Clinicopathologic Features and Prognosis of Female Early Breast Cancer With HER2 Low Expression: A Propensity Score Matched Analysis

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ABSTRACT

BACKGROUND: Metastatic breast cancer (MBC) patients with low expression of human epidermal growth factor 2 (HER2) have been proven to benefit from HER2 targeted therapy. We aimed to determine how HER2-low status affected survival and metastatic risk as well as how it affected pathological complete response (pCR) in neoadjuvant chemotherapy (NAC) patients.

METHODS: According to the results of immunohistochemistry (IHC) and in situ hybridization (ISH) testing, 321 female patients were sorted into HER2-low (IHC 1+/2+ with ISH negative) and HER2-zero (IHC 0) groups using propensity score matching (PSM). Overall survival (OS), disease-free survival (DFS), and distant disease-free survival (DDFS) were compared for both groups, while pCR was only analyzed for NAC patients.

RESULTS: In total, 97 patients in each group after PSM were included. We discovered that pCR was not associated with HER2 expression status in 45 patients who underwent NAC. Five-year OS in the HER2-low group was significantly higher (98.99%) than in the HER2-zero group (95.87%, $P = .044$); however, this difference was not reflected in the 5-year DFS (90.61 vs 90.52%, $P = .868$) and 5-year DDFS (93.67 vs 91.53%, $P = .757$). Meanwhile, multivariate analysis revealed that HER2-low expression could indicate better OS ($P = .047$, hazard ratios [HRs] = 16.121, 95% confidence interval [CI] = 1.035-251.046), but it had no prognostic value for DFS or DDFS.

CONCLUSION: When compared with HER2-zero expression, HER2-low expression was not connected to pCR and could not modify metastasis risk in female patients with early-stage breast cancer (BC), but it may prolong patient survival.

KEYWORDS: HER2-low expression, breast cancer, prognosis, pathological complete response, antibody-drug conjugates

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Introduction

The *ERBB2* gene encodes the transmembrane tyrosine kinase receptor known as human epidermal growth factor 2 (HER2),[1] which primarily depends on dimerization (homologous/heterologous) with the other 3 family members to activate the downstream signaling pathways and control proliferation, invasion, migration, and survival.[1,2] Because HER2 overexpression is associated with invasive biological activity and a poor prognosis in breast cancer (BC) patients,[3] it also presents a novel treatment method, anti-HER2 therapy, which has benefited the prognosis of BC patients with overexpressed HER2 in recent decades.[4,5]

Breast cancer is classified based on their HER2 status, either as HER2-positive or as HER2-negative, which assists health care professionals in determining appropriate therapeutic interventions. The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) update 2018 showed that only HER2-amplified tumors could benefit from HER2-targeted therapy.[6] Some new HER2-targeted antibody-drug conjugates have been demonstrated in several studies to offer potential antitumor activity in HER2-low BC. Trastuzumab deruxtecan (T-DXd, DS-8201a) was assessed in advanced BC patients with HER2-low status, and results from the phase Ib research showed a significant 37% objective response rate (ORR) and 10.4 months of median duration of response (DOR).[7] It has a higher drug antibody ratio than T-DM1 (approximately 7 to 8 vs 3.5), along with a robust bystander effect, and its payload released after cleavage by lysosomal cathepsins can inhibit the surrounding tumor cells regardless of HER2 expression status.[2,8,9] Furthermore, in the DESTINY BREAST 03 phase III clinical trial, T-DXd outperformed T-DM1 in terms of survival benefit for patients with HER2-positive metastatic breast cancer (MBC).[10] Similarly, a phase I study of trastuzumab duocarmazine

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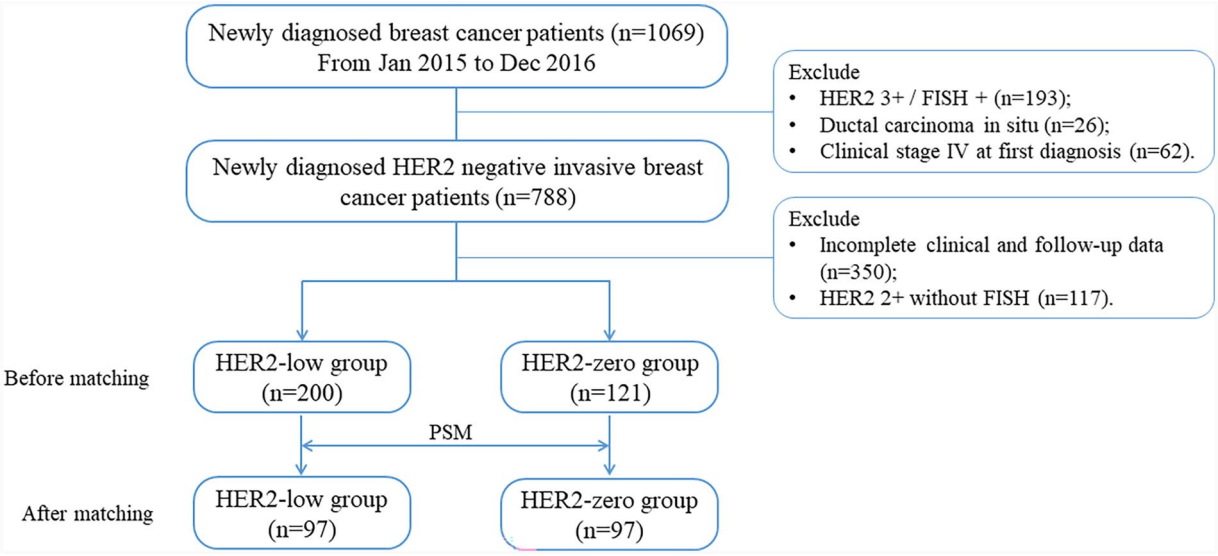

Figure 1. Patient selection flow chart. FISH indicates fluorescence in situ hybridization; HER2, human epidermal growth factor 2.

(SYD985) showed that in patients with advanced HER2-low BC, ORR was 28% and 40% in the hormone receptor (HR)-positive and HR-negative BC groups, respectively.[11] Relevant results from the DESTINY BREAST 04 phase III trial revealed that in patients with HER2-low MBC, independent of HR status, patients treated with T-DXd had significantly longer progression-free survival (PFS) and overall survival (OS) compared with the physician's choice of chemotherapy.[12] These trials demonstrate that, even in the absence of a therapeutic target, patients with HER2-low BC may benefit from HER2-targeted therapy, like SYD985 and T-DXd.

Approximately half of patients previously diagnosed with HER2-negative BC actually have HER2-low expression status,[13-15] and the clinical trials described above confirm the potential to improve the prognosis of patients with HER2-low expression. Currently, the main methods for detecting HER2 expression levels are immunohistochemistry (IHC) and in situ hybridization (ISH), and Supplementary Figure 1 shows microscopic photographs of different HER2 protein expression levels in invasive BC patients detected using IHC and ISH. Comparing with HER2 0, HER2-low (IHC 1+/2+ with a negative ISH) had function on patients' prognosis was a controversial topic. Some studies suggested HER2-low could give a clue to better prognosis[16-18] while others had not.[14,19-21] The aim of this study was to preliminary analyze the differences in pathological complete response (pCR), risk of metastasis, and survival prognosis between female patients with early-stage BC with HER2-low expression and HER2-zero expression in our center.

Patients and Methods

Patient eligibility

From January 2015 to December 2016, all newly diagnosed patients with HER2-negative BC at the Third Affiliated Hospital of Kunming Medical University & Yunnan Cancer Hospital were included in this single-center retrospective analysis. The last follow-up was in December, 2021. A total of 321 patients participated in this study based on HER2 status at baseline before any antitumor therapy. Patients were included if female sex, age ⩾ 18 years, pathologically confirmed HER2-negative BC, received adjuvant or neoadjuvant chemotherapy (NAC) for more than 4 cycles, and clinical stage I-III at diagnosis. The exclusion criteria were a history of synchronous or metachronous BC, insufficient clinicopathologic and follow-up data, as well as no R0 surgical resection previously. Figure 1 shows the flowchart of patient selection in this study.

Data collection and survival outcome assessment

The local Institutional Internal Ethics Review Board approved this study. Age at diagnosis, menopausal status, HR, Ki67, HER2 status, histological type, clinical stage at diagnosis, chemotherapy, surgery, radiotherapy, and endocrine therapy were all collected from patient medical records, pathological reports, and follow-up checks. The time from pathology diagnosis to death from any cause was defined as OS. The time from surgery to disease recurrence, metastasis, or death from any cause was defined as disease-free survival (DFS). The time from surgery to distant metastases or death from any cause was defined as distance disease-free survival (DDFS). The time of pathological diagnosis, surgery, recurrence, and metastasis were collected to calculate DFS, DDFS, and OS. Response Evaluation Criteria in Solid Tumors (RECIST1.1)[22] were used to assess the tumor response after NAC. No invasive cancer was found in the breast and axillary lymph nodes at the time of surgery (ypT0/is ypN0), which is the definition of pathological complete response (pCR).

The ASCO/CAP criteria were followed while determining HER2 status with standard antibodies and technologies. A

primary tumor with an IHC score of 1+ or 2+ with ISH negative was categorized as HER2-low, whereas HER2-zero was categorized as an IHC score of 0. HR-positive primary tumors were classified as estrogen receptor (ER) and/or progesterone receptor (PR) ⩾ 1%, while HR-negative tumors had ER and PR < 1%.

Statistical analysis

Propensity score matching (PSM) was applied to balance the confounding factors between the HER2-zero and HER2-low groups, using a logistic regression model to derive a propensity score for each individual based on age at diagnosis, menopausal status, type of pathology, clinical stage, hormone receptor status, Ki67 (<15% vs ⩾15%), breast and axillary surgery, chemotherapy, radiotherapy, and endocrine therapy, and then the 2 groups of patients were matched 1:1 in a ratio of caliber value of 0.02. The clinicopathologic characteristics were represented using descriptive statistics such as the median, range, or percentage of patients. Pearson χ^2 or Fisher exact tests were used to compare the differences between the HER2-low and HER2-zero groups. To investigate the differences, continuous variables (age at diagnosis) with normal distributions were assessed using a *t* test, while continuous variables with non-normal distributions were tested using the Wilcoxon rank test. The Kaplan-Meier method was used to plot the survival curves, and log-rank analysis was utilized to compare the OS, DFS, and DDFS subgroups. All Cox multiple regression model analyses controlled for age at diagnosis, menopausal status, hormone receptor status, HER2 status, clinical stage, chemotherapy, and radiotherapy, and used this regression model to calculate hazard ratios (HRs) and 95% confidence intervals (CI) for time-to-event endpoints. The statistical significance value was set at $P < .05$ (2-sided). SPSS 25.0 (https://www.ibm.com/products/spss-statistics) and GraphPad Prism 8.0.2 (https://www.graphpad.com/) were used for all statistical analyses and figure drawing.

Results

Patient clinicopathologic characteristics

A total of 321 patients who were screened for this study met the inclusion and exclusion criteria. Since uneven baseline characteristics can have a significant impact on the efficacy of NAC and survival outcomes, we performed a 1:1 PSM analysis with 97 pairs of patients to maximize the elimination of those changes (Table 1). Prior to PSM matching, most patients were HR-positive, premenopausal, had invasive ductal carcinoma, and were in clinical stage II disease, unrelated to their HER2 status. The median follow-up time of this study was 71.37 months (14.27-83.83 months). Propensity score matching balanced the differences in the HR status (before PSM, $P = .001$; after PSM, $P = .869$), radiotherapy (before PSM, $P = .031$; after PSM, $P = .500$), and endocrine therapy (before PSM, $P = .001$; after PSM, $P = .749$) results between the 2 groups at baseline.

HER2-low expression and efficacy of NAC

In the entire treatment cohort, 79 patients received NAC. After PSM, 24 and 21 patients in the HER2-low and HER2-zero groups received NAC, respectively. Table 2 shows that after matching, the ORR was higher in the HER2-zero group than in the HER2-low group (71.43% vs 58.33%), but there was no statistically significant difference ($P = .360$).

After PSM, 8 (17.78%, 8/45) patients achieved pCR. Figure 1 shows that 23.81% (5/21) of patients with HR+/HER2-low, 10.53% (2/19) with HR+/HER2-zero ($P = .412$), 0% (0/3) with HR–/HER2-low, and 50% (1/2) with HR–/HER2-zero ($P = .400$) achieved pCR, indicating that low expression of HER2 had no effect on pCR in the same HR status, which was also revealed using data without PSM (Supplementary Figure 2).

Survival analyses

Of the 194 patients (97 patients for each group), all were included in the OS analysis, while 2 patients were not included in the DFS and DDFS analyses because of data loss. As of December, 2021, in HER2-low and HER2-zero groups, OS events had occurred in 1/97 and 8/97, DFS in 11/97 and 11/95, and DDFS in 10/97 and 8/95 patients. Because of the short follow-up period, no median time-to-event endpoints were reached. Nevertheless, we could still observe the differences between the subgroups.

The HER2-low group had a considerably higher 5-year OS of 98.99% versus 95.87% ($P = .044$; Figure 3A), DFS of 90.61% versus 90.52% ($P = .868$; Figure 3B), and DDFS of 93.67% versus 91.53% ($P = .757$; Figure 3C) than the HER2-zero group. In the overall population, the 5-year OS/DFS/DDFS rate of HR-positive group was higher than that of HR-negative group (99.31% vs 91.84%, $P = .03$; 93.63% vs 81.63%, $P = .061$; and 94.33% vs 87.52%, $P = .367$, respectively; Supplementary Figure 3). Meanwhile, the 5-year OS/DFS of the HER2-low group was higher compared with HER2-zero group under the same HR status; however, this difference was not observed in the 5-year DDFS (Figure 4). The clinical stage at diagnosis has a significant impact on prognosis. Patients with clinical stage III disease had lowest survival outcomes than patients with stage I and II cancer, with 5-year OS/DFS/DDFS rates of 87.50%, 66.67%, and 72.46%, respectively (Supplementary Figure 4).

In subsequent univariate and multivariate analyses, HER2-low expression was found to be an independent predictor of improved OS ($P = .047$, HRs = 16.121, 95% CI = 1.035-251.046; Table 3), but it was not associated with DFS or DDFS (Table 4). After balancing the baseline characteristics, positive HR status emerged as an independent predictor of better OS ($P = .009$, HRs = 90.900, 95% CI = 3.103-2662.854; Table 3),

Table 1. Clinicopathologic characteristics at baseline stratified by HER2 status before and after PSM.

CHARACTERISTIC	BEFORE PSM				*P* VALUE	AFTER PSM				*P* VALUE
	HER2-ZERO		HER2-LOW			HER2-ZERO		HER2-LOW		
	(N = 121)		(N = 200)			(N = 97)		(N = 97)		
	N	%	N	%		N	%	N	%	
Age, years, median (range)	46 (25-73)		46 (20-74)		.995	47 (25-73)		47 (20-72)		.965
Age, years					.454					.884
<50	73	60.33	129	64.50		56	57.73	57	58.76	
⩾50	48	39.67	71	35.50		41	42.27	40	41.24	
Menopausal status					.230					.435
Premenopausal	82	67.77	148	74.00		32	32.99	27	27.84	
Postmenopausal	39	32.23	52	26.00		65	67.01	70	72.16	
Histological type					.436					.602
Ductal	111	91.74	188	94.00		88	90.72	90	92.78	
Others	10	8.26	12	6.00		9	9.28	7	7.22	
Hormone receptor status					**.001**					.869
Positive	81	66.94	165	82.50		73	75.26	72	74.23	
Negative	40	33.06	35	17.50		24	24.74	25	25.77	
Ki67 (%)[a]					.480					.564
<15	45	37.50	83	41.50		42	43.30	46	47.42	
⩾15	75	62.50	117	58.50		55	56.70	51	52.58	
Clinical stage at diagnosis					.109					.022
I	32	26.45	51	25.50		30	30.93	19	19.59	
II	61	50.41	120	60.00		47	48.45	66	68.04	
III	28	23.14	29	14.50		20	20.62	12	12.37	
Chemotherapy					.163					.610
NAC	35	28.93	44	22.00		21	21.65	24	24.74	
AC	86	71.07	156	78.00		76	78.35	73	75.26	
Number of chemotherapy cycle					.402					.598
4	26	21.49	53	26.50		20	20.62	22	22.68	
6	34	28.10	61	30.50		26	26.80	20	20.62	
8	61	50.41	86	43.00		51	52.58	55	56.70	
Breast surgery					.234					.379
Mastectomy	101	83.47	156	78.00		79	81.44	74	76.29	
Breast conserving	20	16.53	44	22.00		18	18.56	23	23.71	
Axillary surgery					.971					.863
ALND	97	80.17	160	80.00		76	78.35	75	77.32	
SLNB	24	19.83	40	20.00		21	21.65	22	22.68	

(Continued)

Table 1. (Continued)

CHARACTERISTIC	BEFORE PSM				*P* VALUE	AFTER PSM				*P* VALUE
	HER2-ZERO		HER2-LOW			HER2-ZERO		HER2-LOW		
	(N = 121)		(N = 200)			(N = 97)		(N = 97)		
	N	%	N	%		N	%	N	%	
Radiotherapy					**.031**					.500
Yes	79	65.29	106	53.00		72	74.23	76	78.35	
No	42	34.71	94	47.00		25	25.77	21	21.65	
Endocrine therapy					**.004**					.749
Yes	78	64.46	158	79.00		71	73.20	69	71.13	
no	43	35.54	42	21.00		26	26.80	28	28.87	

Abbreviations: AC, adjuvant chemotherapy; ALND, axillary lymph-node dissection; HER2, human epidermal growth factor 2; NAC, neoadjuvant chemotherapy; PSM, propensity score matching; SLNB, sentinel lymph-node biopsy.
Boldfaced values: $P < .05$, the difference is statistically significant.
[a]One patient was missing the data of Ki67.

Table 2. Tumor response of patients with NAC before and after PSM.

RESPONSE	BEFORE PSM				*P* VALUE	AFTER PSM				*P* VALUE
	HER2-LOW[a]		HER2-ZERO			HER2-LOW[a]		HER2-ZERO		
	N = 44; N, %		N = 35; N, %			N = 24; N, %		N = 21; N, %		
CR	1	2.27%	2	5.71%		0	0.00%	1	4.76%	
PR	27	61.36%	20	57.14%		14	58.33%	14	66.67%	
SD	14	31.82%	12	34.29%		9	37.50%	6	28.57%	
PD	1	2.27%	1	2.86%		0	0.00%	0	0.00%	
ORR	28	63.64%	22	62.86%	.836	14	58.33%	15	71.43%	.360

Abbreviations: CR, complete response; HER2, human epidermal growth factor 2; NAC, neoadjuvant chemotherapy; ORR, objective response rate; PD, progressive disease; PR, partial response; PSM, propensity score matching; SD, stable disease.
[a]One patient could not evaluate the tumor response.

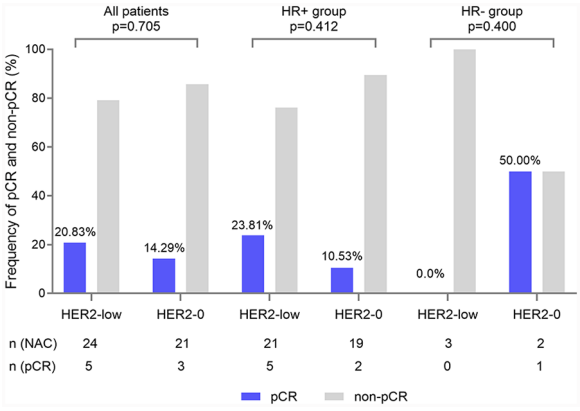

Figure 2. pCR rate in HER2-low and HER2-zero groups after PSM. HER2 indicates human epidermal growth factor 2; NAC, neoadjuvant chemotherapy; pCR, pathological complete response; PSM, propensity score matching.

but this was also not reflected in DFS and DDFS (Table 4). Moreover, patients with clinical stage III disease had significantly worse OS, DFS, and DDFS than those with stage I disease (Tables 3 and 4).

Among the 45 patients who underwent NAC, patients with pCR (n = 8) had a higher 5-year OS/DFS/DDFS rate than patients with non-pCR, although the difference was not statistically significant (Supplementary Figure 5a-c). Since only a few patients achieved pCR in this cohort, we conducted another prognostic analysis in the non-pCR group. The 5-year OS/DFS/DDFS rate was lower in the HER2-zero group than in the HER2-low group (Supplementary Figure 5d-f).

Discussion

Breast cancer is classified according to the clinicopathologic, genetic, and immune features of affected patients to aid in their standard care and personalized treatment. Approximately half of the patients with HER2-negative BC have a HER2-low expression status (IHC 1+ or IHC 2+/ISH negative),[14,15,17] and this subset of patients were considered ineligible for anti-HER2 therapy. In the phase III clinical trial of DESTINY BREAST 04, median PFS was 9.9 and 5.1 months (HRs = 0.50, $P < .001$), and median OS was 23.4 and 16.8 months (HRs = 0.64, $P = .001$) in the trastuzumab deruxtecan and

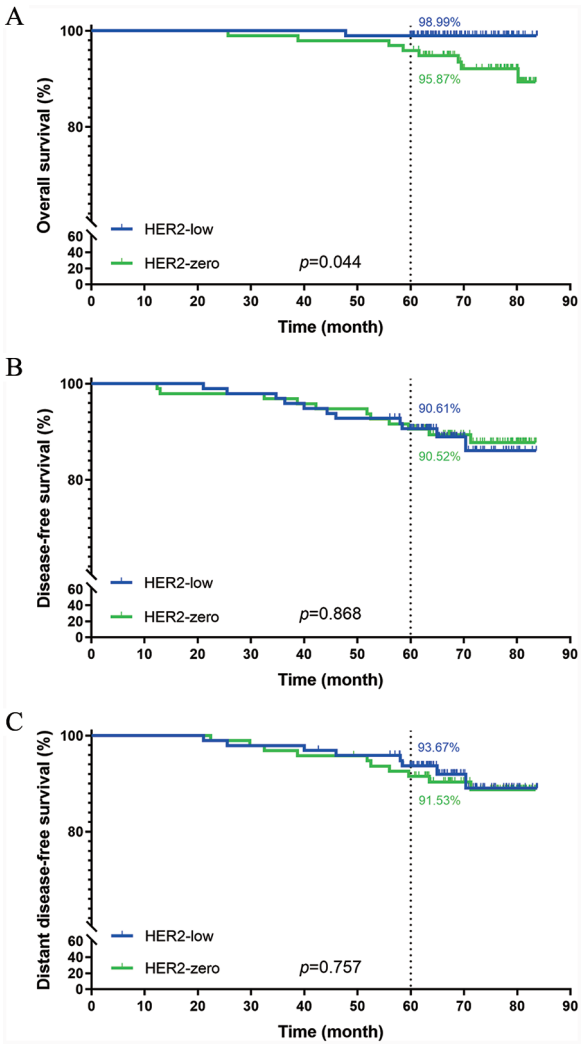

Figure 3 Kaplan-Meier curve and log-rank analyses of overall survival, disease-free survival, and distance disease-free survival in HER2-low and HER2-zero groups. (A) Overall survival. (B) Disease-free survival. (C) Distance disease-free survival. HER2 indicates human epidermal growth factor 2.

physician's drug choice groups, respectively.[12] This trial confirms that there may be additional treatment options for patients with HER2-low expression.

We screened 321 patients with HER2-negative BC who met the inclusion criteria for this retrospective cohort analysis. Before PSM, 62.3% of patients had low HER2 expression. After balancing the baseline differences, we could still observe that HER2-low BC was enriched in the HR+ cohort, which is in line with previous studies.[7,14,21,23] In our NAC cohort, HER2-low status did not correlate with the ORR, pCR, or prognosis after NAC. Due to the small sample size, no difference in pCR rate was observed between the 2 groups, irrespective of HR status. Our preliminary survival analysis revealed that the 5-year OS/DFS/DDFS rates of patients who achieved pCR were numerically superior to those of non-pCR patients, whereas in HER2-low/non-pCR patients, the above values were equally superior to those of HER2-zero/non-pCR patients, although the differences were not statistically significant. Some researchers suggest that HER2-low positivity did not predict pCR and survival outcome after NAC, but those with HR-positive status and pCR have significantly better OS than other patients (HRs = 0.22, $P < .0001$; HRs = 1.42, $P < .0001$, respectively) and relapse-free survival (HRs = 0.23, $P < .0001$; HRs = 1.61, $P < .0001$, respectively).[19] This differs from the results of Denkert et al's[16] pooled analysis of neoadjuvant prospective clinical trials, in which patients with HER2-low expression had a significantly lower pCR rate than HER2-zero patients (29.2% vs 39.0%, $P = .0002$), and HR-patients had a higher pCR than HR+ patients regardless of HER2 status, and superior survival of the HER2-low group compared with the HER2-zero group was observed only in patients with HR–/non-pCR, although it was still much inferior to that of patients who achieved pCR. Taken together with the results of previous studies and our preliminary studies, HER2-low expression may confer long-term benefits to patients with pCR and non-PCR. In BC patients with HER2-low status after NAC, the impact of this status on pCR and long-term survival outcomes is unclear. To determine whether the use of T-Dxd for NAC in HER2-low BC can change the current situation, 2 related clinical trials have enrolled patients. One used DS-8201a alone or in combination with anastrozole for the treatment of early-stage HER2 low/HR+ BC (NCT04553770), while the other used DS-8201a alone or in combination with THP (paclitaxel plus trastuzumab and pertuzumab) or standard therapy (ddAC-THP) for HER2-positive high-risk early BC (DESTINY BREAST 11, NCT05113251).

Regarding the effect on survival, there was no statistically difference in the 5-year DFS/DDFS rate between the 2 groups, but the 5-year OS rate in HER2-low group was considerably higher than in HER2-zero group ($P = .047$, HRs = 16.121, 95% CI = 1.035-251.046). Multivariate analysis indicated the same result: patients with HER2-low BC had a better OS than HER2-zero BC, but there was no difference in DFS/DDFS. Several studies have shown inconclusive associations between HER2-low expression and prognosis of patients with HER2-negative BC. Our results were similar to those of Denkert 2021 that patients with HER2-low expression had a better survival outcome than HER2-zero expression patients (3-year DFS rate: 83.4% vs 76.1%, $P = .0084$; 3-year OS rate: 91.5% vs 85.8%, $P = .0016$, respectively),[16] along with better outcomes as reported in other studies.[17,18] Moreover, some researchers showed that HER2-low status may not be associated with survival prognosis.[14,19-21] HR-positive status was found to be an independent predictor of higher OS in this analysis ($P = .009$, HRs = 90.900, 95% CI = 3.103-2662.854), although there was no significant difference in 5-year OS/DFS/DDFS rates between HER2-low and HER2-zero groups with the same HR status. A large retrospective study showed that HER2-low expression could somewhat affect pCR (adjust OR = 0.89, 95% CI = 0.86-0.92; $P < .001$) and

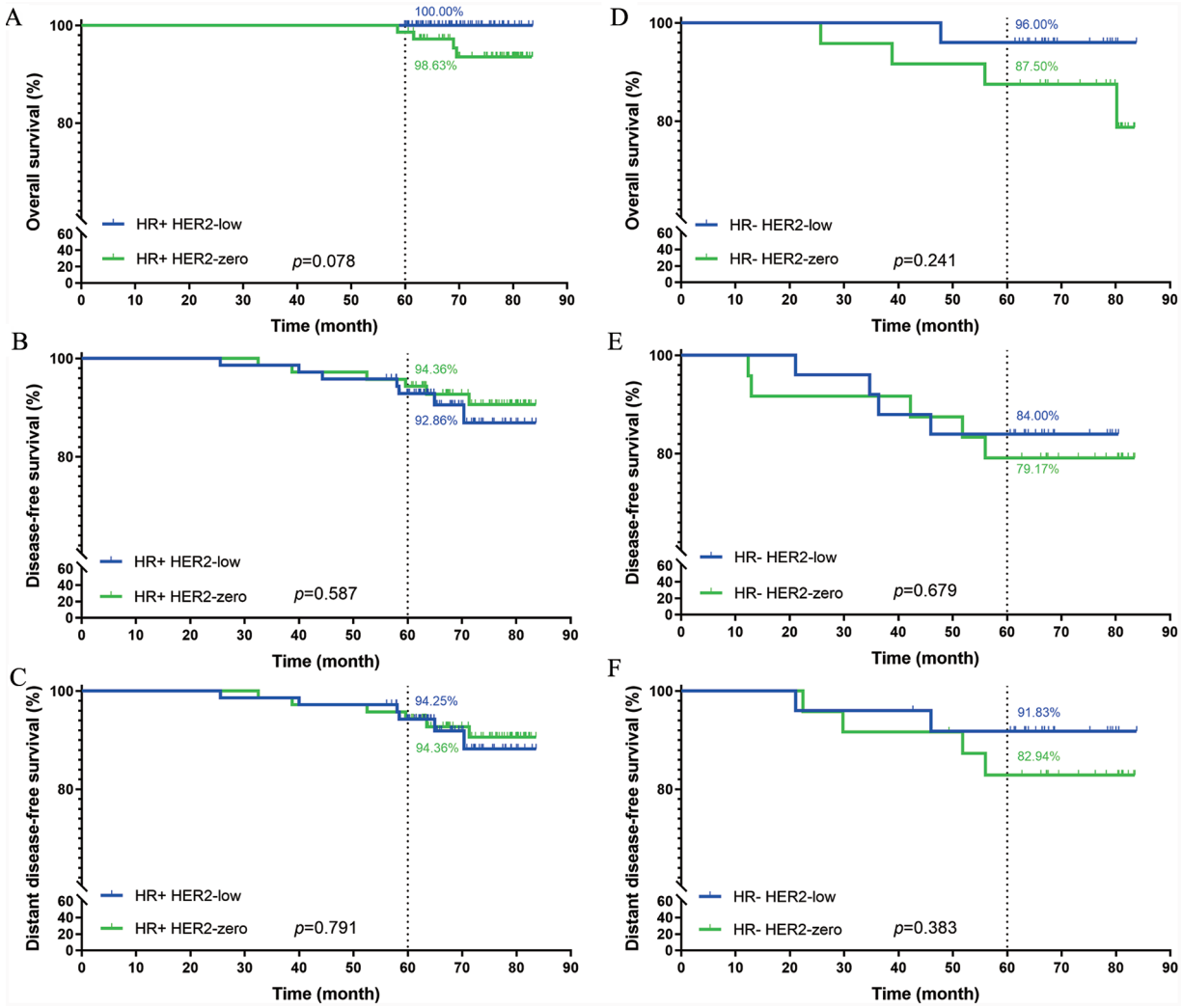

Figure 4. Kaplan-Meier curves and log-rank analyses of overall survival, disease-free survival, and distance disease-free survival of patients stratified based on HER2 and HR status. HR-positive population: (A) Overall survival. (B) Disease-free survival. (C) Distance disease-free survival; HR-negative population: (D) Overall survival. (E) Disease-free survival. (F) Distance disease-free survival. HER2 indicates human epidermal growth factor 2; HR, hormone receptor.

improve OS (adjust HRs = 0.98, 95% CI = 0.97-0.99; $P < .001$), but the effects were all minimal, and the investigators assumed that these differences stemmed primarily from the large sample of the study, the hormone receptor status, and the PAM50 of patients and did not support an intrinsic difference between HER2-low and HER2-zero expression.[24] Like their results, we also found that HER2-low expression could give patients a better OS. But for pCR, our data did not find that HER2-low status had any effect on that. For this reason, we think maybe our data were not large enough to find the difference. We also believe that there was substantial heterogeneity in this cohort of HER2 non-positive BCs due to the different levels of hormone receptor expression and the different treatment regimens and drug doses used in each patient. Therefore, it is difficult to definitively determine the link among HR status, low expression of HER2, and prognosis of survival, which needs to be further explored in randomized controlled trials with large sample sizes and long follow-up time.

We observed a phenomenon of shift in the pathology-reported HER2 status after re-biopsy of locally recurrent lesions or distant metastatic lesions in patients who presented with a recurrent or metastatic event. Among the 31/321 patients who developed recurrent metastases, 2 patients had a change in HER2 status from IHC 0 to IHC 2+ with negative fluorescence in situ hybridization (FISH) results (metastases in the left lung and bilateral ovaries, respectively), 1 patient had a change from IHC 0 to IHC 1+ (contralateral supraclavicular lymph nodes), and 1 patient had a change from IHC 1+ to IHC 0 (contralateral supraclavicular lymph nodes), and no cases of HER2 zero or low expression converted to HER2 overexpression were observed. This phenomenon suggests the instability of HER2 expression. Previous studies have demonstrated that a change in HER2 status occurs in 38% to 66% of patients during the course of the disease from the first biopsy of the primary lesion to re-biopsy of progressive lesions,[20,25] and this shift is mainly due to the

Table 3. Log-rank univariate analysis and Cox multivariate analysis of factors associated with OS after PSM.

CHARACTERISTIC	LOG-RANK	COX MULTIVARIATE ANALYSIS	
	P VALUE	*P* VALUE	HRs (95% CI)
Age (<50 vs ⩾50 years)	.817	.32	3.734 (0.279-50.035)
Menopausal status (post vs pre)	.84	.778	1.440 (0.114-18.250)
HR status (positive vs negative)	**.03**	**.009**	90.900 (3.103-2662.854)
HER2 status (low vs zero)	**.044**	**.047**	16.121 (1.035-251.046)
Ki67 (<15% vs ⩾15%)	.194	.472	2.023 (0.296-13.818)
Clinical stage at diagnosis			
I vs II	.881	.743	0.657 (0.053-8.074)
I vs III	**.012**	**.022**	15.687 (1.491-165.068)
Chemotherapy (NAC vs AC)	.119	.207	0.363 (0.075-1.750)
Radiotherapy (yes vs no)	.131	.128	0.105 (0.006-1.916)

Abbreviations: AC, adjuvant chemotherapy; CI, confidence interval; HER2, human epidermal growth factor 2; HRs, hazard ratios; NAC, neoadjuvant chemotherapy; OS, overall survival; PSM, propensity score matching.
Boldfaced values: *P*<.05, the difference is statistically significant.

Table 4. Log-rank univariate analysis and Cox multivariate analysis of factors associated with DFS and DDFS after PSM.

CHARACTERISTIC	DFS			DDFS		
	LOG-RANK	COX MULTIVARIATE ANALYSIS		LOG-RANK	COX MULTIVARIATE ANALYSIS	
	P VALUE	*P* VALUE	HRS (95% CI)	*P* VALUE	*P* VALUE	HRs (95% CI)
Age (<50 vs ⩾50 years)	.333	.745	0.792 (0.195-3.219)	.465	.477	0.535 (0.096-3.000)
Menopausal status (post vs pre)	.415	.574	1.564 (0.328-7.445)	.79	.884	0.874 (0.142-5.389)
HR status (positive vs negative)	.061	.061	3.852 (0.938-15.830)	.367	.227	2.754 (0.533-14.222)
HER2 status (low vs zero)	.869	.435	0.694 (0.278-1.734)	.757	.915	0.947 (0.353-2.545)
Ki67 (<15% vs ⩾15%)	.363	.974	1.016 (0.385-2.680)	.926	.611	0.765 (0.272-2.150)
Clinical stage at diagnosis						
I vs II	.332	.372	2.043 (0.425-9.813)	.541	0.543	1.647 (0.329-8.246)
I vs III	**<.001**	**.001**	20.407 (4.070-102.319)	**<.001**	**.001**	14.974 (2.906-77.157)
Chemotherapy (NAC vs AC)	.502	.999	0.999 (0.367-2.720)	.911	.555	1.426 (0.439-4.630)
Radiotherapy (yes vs no)	.336	.89	1.105 (0.267-4.566)	.645	.875	1.144 (0.215-6.077)

Abbreviations: AC, adjuvant chemotherapy; CI, confidence interval; DDFS, distant disease-free survival; DFS, disease-free survival; HER2, human epidermal growth factor 2; HRs, hazard ratios; NAC, neoadjuvant chemotherapy; PSM, propensity score matching.
Boldfaced values: *P*<.05, the difference is statistically significant.

interconversion between HER2-zero and HER2-low status, and rarely observed in patients who convert to HER2-positive status. This highlights the need for the re-examination of progressing lesions, as HER2-zero expression may convert into low expression, and this subset of patients may have more treatment options. As shown in a previous prospective study,[16] differences have been observed in the genetic and mutation background between patients with HER2-zero and HER2-low status, which further indicates that we need to pay more attention to the IHC score and ISH results of HER2-negative BC patients.

This study has some limitations that should be discussed. First, this is a single-center retrospective study with a small sample, a single race, and a short follow-up period, and we found that many excluded patients did not undergo FISH due to financial reasons; second, we lacked reconfirmation of HER2 IHC results, which inevitably led to discrepancies in the pathology reports of some patients due to the pathologists'

experience; third, we were unable to collect histological grade and quantitative ER and PR data of the tumor because some pathology reports were incomplete. Therefore, we have to discuss the findings of this study with more caution. However, our findings revealed that HER2 low expression could improve the OS of female early BC patients, but whether HER2 low status could be a new molecular subtype is still being debated. Although some pathologists believe that it is easy to discern IHC 0 from IHC 1+ with current staining techniques;[26] in the future, we still need more accurate pathological detection methods as well as standardized pathological scoring guidelines to accurately distinguish the HER2 expression status, especially IHC 0 from IHC 1+, to develop new treatment options to improve the prognosis of BC patients.

Conclusion

Female patients with early invasive BC with HER2-low expression or HER2-zero expression have no difference in pCR or metastasis risk, but patients with low HER2 expression may have better long-term survival rates. However, whether HER2 low expression can be considered a new molecular subtype is still being debated, which will necessitate large-scale clinical trials and precise pathological confirmation.

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Lanyi Dai: Investigation; Writing—Original Draft; Formal analysis.
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Yiyin Tang: Resources; Writing—Review & Editing.
Dedian Chen: Supervision; Funding acquisition.
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All authors have read and approved the final version.

Availability of Data and Materials

The data sets generated during the current study are available from the corresponding author on reasonable request.

Consent for Publication

Not applicable.

Ethics Approval and Consent to Participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by The Third Affiliated Hospital of Kunming Medical University Institution Review Board of no. KYLX2022156 and individual consent for this retrospective analysis was waived.

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Supplemental Material

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