Am J Cancer Res 2024;14(8):3859-3872
www.ajcr.us /ISSN:2156-6976/ajcr0157029

Original Article

Evolution and prognostic significance of HER-2 conversion from primary to residual disease in HER-2 negative patients with breast cancer after neoadjuvant chemotherapy

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Received April 5, 2024; Accepted August 7, 2024; Epub August 25, 2024; Published August 30, 2024

Abstract: This study aimed to analyze HER-2 zero or HER-2 low conversion in HER-2 negative patients after neoadjuvant chemotherapy (NAC) and evaluate its prognostic significance. HER-2 negative patients with breast cancer with residual disease after NAC and paired pre- and post-therapeutic HER-2 testing results were analyzed retrospectively. HER-2 low, defined as immunohistochemistry (IHC) scores of 1+ or 2+/in situ hybridization (ISH), were not amplified. HER-2 zero is defined as an IHC score of 0. A total of 571 patients were enrolled, including primary HER-2 zero (n=201, 35.2%) and HER-2 low (n=370, 64.8%). The overall HER-2 change rate was 32.4%. Multivariable logistic regression showed that patients with hormone receptor-positive status before NAC was significantly associated with the conversion of HER-2 zero to low (OR=3.436, $P < 0.0001$). The median follow-up time was 50.0 months. In patients who are primary HER-2 zero, HER-2 zero to low was significantly associated with better disease-free survival (DFS) than constant HER-2 zero (HR=0.49, $P=0.01$) after adjustment (4-year DFS 80.1% vs 55.7%, Log-rank $P=0.033$). Subgroup analysis revealed that among patients who are primary HER-2 zero with hormone receptor-positive, HER-2 zero to low had a significantly better DFS than constant HER-2 zero (Log-rank $P=0.037$). In contrast, patients with hormone receptor-negative status did not. In conclusion, almost one-third of patients who are HER-2 negative underwent HER-2 zero or HER-2 low conversion after NAC. HER-2 zero to low conversion was associated with better DFS in patients who are HER-2 zero. These results provide a valuable reference for the potential application of anti-HER-2 ADC in an adjuvant setting for patients with residual disease after NAC.

Keywords: Breast cancer, neoadjuvant chemotherapy, HER-2 low, HER-2 zero, HER-2 conversion, prognosis

Introduction

Breast cancer is one of the most common cancers and a leading cause of cancer-related deaths in women worldwide [1]. It is a highly heterogeneous disease with significant biological diversity and different clinicopathological features and prognosis [2]. In clinical practice, breast cancer can be molecularly classified based on the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) status. For HER-2 status, a dichotomy is typically used (positive or negative), and HER-2-positive patients can use HER-2-targeting drugs such as trastuzumab [3]. With the development of anti-HER-2 antibody-drug conjugates (ADC), trastuzumab deruxtecan showed significant efficacy in patients with HER-2 low, defined as a score of 1+ on immunohistochemistry (IHC) or as a score of 2+ and negative results on in situ hybridization (ISH) [4].

HER-2 low breast cancer, accounting for 45% to 55% of breast cancer patients, has emerged as a novel therapeutic target for anti-HER-2 ADC, but its prognostic significance remains unclear [5, 6]. HER-2 status can shift during treatment,

and there are disparities between primary and recurrent breast cancers [7-10]. Notably, alterations in HER-2 status between positive and negative have been observed after neoadjuvant chemotherapy (NAC) [11, 12]. Niikura et al. reported that 3.4% of patients initially diagnosed as HER-2 negative exhibited HER-2 positive status after NAC, while 21.4% of patients had HER-2 conversion from positive to negative status [13]. However, most studies have shown that alterations in HER-2 status between positive and negative do not affect patient prognosis [14-16]. Currently, few studies are investigating the conversion of HER-2 status between HER-2 low and zero among patients who are HER-2 negative pre- or post-NAC. Moreover, the results regarding its prognostic impact from existing studies are inconsistent [17, 18].

Our study aimed to analyze the conversion of HER-2 status between low and zero in primary HER-2 negative breast cancer after NAC, evaluate the factors associated with this transition, and explore its impact on prognosis.

Method

Study population

This multicenter retrospective study was performed at three hospitals in China (Cancer Hospital, Chinese Academy of Medical Sciences, Beijing Chaoyang District Sanhuan Cancer Hospital, and Cancer Hospital of Huanxing, China). Patients were recruited if they met the following inclusion criteria: 1) women aged > 18 years with pathologically diagnosed early invasive breast cancer (clinical stage I-III); 2) histologically confirmed primary HER-2 negative; 3) treated with NAC; 4) pathologically assessed residual disease after surgery, defined as invasive carcinoma of the breast or positive axillary nodes; 5) HER-2 status of the residual disease. Patients were excluded if any of the following conditions were present: 1) recurrent or metastatic breast cancer; 2) pathologically complete response after surgery (ypT0/TisypN0); 3) no survival follow-up information.

A total of 571 patients who were HER-2 negative receiving NAC with paired primary and residual disease between July 2008 and August 2022 were included in the study. A flowchart is presented in Supplementary Figure 1.

Data collection and outcomes

Clinical and pathological data, including age, histopathology, tumor size, nodal status, stage, ER, PR, and HER-2 status were extracted. We also collected neoadjuvant treatment information from inpatient and outpatient medical records. Clinical and pathological stages were based on the seventh edition of the American Joint Committee on Cancer Staging Manual (AJCC 7th edition). Survival data were acquired from medical records or telephone calls.

Pathology definition

The 2020 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines were used to define ER and PR, with a threshold of 1% of nuclei staining by IHC for the positive (≥ 1%) [19]. Hormone receptor positivity (HR+) was defined as ER and PR positivity, ER positivity and PR negativity, or ER negativity and PR positivity. Hormone receptor-negative (HR-) status was defined as ER- or PR-negative. HER-2 negative was defined according to the 2018 ASCO/CAP Guidelines for tumors with IHC scores of 0, 1+, or 2+/ISH not amplified [3]. HER-2 low was defined as an IHC score of 1+ or 2+/ISH not amplified, and HER-2 zero was defined as an IHC score of 0, according to the 2023 European Society for Medical Oncology expert consensus [20].

Survival definitions

The primary survival endpoint was disease-free survival (DFS), which was defined as the time from surgery to the first appearance of one of the following invasive events: locoregional recurrence, distant metastasis, new contralateral or ipsilateral breast cancer, a second primary malignancy, or death from any cause. The secondary endpoint was overall survival (OS), defined as the time from neoadjuvant chemotherapy to death or the last follow-up [21].

Statistical analysis

Characteristics variables were shown in the table. The chi-square test or the Fisher exact test was used to compare categorical variables (n/%), whereas the Student's *t* test was performed to compare continuous variables (mean ± SD). Sankey plots were used to describe the evolution of HER-2 expression. A Cohen kappa

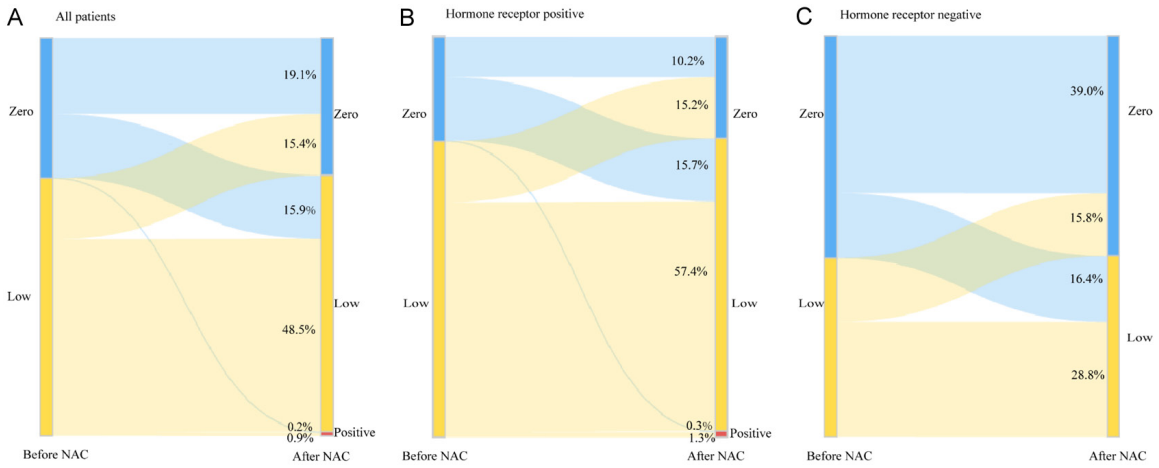

Figure 1. The evolution of HER-2 expression from primary to residual disease. A. The evolution in overall patients; B. The evolution in hormone receptor positive patients; C. The evolution in hormone receptor negative patients.

analysis was used to assess the concordance of HER-2 status between the primary and residual diseases. Bivariable logistic regression was used to conduct a univariate analysis of the factors related to HER-2 changes. Factors with univariate $P < 0.05$ were included in the multivariate logistic models, and the corresponding results were expressed in the table. Survival outcomes, including DFS and OS, were estimated using the Kaplan-Meier (K-M) curve and compared using the log-rank test. Multivariate Cox regression models were used to identify independent factors associated with survival outcomes, as shown in the table. All tests were two-sided, and a P value < 0.05 was considered statistically significant. Statistical analysis was conducted using SPSS version 26.0 and R software version 4.2.0.

Results

HER-2 changes from primary to residual disease

The changes in HER-2 expression from primary to residual disease in patients who are HER-2 negative are presented in **Figure 1A**. A total of 571 patients with primary HER-2 negative breast cancer with residual disease after NAC were enrolled, including primary HER-2 zero (n=201, 35.2%) and HER-2 low (n=370, 64.8%). The overall HER-2 change rate was 32.4%, HER-2 zero to low was 15.9% (n=91), HER-2 low to zero was 15.4% (n=88), and HER-2 negative to positive changes were 1.1% (n=6). The Cohen kappa value was 0.297. Among the 201 patients with no HER-2 zero before NAC, 45.3% (n=91) of patients showed HER-2 low after NAC. Only 1 (0.4%) patient's HER-2 status transferred to positive. Among the 370 patients who were HER-2 low before NAC, 23.8% (n=88) showed HER-2 changes (low to zero) after NAC. Additionally, 5 (1.4%) patients developed a HER-2 positive status.

A total of 394 (69.0%) patients were HR+ and 177 (31.0%) were HR-. Changes in HER-2 expression according to hormone receptor status are presented in **Figure 1B** and **1C**. In the HR+ cohort, the HER-2 change rate was 32.5% (n=128); 15.7% (n=62) of patients showed HER-2 zero transfer to HER-2 low, and HER-2 low to HER-2 zero was 15.2% (n=60). A total of 1.6% of the patients (n=6) had a HER-2 status conversion from negative to positive. The Cohen kappa value was 0.175. Similarly, in the HR- cohort, 33.2% (n=57) of the patients showed HER-2 transition. Of these patients, 16.4% (n=29) showed a conversion from HER-2 zero to HER-2 low, and 15.8% (n=28) showed a switch in the opposite direction. The Cohen kappa value was 0.349.

Baseline characteristics

The baseline characteristics of the overall population and groups are presented in **Table 1**. In the HER-2 zero before NAC cohort, patients with HER-2 changes (zero to low) were likely to be hormone receptor-positive (68.1% vs 36.7%, $P < 0.0001$) and had a higher proportion of patients with Ki-67 ≤ 20 (Ki-67 ≤ 20, 27.5% vs

Table 1. Baseline characteristics of the overall population

Characteristic	HER-2 zero before NAC			HER-2 low before NAC		
	Constantly HER-2 zero N=109	HER-2-zero to HER-2-low N=91	*p*-value	HER-2-low to HER-2-zero N=88	Constantly HER-2 low N=277	*p*-value
Age at diagnosis						
Median (range)	47 (25-75)	46 (26-75)		45 (21-74)	48 (24-77)	
≤ 35	18 (16.5)	19 (20.9)	0.724	16 (18.2)	42 (15.2)	0.368
36-49	44 (40.4)	34 (37.4)		37 (42.0)	101 (36.5)	
≥ 50	47 (43.1)	38 (41.8)		35 (39.8)	134 (48.4)	
Menstruation status			0.906			0.329
Premenopausal	71 (65.1)	60 (65.9)		56 (63.6)	160 (57.8)	
Postmenopausal	38 (34.9)	31 (34.1)		32 (36.4)	117 (42.2)	
Clinical T stage			0.404			0.789
0-2	73 (67.0)	63 (69.2)		51 (57.9)	165 (59.6)	
3-4	36 (33.0)	28 (30.8)		37 (42.1)	112 (40.4)	
Clinical N stage			0.746			0.663
0	28 (25.7)	23 (25.3)		15 (17.0)	40 (14.4)	
1	32 (29.4)	33 (36.3)		18 (20.5)	74 (26.7)	
2	28 (25.7)	20 (22.0)		35 (39.8)	100 (36.1)	
3	21 (19.3)	15 (16.5)		20 (22.7)	63 (22.7)	
Clinical stage			0.267			0.957
II	43 (39.5)	43 (47.3)		26 (29.6)	81 (29.2)	
III	66 (60.5)	48 (52.7)		62 (70.4)	196 (70.8)	
HR before NAC			< 0.0001			0.008
Negative	69 (63.3)	29 (31.9)		28 (31.8)	51 (18.4)	
Positive	40 (36.7)	62 (68.1)		60 (68.2)	226 (81.6)	
Ki-67 before NAC			0.035			0.030
≤ 20	17 (15.6)	25 (27.5)		18 (20.5)	94 (33.9)	
> 20	87 (79.8)	61 (67.0)		65 (73.9)	180 (65.0)	
Missing	5 (4.6)	5 (5.5)		5 (5.7)	3 (1.1)	
Breast surgery			0.688			0.124
Mastectomy	95 (87.2)	81 (89.0)		75 (85.2)	252 (91.0)	
Breast-conserving	14 (12.8)	10 (11.0)		13 (14.8)	25 (9.0)	
Axillary node surgery			0.962			0.075
ALND	98 (89.9)	82 (90.1)		81 (92.0)	269 (97.1)	
SLNB	11 (10.1)	9 (9.9)		7 (8.0)	8 (2.9)	
Miller-Payne grade			0.598			0.409
1-2	42 (38.5)	29 (31.9)		34 (38.6)	107 (37.6)	
3	45 (41.3)	46 (50.5)		36 (40.9)	127 (45.8)	
4	15 (13.8)	10 (11.0)		13 (14.8)	34 (12.3)	
5	4 (3.7)	3 (3.3)		3 (3.4)	3 (1.1)	
Missing	3 (2.8)	3 (3.3)		2 (2.3)	6 (2.2)	
Pathologic T stage			0.581			0.266
ypT0	4 (3.7)	3 (3.3)		3 (3.4)	5 (1.8)	
ypT1	55 (50.5)	42 (46.2)		34 (38.6)	127 (45.8)	
ypT2	40 (36.7)	41 (45.1)		44 (50.0)	112 (40.4)	
ypT3-4	10 (9.2)	5 (5.5)		7 (8.0)	33 (11.9)	
Pathologic N stage			0.283			0.205
ypN0	51 (46.8)	32 (35.2)		24 (27.3)	53 (19.1)	
ypN1	26 (23.9)	32 (35.2)		19 (21.6)	81 (29.2)	
ypN2	14 (12.8)	12 (13.2)		15 (17.0)	60 (21.7)	
ypN3	18 (16.5)	15 (16.5)		30 (34.1)	83 (30.0)	

ypTNM stage			0.232			0.443
I	34 (31.2)	19 (20.9)		15 (17.0)	34 (11.9)	
II	42 (38.5)	43 (47.3)		27 (30.7)	95 (34.3)	
III	33 (30.3)	29 (31.9)		46 (52.3)	149 (53.8)	
HR after NAC			< 0.0001			< 0.0001
Negative	79 (72.5)	32 (35.2)		40 (45.5)	61 (22.0)	
Positive	30 (27.5)	59 (64.8)		48 (54.5)	216 (78.0)	
Ki-67 after NAC			0.001			0.058
≤ 20	33 (30.3)	49 (53.8)		44 (50.0)	171 (61.7)	
> 20	75 (68.8)	41 (45.1)		42 (47.7)	102 (36.8)	
Missing	1 (0.9)	1 (1.1)		2 (2.3)	4 (1.4)	
Neoadjuvant therapy*			0.001			0.056
Anthracycline and taxane	42 (38.5)	59 (64.8)		52 (59.1)	196 (70.8)	
Taxane and platinum	53 (48.6)	25 (27.5)		25 (28.4)	47 (17.0)	
Anthracycline, taxane and platinum	7 (6.4)	0 (0.0)		2 (2.3)	3 (1.1)	
Anthracycline or taxane	5 (4.6)	5 (5.5)		7 (8.0)	18 (6.5)	
Endocrine therapy	0 (0.0)	0 (0.0)		0 (0.0)	6 (2.2)	
Others	2 (1.8)	2 (2.2)		2 (2.3)	7 (2.5)	

We treated missing data as censored when performing chi-square analysis. *The rows of Anthracycline, taxane and platinum, Anthracycline or taxane, Endocrine therapy and others were combined for χ^2 test. Abbreviations: HR, hormone receptor; Ki67, Ki67 index; SLNB, Sentinel lymph node biopsy; ALND, Axillary lymph node dissection; NAC, neoadjuvant chemotherapy.

15.6%, *P*=0.035). Among patients with HER-2 low before NAC, a higher proportion of hormone receptor-negative tumors was observed in HER-2-changed patients (low to zero) (31.8% vs 18.4%, *P*=0.008), and the Ki-67 level was relatively high in patients with HER-2 changes (Ki-76 > 20, 73.9% vs 65.0%, *P*=0.030). Regarding the NAC regimen, patients with constant HER-2 zero were more likely to receive taxane and platinum regimens than patients with HER-2 zero to low (48.6% vs 27.5%, *P*=0.001). No significant differences were observed in other factors among the patients.

The baseline characteristics of the six patients with HER-2 conversion from negative to positive are presented in Supplementary Table 1. All the patients were hormone receptor-positive before and after NAC. The Ki-67 index of the residual disease in these patients was < 20.

Factors associated with HER-2 changes

Bivariable logistic regression analyses were used to evaluate factors associated with HER-2 changes. Among the patients with HER-2 zero before NAC, univariable analysis showed that HR+ before NAC, Ki-67 ≤ 20 before NAC, and anthracycline and taxane neoadjuvant therapy were found to be significantly associated with HER-2 changes (zero to low). After multivariable adjustment, the results showed that HR+ before NAC was related to the conversion of HER-2 zero to HER-2 low (odds ratio [OR] 3.436; *P* < 0.0001; **Table 2**).

In contrast to the HER-2 zero group, univariable analysis in patients with HER-2 low before NAC showed that HR before NAC, Ki-67 > 20 before NAC, and those who received taxane and platinum neoadjuvant therapy were more likely to have HER-2 transition (low to zero). However, multivariable logistic regression analyses showed that none of the variables were significantly association with HER-2 changes (low to zero), including HR status (OR 0.755; *P*=0.409; Supplementary Table 2).

Survival outcomes and prognostic factors

At the data cutoff of September 19, 2023, the median follow-up time was 50.0 months (interquartile range, 26.0-76.0 months). A total of 172 DFS and 72 OS events were observed. According to HER-2 status before NAC, the 4-year DFS rates for patients with HER-2 zero and HER-2 low were 67.0% and 69.5%, respectively (Log-rank *P*=0.997). The 4-year OS rates were 79.4% and 88.6%, respectively (Log-rank *P*=0.135) (Supplementary Figure 2).

Univariate and multivariate analyses of DFS and OS were performed separately for patients with HER-2 zero and HER-2 low status before

Table 2. Bivariable logistic regression analysis of HER-2 status after NAC (HER-2 low versus HER-2 zero) among the patients with HER-2 zero before NAC

Characteristic	Univariable			Multivariable		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age at diagnosis						
≤ 35	1					
36-49	0.732	0.334-1.605	0.436			
≥ 50	0.766	0.353-1.660	0.499			
Menstruation status						
Premenopausal						
Postmenopausal	0.965	0.537-1.734	0.906			
Clinical T stage						
0-2	1		0.733			
3-4	0.901	0.496-1.639				
Clinical N stage						
0	1					
1	1.255	0.602-2.619	0.544			
2	0.870	0.392-1.927	0.731			
3	0.870	0.367-2.059	0.751			
HR before NAC						
Negative	1			1		
Positive	3.688	2.048-6.642	< 0.0001	3.436	1.746-6.762	< 0.0001
Ki-67 before NAC						
≤ 20	1			1		
> 20	0.477	0.237-0.958	0.037	0.882	0.405-1.922	0.752
Neoadjuvant therapy						
Anthracycline and taxane	1			1		
Taxane and platinum	0.336	0.181-0.623	0.001	0.498	0.248-1.000	0.050
Others	0.356	0.132-0.958	0.041	0.381	0.135-1.080	0.070

NAC. In the HER-2 zero group, the pathological N stage was an independent prognostic factor for DFS and OS. Furthermore, HER-2 zero to low was significantly associated with better DFS (hazard ratio [HR] 0.49; *P*=0.01; **Table 3**) after adjustment.

In the HER-2 low group, pathological N stage and hormone receptor status were independent prognostic factors for DFS and OS. Furthermore, HER-2 changes (low to zero) did not have a prognostic effect on survival outcomes after adjustment (Supplementary Table 3).

Prognostic significance of HER-2 changes

To evaluate the potential prognostic impact of HER-2 changes from primary to residual disease, we compared survival outcomes between groups according to HER-2 status pre- and post-NAC, as well as hormone receptor status. In the HER-2 zero before NAC cohort, the 4-year DFS rate of patients with HER-2 changes (zero to low) was significantly better than that of patients with HER-2 constant zero (80.1% vs 55.7%, Log-rank *P*=0.033). A similar result was observed for OS outcomes; the 4-year OS rates were 85.5% and 74.2%, respectively (Log-rank *P*=0.048). We conducted further analyses stratified by hormone receptor status before NAC. In the HR- subgroup, there were no significant differences in DFS and OS between the two groups. However, in the HR+ subgroup, patients with HER-2 low in residual disease had significantly better DFS than those with constant HER-2 zero (Log-rank *P*=0.037; **Figure 2**).

In the HER-2 low before the NAC cohort, there were no significant differences in survival outcomes between patients with HER-2 changes

Table 3. Univariable and multivariable analyses of patients with HER-2 zero before NAC

Characteristic	Disease-free survival						Overall survival					
	Univariable			Multivariable			Univariable			Multivariable		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age at diagnosis												
≤ 35	1		0.826				1		0.496			
36-49	0.924	0.452-1.888	0.828				1.093	0.373-3.2	0.871			
≥ 50	1.104	0.560-2.173	0.776				1.618	0.593-4.418	0.348			
Menstruation status												
Premenopausal	1						1					
Postmenopausal	1.228	0.732-2.062	0.437				1.325	0.643-2.73	0.445			
Clinical T stage												
0-2	1			1			1					
3-4	1.738	1.045-2.890	0.033	1.155	0.650-2.051	0.624	1.893	0.935-3.830	0.076			
Clinical N stage												
Negative	1			1			1					
Positive	2.447	1.163-5.149	0.018	1.646	0.715-3.793	0.242	1.585	0.650-3.866	0.311			
Ki-67 before NAC												
≤ 20							1					
> 20	1.442	0.745-2.792	0.278				1.587	0.601-4.195	0.352			
NAC regimen												
Anthracycline and taxane	1		0.685				1		0.168			
Taxane and platinum	0.876	0.51-1.504	0.631				1.273	0.581-2.791	0.547			
Others	1.271	0.561-2.88	0.566				2.537	0.964-6.678	0.059			
Miller-Payne grade												
1-2	1		0.708				1		0.382			
3	0.94	0.55-1.606	0.822				1.008	0.472-2.153	0.984			
4	0.565	0.216-1.478	0.245				0.501	0.112-2.24	0.366			
5	1.015	0.239-4.305	0.983				2.828	0.627-12.759	0.176			
Pathologic T stage												
ypT0	1		0.04			0.308	1		0.09	1		0.091
ypT1	0.573	0.134-2.459	0.454	0.948	0.214-4.207	0.944	0.208	0.045-0.957	0.044	1.127	0.131-9.675	0.913
ypT2	1.062	0.253-4.461	0.935	1.326	0.313-5.619	0.702	0.401	0.091-1.77	0.228	2.058	0.25-16.953	0.503
ypT3-4	1.707	0.362-8.058	0.499	2.243	0.458-10.986	0.319	0.631	0.115-3.467	0.596	5.166	0.509-52.464	0.165

Pathologic N stage												
ypN0	1		0.002			0.029	1		0.126	1		0.003
ypN1	1.921	1.015-3.638	0.045	1.906	0.929-3.907	0.078	1.046	0.405-2.702	0.926	1.633	0.606-4.4	0.332
ypN2	0.869	0.294-2.569	0.799	0.690	0.224-2.127	0.519	1.027	0.286-3.685	0.968	2.28	0.611-8.509	0.22
ypN3	3.257	1.701-6.237	< 0.001	2.521	1.173-5.418	0.018	2.555	1.083-6.024	0.032	6.91	2.447-19.517	< 0.0001
HR after NAC												
Negative	1						1			1		
Positive	0.696	0.414-1.17	0.172				0.377	0.169-0.844	0.018	0.561	0.201-1.572	0.272
Ki-67 after NAC												
≤ 20	1						1			1		
> 20	1.489	0.873-2.54	0.144				5.271	1.832-15.162	0.002	7.973	2.268-28.028	0.001
HER-2 status												
Constant zero	1						1			1		
Zero to low	0.57	0.337-0.964	0.036	0.490	0.285-0.843	0.010	0.477	0.224-1.014	0.054	0.51	0.219-1.184	0.117

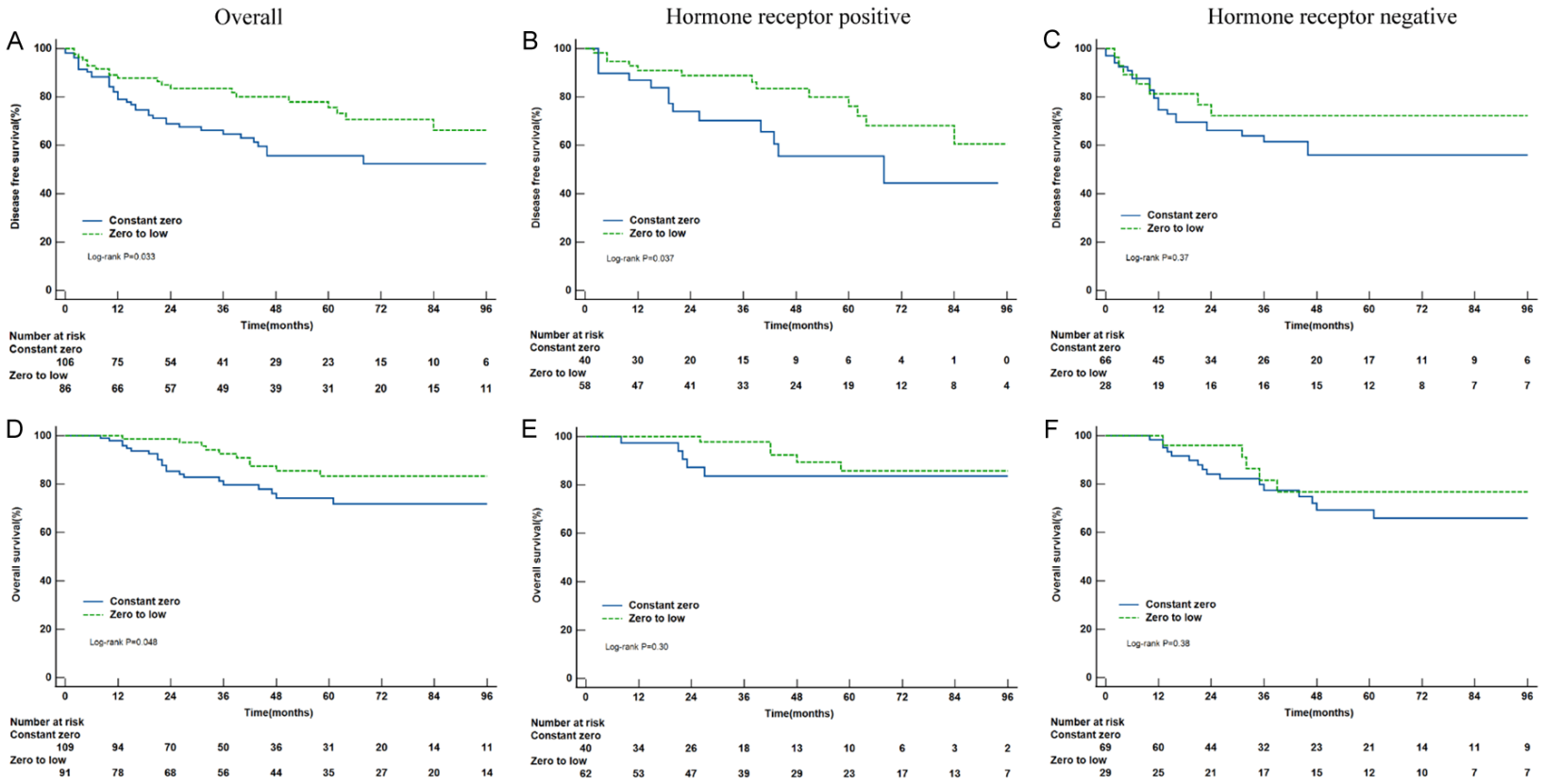

Figure 2. Kaplan-Meier curve showing disease-free survival (DFS) and overall survival (OS) of patients with HER-2 zero before neoadjuvant chemotherapy (NAC) according to HER-2 changes. A. DFS of overall patients with HER-2 constant zero and HER-2 zero to low; B. DFS of hormone receptor positive patients with HER-2 constant zero and HER-2 zero to low; C. DFS of hormone receptor negative patients with HER-2 constant zero and HER-2 zero to low; D. OS of overall patients with HER-2 constant zero and HER-2 zero to low; E. OS of hormone receptor positive patients with HER-2 constant zero and HER-2 zero to low; F. OS of hormone receptor negative patients with HER-2 constant zero and HER-2 zero to low.

(low to zero) and constant HER-2 low status. The 4-year estimated DFS rates were 64.6% and 71.8%, respectively, and the 4-year OS rates were 87.5% and 89.3%. However, when stratified according to hormone receptor status, no differences were observed in terms of OS and DFS in the HR+ and HR- subgroups (**Figure 3**).

Discussion

Our study confirmed that HER-2 status is unstable and prone to change in primary patients who are HER-2 negative after NAC. Moreover, the alteration of HER-2 from zero to low exhibited a favorable impact on prognosis among patients with primary HER-2 zero, especially the HR+ subgroup.

The status of HER-2 may undergo alterations before and after NAC, as reported in previous studies. In a study conducted by Kang et al., the discordance rate of HER-2 status among patients who are HER-2 negative who received NAC was 36.5% [17]. Our study demonstrated an overall HER-2 change rate of 32.4%, which aligns closely with their results. Miglietta et al. observed the conversion of HER-2 from primary to residual disease after NAC. However, the study included patients who were both HER-2 positive and HER-2 negative, with a prevalence of 8.9% for HER-2 zero to low and 14.8% for HER-2 low to zero [18]. This finding underscores the necessity of retesting the HER-2 status of residual disease after NAC, as it can significantly impact treatment decisions. For instance, anti-HER-2 targeted therapy can only be selected if the HER-2 status is converted to positive.

However, the specific mechanisms underlying this change remains unclear. This may be due to the temporal heterogeneity of HER-2 expression, the influence of treatment, or inconsistencies in the pathologists' interpretations. Most experts agree with the heterogeneity hypothesis in explaining changes in HER-2 expression [22]. Previous research has reported that higher estrogen receptor expression is associated with increased rates of HER-2 low disease [23, 24]. Our results demonstrate a correlation between the conversion of HER-2 from zero to low and HR+. In another study, patients with HR+ showed a higher probability of HER-2 transition (zero to low) than those with HR- [17].

An increasing number of studies have indicated that there is no prognostic or biological difference between HER-2 low and HER-2 zero breast cancers, and HER-2 low should not be considered an independent subtype [23, 25]. The results of our study demonstrated that the alteration of HER-2 from zero to low confers a favorable prognosis among patients with primary HER-2 zero. To date, only one study independently reported the prognostic significance of HER-2 changes after NAC in primary HER-2 negative breast cancer. They reported that HER-2 low to HER-2 zero was associated with better OS and DFS compared to those with constant HER-2 zero regardless of the estrogen receptor [17]. Two additional studies included patients with HER-2 negative and HER-2 positive who underwent NAC. Miglietta et al. reported no DFS difference between the HER-2 concordant group and HER-2 changed group (concordant HER-2 zero vs HER-2 zero to low, P=0.77; concordant HER-2 low vs HER-2 low to zero, P=0.23) [18]. Zhu et al. found that among patients who are ER-positive, patients who are HER-2 zero to low had the lowest RFI, whereas constant patients who are HER-2 low had the highest RFI [26]. The different conclusions drawn from these studies may be due to the diverse populations and analytical perspectives. Moreover, retrospective analysis of HER-2 status has unavoidable biases and limitations making it difficult to reach a definitive conclusion.

Our results showed that the expression of HER-2 from zero to low had a better prognosis than those patients with a constant HER-2 of zero. Subgroup analysis revealed significant differences in the hormone receptor-positive subgroups. As indicated in previous reports, among hormone receptor-positive patients, patients with HER-2 low had better survival than patients with HER-2 zero [6, 27]. This finding can be interpreted in terms of molecular mechanisms. Higher expression of luminal-related genes was found in patients who are HER-2 low, which may improve their response to endocrine therapy [28]. Other researchers have also demonstrated that HER-2 low tumors show less alteration in 17q peaks than HER-2 zero tumors among hormone receptor-positive patients, which may improve prognosis [22]. While our study provides original insights, it also underscores the need for further investigation to elu-

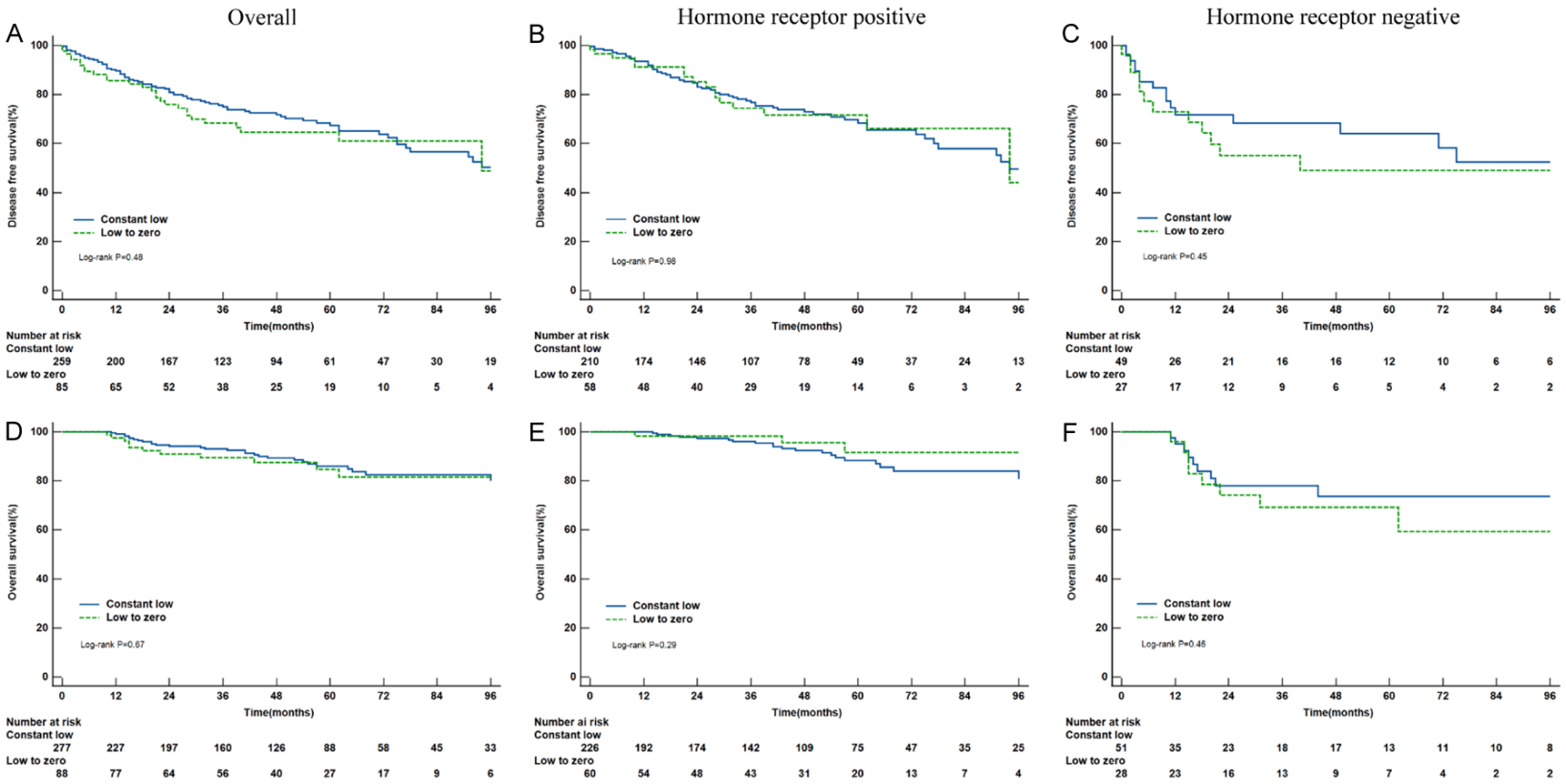

Figure 3. Kaplan-Meier curve showing disease-free survival (DFS) and overall survival (OS) of patients with HER-2 low before neoadjuvant chemotherapy (NAC) according to HER-2 changes. A. DFS of overall patients with HER-2 constant low and HER-2 low to zero; B. DFS of hormone receptor positive patients with HER-2 constant low and HER-2 low to zero; C. DFS of hormone receptor negative patients with HER-2 constant low and HER-2 low to zero; D. OS of overall patients with HER-2 constant low and HER-2 low to zero; E. OS of hormone receptor positive patients with HER-2 constant low and HER-2 low to zero; F. OS of hormone receptor negative patients with HER-2 constant low and HER-2 low to zero.

cidate the prognostic significance of HER-2 changes.

Patients with residual disease after NAC usually have a poor prognosis [29]. In contrast, adjuvant therapy for these patients can reduce the risk of recurrence and improve prognosis, such as adjuvant capecitabine for triple-negative breast cancer (TNBC) patients [30], adjuvant olaparib for patients with BRCA mutations [31], and trastuzumab emtansine for patients who are HER-2 positive [32]. Ongoing studies have explored the efficacy of ADC, such as datopotamab deruxtecan and sacituzumab govitecan, as adjuvant therapy for patients with non-pathological complete response TNBC after NAC. Currently, HER-2 low has emerged as a novel target, and anti-HER-2 ADC has not been approved for HER-2 low early breast cancer. Therefore, further clinical trials are warranted to explore the potential application of anti-HER-2 ADC in patients who are HER-2 low with residual disease after NAC. Our findings serve as a crucial point of reference for patients with constant HER-2 low and those exhibiting HER-2 low conversion, suggesting that both groups can be candidates and are likely to derive equal benefits from anti-HER-2 ADC therapy.

This study's findings should be considered in light of these limitations. First, this was a retrospective study with unavoidable diagnoses and selection errors, and the comparison of survival endpoints may have been statistically underpowered. Second, there are subjective discrepancies in the current interpretation of HER-2, and the central laboratory did not review HER-2 status. Third, we should be aware of the heterogeneity of core needle biopsy, as it cannot provide a comprehensive representation of breast cancer HER-2 status. However, our research remains innovative in the investigation of changes in HER-2 expression within a neoadjuvant cohort of patients with early breast cancer, which provides valuable insights into the potential application of anti-HER-2 ADC in the adjuvant setting among patients with residual disease after NAC.

Conclusions

In conclusion, our study revealed the instability of HER-2 expression from primary breast cancer to residual disease. This finding underscores the necessity of retesting HER-2 status in residual disease for the potential application of anti-HER-2 ADC in the adjuvant setting among patients with residual disease after NAC. Furthermore, the expression of HER-2 from zero to low had a positive prognostic effect on survival in patients with primary HER-2 zero which needs to be further verified in a large cohort.

Acknowledgements

The authors wish to thank all the study participants and research staff who participated in this work. This work was supported by the Beijing Hope Run Special Fund of Cancer Foundation of China (LC2019L06).

Informed consent has been obtained from the study participants prior to study commencement.

Disclosure of conflict of interest

None.

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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 2021; 71: 209-249.

[2] Waks AG and Winer EP. Breast cancer treatment: a review. JAMA 2019; 321: 288-300.

[3] Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, Jenkins RB, Press MF, Spears PA, Vance GH, Viale G, McShane LM and Dowsett M. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. J Clin Oncol 2018; 36: 2105-2122.

[4] Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, Tsurutani J, Ueno NT, Prat A, Chae YS, Lee KS, Niikura N, Park YH, Xu B, Wang X,

Gil-Gil M, Li W, Pierga JY, Im SA, Moore HCF, Rugo HS, Yerushalmi R, Zagouri F, Gombos A, Kim SB, Liu Q, Luo T, Saura C, Schmid P, Sun T, Gambhire D, Yung L, Wang Y, Singh J, Vitazka P, Meinhardt G, Harbeck N and Cameron DA; DESTINY-Breast04 Trial Investigators. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med 2022; 387: 9-20.

[5] Tarantino P, Hamilton E, Tolaney SM, Cortes J, Morganti S, Ferraro E, Marra A, Viale G, Trapani D, Cardoso F, Penault-Llorca F, Viale G, Andrè F and Curigliano G. HER2-low breast cancer: pathological and clinical landscape. J Clin Oncol 2020; 38: 1951-1962.

[6] Li Y, Abudureheiyimu N, Mo H, Guan X, Lin S, Wang Z, Chen Y, Chen S, Li Q, Cai R, Wang J, Luo Y, Fan Y, Yuan P, Zhang P, Li Q, Ma F and Xu B. In real life, low-level HER2 expression may be associated with better outcome in HER2-negative breast cancer: a study of the national cancer center, China. Front Oncol 2022; 11: 774577.

[7] Cai M, Li M, Lv H, Zhou S, Xu X, Shui R and Yang W. HER2-low breast cancer: evolution of HER2 expression from primary tumor to distant metastases. BMC Cancer 2023; 23: 656.

[8] Anderson S, Bartow BB, Siegal GP, Huang X and Wei S. The dynamics of HER2-low expression during breast cancer progression. Breast Cancer Res Treat 2023; 201: 437-446.

[9] Tarantino P, Gandini S, Nicolò E, Trillo P, Giugliano F, Zagami P, Vivanet G, Bellerba F, Trapani D, Marra A, Esposito A, Criscitiello C, Viale G and Curigliano G. Evolution of low HER2 expression between early and advanced-stage breast cancer. Eur J Cancer 2022; 163: 35-43.

[10] Miglietta F, Griguolo G, Bottosso M, Giarratano T, Lo Mele M, Fassan M, Cacciatore M, Genovesi E, De Bartolo D, Vernaci G, Amato O, Conte P, Guarneri V and Dieci MV. Evolution of HER2-low expression from primary to recurrent breast cancer. NPJ Breast Cancer 2021; 7: 137.

[11] Bo J, Yu B, Bi R, Xu X, Cheng Y, Tu X, Bai Q, Yang W and Shui R. Conversion of ER and HER2 status after neoadjuvant therapy in Chinese breast cancer patients. Clin Breast Cancer 2023; 23: 436-446.

[12] Parinyanitikul N, Lei X, Chavez-MacGregor M, Liu S, Mittendorf EA, Litton JK, Woodward W, Zhang AH, Hortobagyi GN, Valero V, Meric-Bernstam F and Gonzalez-Angulo AM. Receptor status change from primary to residual breast cancer after neoadjuvant chemotherapy and analysis of survival outcomes. Clin Breast Cancer 2015; 15: 153-160.

[13] Niikura N, Tomotaki A, Miyata H, Iwamoto T, Kawai M, Anan K, Hayashi N, Aogi K, Ishida T, Masuoka H, Iijima K, Masuda S, Tsugawa K, Kinoshita T, Nakamura S and Tokuda Y. Changes in tumor expression of HER2 and hormone receptors status after neoadjuvant chemotherapy in 21,755 patients from the Japanese breast cancer registry. Ann Oncol 2016; 27: 480-487.

[14] Yoshida A, Hayashi N, Suzuki K, Takimoto M, Nakamura S and Yamauchi H. Change in HER2 status after neoadjuvant chemotherapy and the prognostic impact in patients with primary breast cancer. J Surg Oncol 2017; 116: 1021-1028.

[15] Li C, Fan H, Xiang Q, Xu L, Zhang Z, Liu Q, Zhang T, Ling J, Zhou Y, Zhao X and Cui Y. Prognostic value of receptor status conversion following neoadjuvant chemotherapy in breast cancer patients: a systematic review and meta-analysis. Breast Cancer Res Treat 2019; 178: 497-504.

[16] Ma Y, Zhu M, Lv M, Yuan P, Chen X and Liu Z. Impact of residual disease biomarkers on the prognosis of HER2-positive breast cancer following neoadjuvant therapy. Cancer Med 2023; 12: 11293-11304.

[17] Kang S, Lee SH, Lee HJ, Jeong H, Jeong JH, Kim JE, Ahn JH, Jung KH, Gong G, Kim HH, Lee S, Lee J and Kim SB. Prognostic implications of HER2 changes after neoadjuvant chemotherapy in patients with HER2-zero and HER2-low breast cancer. Eur J Cancer 2023; 191: 112956.

[18] Miglietta F, Griguolo G, Bottosso M, Giarratano T, Lo Mele M, Fassan M, Cacciatore M, Genovesi E, De Bartolo D, Vernaci G, Amato O, Porra F, Conte P, Guarneri V and Dieci MV. HER2-low-positive breast cancer: evolution from primary tumor to residual disease after neoadjuvant treatment. NPJ Breast Cancer 2022; 8: 66.

[19] Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, Perou CM, Regan MM, Rimm DL, Symmans WF, Torlakovic EE, Varella L, Viale G, Weisberg TF, McShane LM and Wolff AC. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. J Clin Oncol 2020; 38: 1346-1366.

[20] Tarantino P, Viale G, Press MF, Hu X, Penault-Llorca F, Bardia A, Batistatou A, Burstein HJ, Carey LA, Cortes J, Denkert C, Diéras V, Jacot W, Koutras AK, Lebeau A, Loibl S, Modi S, Mosele MF, Provenzano E, Pruneri G, Reis-Filho JS, Rojo F, Salgado R, Schmid P, Schnitt SJ, Tolaney SM, Trapani D, Vincent-Salomon A, Wolff AC, Pentheroudakis G, André F and Curigliano G. ESMO expert consensus statements (ECS) on the definition, diagnosis, and management of HER2-low breast cancer. Ann Oncol 2023; 34: 645-659.

[21] Litton JK, Regan MM, Pusztai L, Rugo HS, Tolaney SM, Garrett-Mayer E, Amiri-Kordestani L, Basho RK, Best AF, Boileau JF, Denkert C, Foster JC, Harbeck N, Jacene HA, King TA, Mason G, O'Sullivan CC, Prowell TM, Richardson AL, Sepulveda KA, Smith ML, Tjoe JA, Turashvili G, Woodward WA, Butler LP, Schwartz EI and Korde LA. Standardized definitions for efficacy end points in neoadjuvant breast cancer clinical trials: NeoSTEEP. J Clin Oncol 2023; 41: 4433-4442.

[22] Dai LJ, Ma D, Xu YZ, Li M, Li YW, Xiao Y, Jin X, Wu SY, Zhao YX, Wang H, Yang WT, Jiang YZ and Shao ZM. Molecular features and clinical implications of the heterogeneity in Chinese patients with HER2-low breast cancer. Nat Commun 2023; 14: 5112.

[23] Tarantino P, Jin Q, Tayob N, Jeselsohn RM, Schnitt SJ, Vincuilla J, Parker T, Tyekucheva S, Li T, Lin NU, Hughes ME, Weiss AC, King TA, Mittendorf EA, Curigliano G and Tolaney SM. Prognostic and biologic significance of ERBB2-low expression in early-stage breast cancer. JAMA Oncol 2022; 8: 1177-1183.

[24] Peiffer DS, Zhao F, Chen N, Hahn OM, Nanda R, Olopade OI, Huo D and Howard FM. Clinicopathologic characteristics and prognosis of ERBB2-low breast cancer among patients in the national cancer database. JAMA Oncol 2023; 9: 500-510.

[25] Jin J, Li B, Cao J, Li T, Zhang J, Cao J, Zhao M, Wang L, Wang B, Tao Z and Hu X. Analysis of clinical features, genomic landscapes and survival outcomes in HER2-low breast cancer. J Transl Med 2023; 21: 360.

[26] Zhu S, Lu Y, Fei X, Shen K and Chen X. Pathological complete response, category change, and prognostic significance of HER2-low breast cancer receiving neoadjuvant treatment: a multicenter analysis of 2489 cases. Br J Cancer 2023; 129: 1274-1283.

[27] Almstedt K, Heimes AS, Kappenberg F, Battista MJ, Lehr HA, Krajnak S, Lebrecht A, Gehrmann M, Stewen K, Brenner W, Weikel W, Rahnenführer J, Hengstler JG, Hasenburg A and Schmidt M. Long-term prognostic significance of HER2-low and HER2-zero in node-negative breast cancer. Eur J Cancer 2022; 173: 10-19.

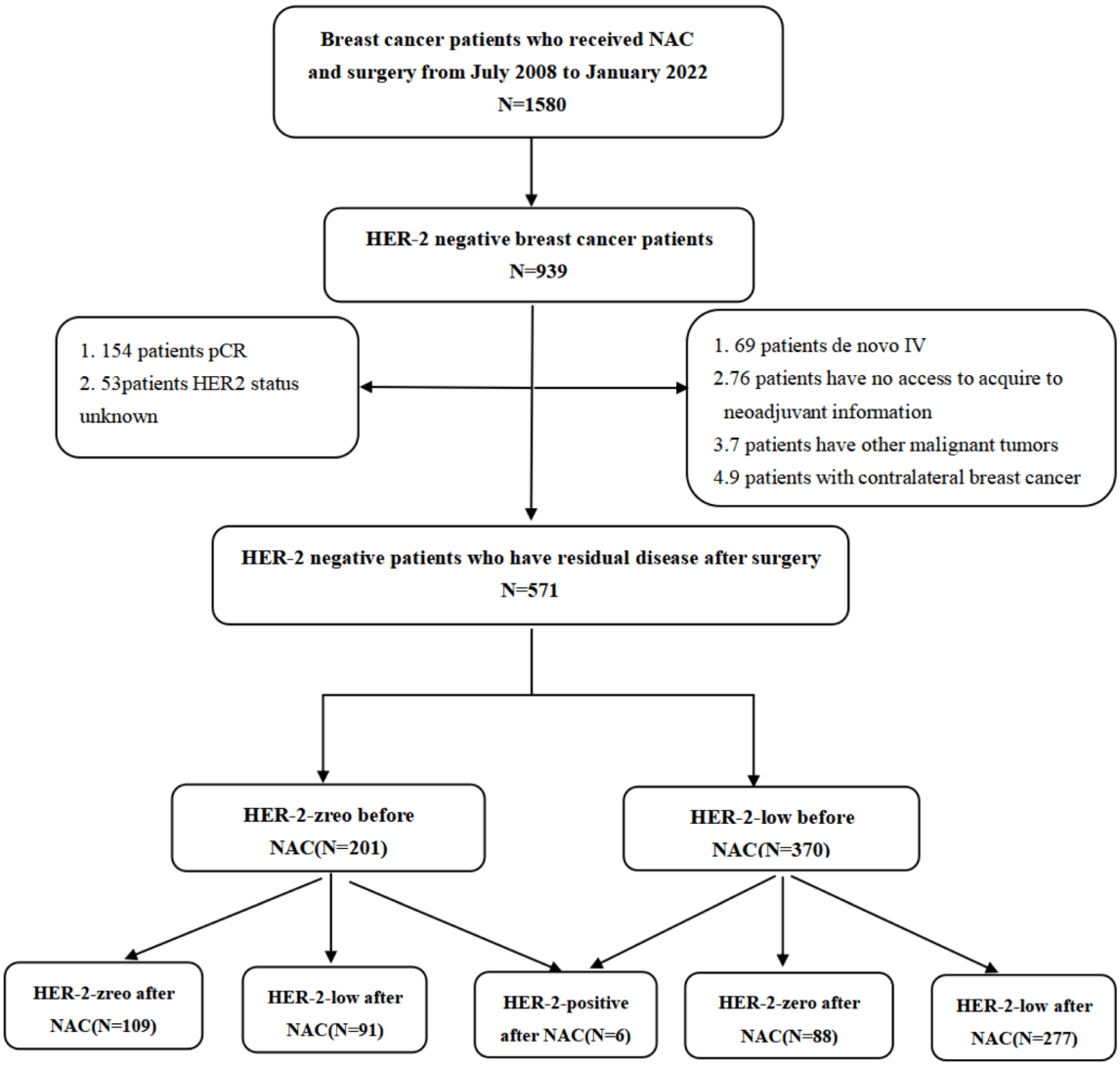
[28] Schettini F, Chic N, Brasó-Maristany F, Paré L, Pascual T, Conte B, Martínez-Sáez O, Adamo B, Vidal M, Barnadas E, Fernández-Martinez A, González-Farre B, Sanfeliu E, Cejalvo JM, Perrone G, Sabarese G, Zalfa F, Peg V, Fasani R, Villagrasa P, Gavilá J, Barrios CH, Lluch A, Martín M, Locci M, De Placido S and Prat A. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. NPJ Breast Cancer 2021; 7: 1.

[29] Von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, Gerber B, Eiermann W, Hilfrich J, Huober J, Jackisch C, Kaufmann M, Konecny GE, Denkert C, Nekljudova V, Mehta K and Loibl S. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012; 30: 1796-1804.

[30] Masuda N, Lee SJ, Ohtani S, Im YH, Lee ES, Yokota I, Kuroi K, Im SA, Park BW, Kim SB, Yanagita Y, Ohno S, Takao S, Aogi K, Iwata H, Jeong J, Kim A, Park KH, Sasano H, Ohashi Y and Toi M. Adjuvant capecitabine for breast cancer after preoperative chemotherapy. N Engl J Med 2017; 376: 2147-2159.

[31] Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, Gelber RD, De Azambuja E, Fielding A, Balmaña J, Domchek SM, Gelmon KA, Hollingsworth SJ, Korde LA, Linderholm B, Bandos H, Senkus E, Suga JM, Shao Z, Pippas AW, Nowecki Z, Huzarski T, Ganz PA, Lucas PC, Baker N, Loibl S, McConnell R, Piccart M, Schmutzler R, Steger GG, Costantino JP, Arahmani A, Wolmark N, McFadden E, Karantza V, Lakhani SR, Yothers G, Campbell C and Geyer CE Jr; OlympiA Clinical Trial Steering Committee and Investigators. Adjuvant olaparib for patients with *BRCA1*- or *BRCA2*-mutated breast cancer. N Engl J Med 2021; 384: 2394-2405.

[32] Von Minckwitz G, Huang CS, Mano MS, Loibl S, Mamounas EP, Untch M, Wolmark N, Rastogi P, Schneeweiss A, Redondo A, Fischer HH, Jacot W, Conlin AK, Arce-Salinas C, Wapnir IL, Jackisch C, DiGiovanna MP, Fasching PA, Crown JP, Wülfing P, Shao Z, Rota Caremoli E, Wu H, Lam LH, Tesarowski D, Smitt M, Douthwaite H, Singel SM and Geyer CE Jr; KATHERINE Investigators. Trastuzumab emtansine for residual invasive HER2-positive breast cancer. N Engl J Med 2019; 380: 617-628.



Supplementary Figure 1. Flowchart of patient selection.

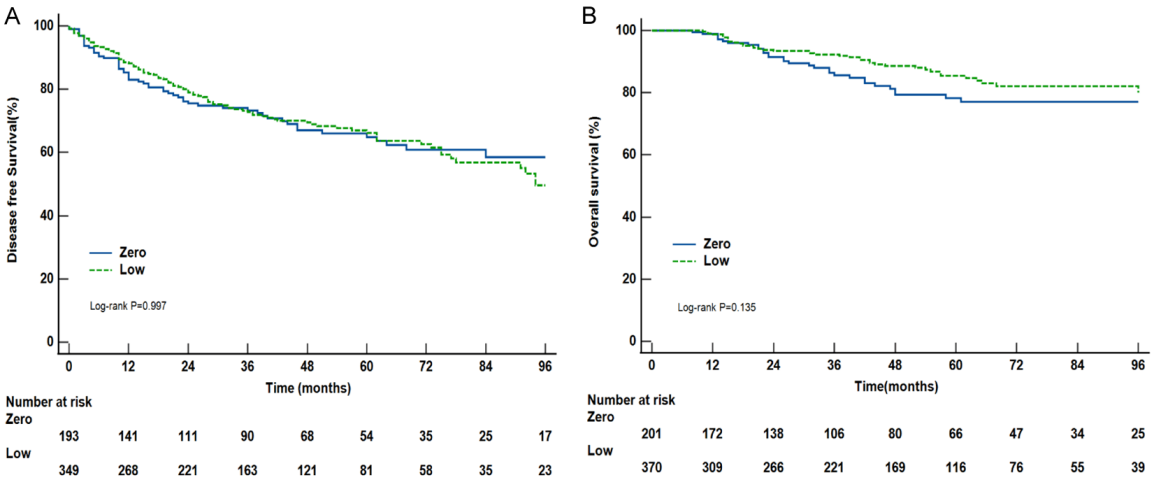
Supplementary Table 1. Baseline characteristics of patients with HER-2 positive tumors after NAC

Characteristic	N=6
Menstruation status	
Premenopausal	4
Postmenopausal	2
Clinical T stage	
2	3
3	2
4	1
Clinical N stage	
0	1
1	1
2	3
3	1

Clinical stage	
IIA	1
IIB	1
IIIA	2
IIIB	1
IIIC	1
HER-2 status before NAC	
Zero	1
Low	5
HR before NAC	
Negative	0
Positive	6
Ki-67 before NAC	
≤ 20	3
> 20	3
Missing	
Breast surgery	
Mastectomy	3
Breast-conserving	3
Axillary node surgery	
ALND	5
SLNB	1
Miller-Payne grade	
2	2
3	4
Pathologic T stage	
ypT1	2
ypT2	4
Pathologic N stage	
ypN0	1
ypN1	2
ypN2	1
ypN3	2
ypTNM stage	
I	1
II	2
III	3
HR after NAC	
Negative	0
Positive	6
HER-2 after NAC	
2+/FISH+	5
3+	1
Ki-67 after NAC	
≤ 20	6
> 20	0
Missing	
Neoadjuvant therapy	
Anthracycline and taxane	5
Endocrine therapy	1

Supplementary Table 2. Bivariable logistic regression analysis of HER-2 status after NAC (HER-2 zero versus HER-2 low) among the patients with HER-2 low before NAC

Characteristic	Univariable			Multivariable		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age at diagnosis						
≤ 35	1					
36-49	0.962	0.483-1.913	0.911			
≥ 50	0.686	0.345-1.361	0.281			
Menstruation status						
Premenopausal	1					
Postmenopausal	0.781	0.476-1.283	0.329			
Clinical T stage						
0-2	1					
3-4	1.069	0.657-1.739	0.789			
Clinical N stage						
0	1					
1	0.649	0.296-1.423	0.280			
2	0.933	0.460-1.893	0.848			
3	0.847	0.389-1.843	0.675			
HR before NAC						
Negative	1			1		
Positive	0.484	0.281-0.831	0.009	0.755	0.388-1.471	0.409
Ki-67 before NAC						
≤ 20	1			1		
> 20	1.886	1.057-3.363	0.032	1.698	0.939-3.068	0.080
Neoadjuvant therapy						
Anthracycline and taxane	1			1		
Taxane and platinum	2.005	1.130-3.558	0.017	1.471	0.733-2.953	0.278
Others	1.219	0.579-2.570	0.602	1.243	0.585-2.638	0.571



Supplementary Figure 2. Kaplan-Meier curve showing disease-free survival (DFS) and overall survival (OS) of overall patients according to HER-2 status before NAC. A. DFS of patients with HER-2 zero and HER-2 low. B. OS of patients with HER-2 zero and HER-2 low.

Supplementary Table 3. Univariable and multivariable analyses of patients with HER-2 low before NAC

Characteristic	Disease-free survival						Overall survival					
	Univariable			Multivariable			Univariable			Multivariable		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age at diagnosis												
≤ 35	1		0.957				1		0.857			
36-49	1.08	0.617-1.891	0.788				0.839	0.359-1.962	0.686			
≥ 50	1.032	0.594-1.793	0.011				0.789	0.340-1.829	0.580			
Menstruation status												
Premenopausal	1						1					
Postmenopausal	0.878	0.598-1.289	0.507				0.512	0.256-1.022	0.058			
Clinical T stage												
0-2	1		< 0.0001	1		0.063	1		0.061			
3-4	2.005	1.375-2.923		1.502	0.977-2.308		1.803	0.973-3.340				
Clinical N stage												
Negative	1		0.069				1		0.073			
Positive	1.746	0.958-3.184					3.677	0.887-15.241				
Ki-67 before NAC												
≤ 20	1						1					
> 20	1.283	0.841-1.957	0.248				1.794	0.814-3.957	0.147			
Neoadjuvant therapy												
Anthracycline and taxane	1		0.411				1		0.025			
Taxane and platinum	1.295	0.805-2.081	0.286				2.569	1.299-5.079	0.007			
Others	1.322	0.769-2.276	0.313				1.325	0.503-3.485	0.569			
Miller-Payne grade												
1-2	1		0.438				1		0.512			
3	0.849	0.563-1.28	0.435				0.808	0.399-1.636	0.554			
4	0.809	0.427-1.531	0.515				0.976	0.357-2.665	0.962			
5	2.461	0.593-10.205	0.215				3.773	0.493-28.901	0.201			
Pathologic T stage												
ypT0	1		0.004	1		0.188	1		0.001	1		0.003
ypT1	0.348	0.083-1.455	0.148	0.613	0.139-2.701	0.518	0.224	0.029-1.729	0.151	0.723	0.087-6.002	0.764
ypT2	0.455	0.11-1.888	0.278	0.640	0.146-2.815	0.555	0.207	0.027-1.602	0.131	0.513	0.061-4.286	0.538
ypT3-4	0.917	0.213-3.95	0.907	1.117	0.236-5.290	0.889	0.797	0.102-6.218	0.829	2.618	0.292-23.491	0.390

Pathologic N stage												
ypN0	1		< 0.0001	1		< 0.0001	1		0.034	1		0.001
ypN1	1.738	0.873-3.46	0.116	1.964	0.948-4.070	0.069	2.296	0.621-8.48	0.213	4.225	0.894-19.961	0.069
ypN2	2.807	1.395-5.649	0.004	3.115	1.469-6.604	0.003	3.11	0.824-11.742	0.094	5.774	1.136-29.358	0.035
ypN3	3.53	1.873-6.655	< 0.0001	4.202	2.101-8.402	< 0.001	4.888	1.457-16.397	0.01	13.137	2.921-59.084	0.001
HR after NAC												
Negative	1			1	1					1		
Positive	0.552	0.371-0.823	0.003	0.403	0.248-0.656	< 0.0001	0.231	0.125-0.428	< 0.0001	0.096	0.041-0.224	< 0.0001
Ki-67 after NAC												
≤ 20	1			1			1			1		
> 20	1.485	1.015-2.172	0.042	1.426	0.921-2.210	0.112	1.902	1.015-3.567	0.045	0.963	0.446-2.077	0.923
HER-2 status												
Constant zero	1			1			1			1		
Zero to low	1.166	0.757-1.796	0.486	1.067	0.678-1.679	0.778	1.165	0.582-2.333	0.667	1.008	0.487-2.086	0.982