	252 (48.3)	0.320	-
Menstruation	Pre-menopausal	293 (56.1)	-	-
	Post-menopausal	229 (43.9)	0.136	-
BMI	Mean (SD)	24.6 (3.4)	0.287	-
Tumour size	≤ 2cm	65 (12.5)	-	-
	2-5cm	370 (70.9)	**0.011**	**3.22 (1.61–4.85, *p* = 0.014)**
	> 5cm	87 (16.7)	0.848	0.78 (0.19–3.18, *p* = 0.730)
Lymph node	Negative	226 (43.3)	-	-
	Positive	296 (56.7)	**0.038**	1.24 (0.69–2.23, *p* = 0.482)
Grade	I	56 (10.7)	-	-
	II	301 (57.7)	0.299	-
	III	165 (31.6)	0.118	-
Histology	IDC	438 (83.9)	-	-
	non-IDC	84 (16.1)	0.790	-
HR	Negative	213 (40.8)	-	-
	Positive	309 (59.2)	0.547	-
HER2	HER2-0	178 (34.1)	-	-
	HER2-low	344 (65.9)	0.064	0.56 (0.31–1.02, *p* = 0.057)
Ki67	≤ 15%	116 (22.2)	-	-
	> 15%	406 (77.8)	0.402	-
NAT strategy	Anthracycline + Taxane	462 (88.5)	-	-
	Anthracycline	52 (10.0)	**0.004**	**2.50 (1.25–4.98, *p* = 0.009)**
	Taxane	8 (1.5)	0.105	2.59 (0.61–10.96, *p* = 0.196)
NAT Cycle	4–6	311 (59.6)	-	-
	> 6	211 (40.4)	0.486	-
Breast Surgery	Mastectomy	443 (84.9)	-	-
	BCS	79 (15.1)	0.146	-
pCR	pCR	118 (22.6)	-	-
	non-pCR	404 (77.4)	**0.038**	**2.65 (1.12–6.29, *p* = 0.027)**

Abbreviations: *HR* hormone receptor, *IDC* invasive ductal carcinoma, *non-IDC* non-invasive ductal carcinoma, *ER* estrogen receptor, *PR* progesterone receptor, *NAT* neoadjuvant therapy, *pCR* pathologic complete response, *non-pCR* non-pathologic complete response, *CI* Confidence interval, *OS* overall survival

expression should be compared. At the same time, in the study by Li et al., 45,331 patients with early invasive breast cancer were included to explore the potential differences in pCR rates and OS between HER2-low and HER2-0 early HR-positive and triple-negative breast cancer patients in the neoadjuvant chemotherapy setting [26]. The study found that, regardless of HR status, HER2-low patients who did not achieve pCR had better survival compared to HER2-0 patients. Further analysis also revealed that HER2-low patients had lower staging and were more likely to have ductal carcinoma in situ compared to HER2-0 patients. This provides some scientific evidence to explain why HER2-low patients who did not achieve pCR have better survival outcomes.

Besides, the inconsistencies of HER2 expression between the primary lesion at initial diagnosis and residual disease after receiving NAT in patients were analyzed. This research indicated rate of HER2 expression inconsistency in the overall cohort was 30.4%, in which a higher rate in the HR+group (32.0%) can be seen compared to that of the HR- group (29.3%). That inconsistency mainly involved transitions from HER2-0 to HER2-low, which was followed by the transitions from HER2-low to HER2-0, and some individual patients transitioned from HER2-0/low to HER2+. Research has found early

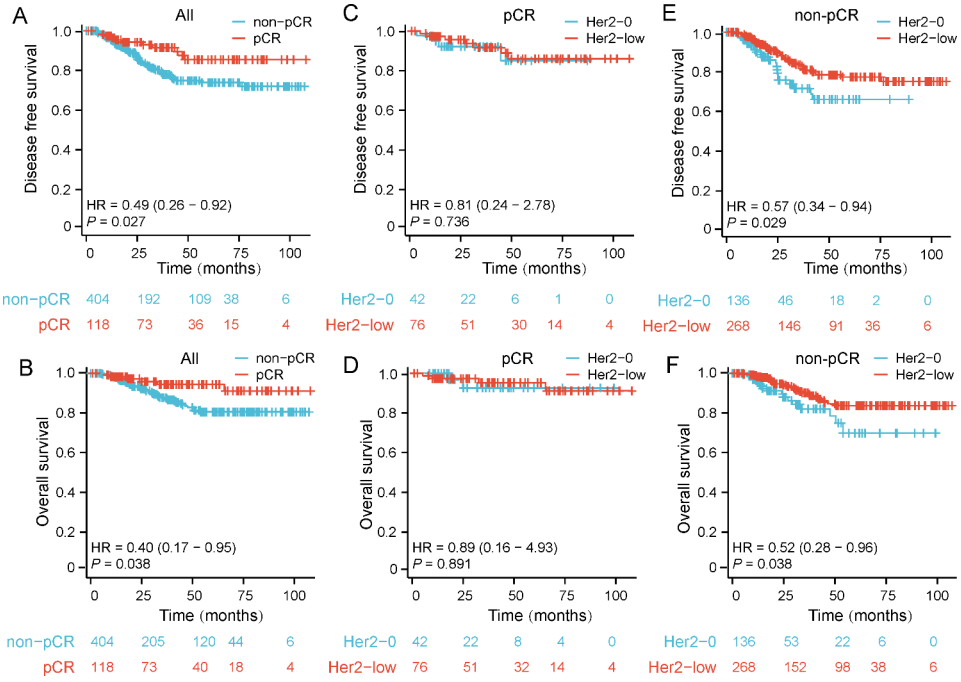


Fig. 4 Kaplan–Meier survival curves in pCR and non-pCR for DFS and for OS. (**A**) The Kaplan–Meier curve for disease-free survival in the pCR and non-pCR patients. (**B**) The Kaplan–Meier curve for overall survival in the pCR and non-pCR patients. (**C**) The Kaplan–Meier curve for disease-free survival in the pCR/HER2-0 and pCR/HER2-low patients. (**D**) The Kaplan–Meier curve for overall survival in the pCR/HER2-0 and pCR/HER2-low patients. (**E**) The Kaplan–Meier curve for o disease-free survival in the non-pCR/HER2-0 and non-pCR/HER2-low patients. (**F**) The Kaplan–Meier curve for overall survival in the non-pCR/HER2-0 and non-pCR/HER2-low patients

breast cancer patients who received NAT between core needle biopsy and surgical excision specimens showed a discrepancy rate of up to 23.13% in HER2-low expression [27]. The other research showed approximately 40% of patients with a baseline phenotype of HER2-0 changed to low HER2 expression after NAT [14]. As put forward by Miglietta et al., the overall inconsistency rate of 38.0% for HER2-low expression existed when conducting an evaluation of the inconsistency of low HER2 expression from primary to recurrent breast cancer [28]. That can be caused by the susceptibility of HER2-low expression tumors to chemotherapy. In addition, it was before found for HR-positive patients, endocrine therapy may induce tumor cell expression of HER2 protein, which brings about tumor cell adaptation and resistance and also causes inconsistency of HER2-low expression [29, 30]. This means inconsistency of HR and/or HER2 status from primary tumors to residual disease after NAT can be a relatively frequent occurrence. Therefore, evaluating and identifying HER2-0/low expression status in both the initial diagnosis of primary lesions and residual disease may enable a proportion of patients to access potentially effective new treatment strategies, whereas these patients might be excluded in accordance with the baseline tumor phenotype.

According to the evidence mentioned before, this research emphasizes the importance of reassessing the molecular biomarker status of tumors, including HER2, in residual disease. In addition, they support that HER2-low classification should be involved as an important influencing factor in this assessment. This study is designed to find suitable patients who may benefit from HER2-targeted therapy in the new ADCs era and provide the correct treatment strategies to the appropriate patient population. For example, significant efficacy exists in the new ADC drug T-DXd when advanced HER2-low expression breast malignancies are treated. In the recent phase 3 clinical trial of T-DXd in advanced HER2-low expression breast cancer, significantly prolonged progression-free survival ($P<0.001$) and overall survival ($P=0.003$) were shown compared to that of the physician's choice of chemotherapy regimen group [6]. Taken together, different non-pCR rates and prognoses existed in HER2-low expression patients than in the HER2-0 patients, and T-DXd in combination with neoadjuvant or adjuvant therapy can effectively improve patients' objective response rates and survival time. Furthermore, applying T-DXd can provide multiple treatment opportunities for the population with changing HER2 status, which can improve treatment strategies and efficiency. However, more clinical research should be conducted to verify those findings.

The analysis still has certain limitations. Firstly, this is single-center retrospective research, and certain

confounding factors and sample size limitations are inevitable. In the future, multicenter studies with larger sample sizes can help to better learn about the characteristics of HER2-low expression breast cancer. In addition, the research mainly focused on patients receiving NAT, and cannot be extrapolated to all malignancies due to tumor heterogeneity and heterogeneity of neoadjuvant chemotherapy. Therefore, further clinical research and more data from adjuvant therapy are still needed to provide more evidence.

Conclusions

In summary, this research can inspire the treatment and prognostic prediction of HER2-low expression breast cancer and partially reveals clinical pathological characteristics of the HER2-low breast cancer subgroup in the breast cancer patients receiving NAT. It can be seen that HER2-low serves as the distinct subtype of breast cancer, which was accompanied by unique survival outcomes. HR expression status can serve as an important molecular marker affecting pCR and survival in HER2-low patients. In HR- patients, better survival can be found in HER2-low patients. Additionally, in non-pCR patients, better DFS and OS were seen in HER2-low than in HER2-0. Finally, significant instability of HER2 expression from primary breast cancer to residual invasive disease was also revealed. This indicates the periodic monitoring of HER2 expression status in residual disease can provide more accurate guidance for the anti-HER2 ADC therapy in the clinical practices for HER2-low patients.

Abbreviations

ADCs	Antibody drug conjugates
IHC	Immunohistochemistry
ISH	In situ hybridization
T-DXd	Fam-trastuzumab deruxtecan-nxki
ESMO	European Society for Medical Oncology
ASCO/CAP	American Society of Clinical Oncology/College of American Pathologists
AJCC	American Joint Committee on Cancer
HR	Hormone receptor
IDC	Invasive ductal carcinoma
ER	Estrogen receptor
PR	Progesterone receptor
NAT	Neoadjuvant therapy
pCR	Pathologic complete response
DFS	Disease-free survival
OS	Overall survival
CI	Confidence interval

Author contributions

Shuai Yan and Weiyang Tao wrote the main manuscript text. Wenxi Zhao, Yuhan Dong, Hongyue Wang and Weiyang Tao contributed to the design and implementation of the research. Shuai Yan, Wenxi Zhao, Shouping Xu and Tong Yu contributed to the analysis of the results. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki, and approved by the independent Ethical Committees of Harbin Medical University Cancer Hospital (The approval date is April 12, 2024.). Given the anonymized nature of the data, the requirement for informed consent was waived by the IEC of Harbin Medical University Cancer Hospital.

Competing interests

The authors declare no competing interests.

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