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Comparison of clinicopathological characteristics, efficacy of neoadjuvant therapy, and prognosis in HER2-low and HER2-ultralow breast cancer

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Abstract

Background This study aims to analyze potential differences in clinicopathology, efficacy of neoadjuvant therapy (NAT), and clinical outcome among HER2-null, HER2-ultralow and HER2-low breast cancers.

Methods Consecutive cases of HER2-negative breast cancer that received NAT were included. They were classified as HER2-null (no staining), HER2-ultralow (incomplete faint staining in ≤ 10% of tumour cells) and HER2-low (HER2-1 + or HER2-2+, in situ hybridisation negative). Subgroup analysis was performed based on the HER2 expression level.

Results Out of 302 patients, 215 (71.19%) were HER2-low, 59 (19.54%) were HER2-ultralow, and 28 (9.27%) were HER2-null. In comparison to the HER2-ultralow group, the HER2-low group exhibited higher expression frequencies of ER ($p < 0.001$), PR ($p < 0.001$), and AR ($p = 0.004$), along with a greater prevalence of the luminal subtype ($p < 0.001$). The HER2-ultralow group also demonstrated a higher prevalence of lymph node metastasis compared to the HER2-null group ($p = 0.026$). Varied rates of pathologic complete response (pCR) were observed among the three subgroups: HER2-null, HER2-ultralow, and HER2-low, with rates of 35.71%, 22.03%, and 12.56%, respectively. Only the HER2-low subgroup exhibited a significant difference compared to HER2-null ($p = 0.001$). Despite variations in pCR rates, the three subgroups exhibited comparable disease-free survival (DFS) ($p = 0.571$). Importantly, we found HER2-low patients with better treatment response (RCB-0/I) exhibited significantly better DFS than those with significant residual disease (RCB-II/III) ($P = 0.036$). The overall rate of HER2 immunohistochemical score discordance was 45.24%, mostly driven by the conversion between HER2-0 and HER2-low phenotype. Notably, 32.19% of cases initially classified as HER2-0 phenotype on baseline biopsy were later reclassified as HER2-low after neoadjuvant therapy, and it is noteworthy that 22 out of these cases (78.57%) originally had an HER2-ultralow status in the pretreatment biopsy sample.

Conclusions Our results demonstrate the distinct clinicopathological features of HER2-low and HER2-ultralow breast tumors and confirm that RCB is an effective predictor of prognosis in HER2-low populations for the first time. Notably,

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our findings demonstrate high instability in both HER2-low and HER2-ultralow expression from the primary baseline biopsy to residual disease after NAT. Furthermore, this study is the first to investigate the clinicopathological feature and the effectiveness of NAT for HER2-ultralow breast cancer.

Keywords Breast cancer, HER2-low, HER2-ultralow, HER2-null, Clinicopathological features, Neoadjuvant therapy

Introduction

The novel HER2-targeted antibody-drug conjugate (ADC) trastuzumab deruxtecan (TDXd) marks the start of a new era in managing HER2-low breast cancer, which is defined as HER2 immunohistochemistry (IHC) 1+or 2+with fluorescence in situ hybridization (FISH) negative. Literature indicates that approximately 55% of all breast cancer cases can be classified as HER2-low [1], with this percentage potentially rising to 65% in hormone receptor (HR)-positive/HER2-negative breast cancer, and to around 15% or more in triple-negative breast cancer (TNBC) [2, 3]. It is increasingly evident that HER2-low breast cancer demonstrates distinctive characteristics in clinicopathologic features, treatment response, and prognosis compared to HER2-negative breast cancer [4–6].

However, the ongoing debate centers around whether HER2-low breast cancer can be regarded as a distinct entity with a clearly defined definition. Subgroup analysis of the DESTINY-Breast04 trial (NCT03734029) has revealed that TDXd offers therapeutic benefits even in cases with relatively low levels of HER2 expression (IHC 1+) [7]. Furthermore, results from the Phase II DAISY study (NCT04132960) have suggested that patients with HER2-ultralow expression, defined as IHC expression>0 and <1+, exhibit objective response to TDXd, implying that the therapeutic effect of TDXd may also apply to patients with HER2-0 expression [8]. The ongoing DESTINY-Breast06 trial (NCT04494425) aims to delve further into the therapeutic benefits of TDXd in patients with HER2-low expression, subcategorized as IHC 0–1+ (defined as faint or nearly invisible membrane staining in 10% or fewer tumor cells), IHC 1+, and 2+with FISH negative [9]. As such, it becomes evident that a reconsideration of the lower limit for HER2 low expression may be necessary, as the current definition may not accurately delineate the therapeutic boundaries of TDXd. Consequently, further clinical research is urgently needed to expound and elucidate the clinical and pathological characteristics of these cases.

In 2020, the FDA approved the use of pathological complete response (pCR) as an alternative endpoint for neoadjuvant therapy (NAT) studies in high-risk breast cancer. However, there has been controversy about whether pCR translates into survival benefits. At the same time, the residual cancer burden (RCB), a pathologic evaluation system introduced in 2007, has garnered clinical validation with long-term follow-up data, confirming its substantial clinical relevance [10]. Recent research has revealed that RCB serves as a robust predictor of recurrence and metastasis risk in breast cancer patients post-NAT, establishing itself as a highly reliable prognostic indicator [11, 12]. However, it is worth noting that previous studies did not account for the newly identified categories of HER2-low and HER2-ultralow, indicating a gap in understanding the full scope of RCB's prognostic capabilities in these specific patient populations.

In this study, we conducted a retrospective analysis of clinical and pathological data from 302 HER2-negative breast cancer patients who underwent NAT. The study cohort was classified into three subtypes based on the tumor's HER2 status: HER2-null (no HER2 staining detected), HER2-ultralow (IHC score>no staining and <1+), and HER2-low (HER2 IHC score of 1+or 2+/ISH not amplified) [13]. This classification aimed to assess the potential of HER2-low and HER2-ultralow as independent subtypes of breast cancer. The comparison and thorough examination of the clinical and pathological characteristics, neoadjuvant treatment efficacy, and survival data of the three patient groups were conducted to support this evaluation. The clinicopathologic characteristics, efficacy of NAT, and survival data of the patients in the three groups were studied and compared extensively to further investigate the feasibility of treating the HER2-low and HER2-ultralow expression subtypes as independent breast cancer subtypes. This is the initial clinical study involving HER2-ultralow breast cancer patients receiving NAT.

Materials and methods

Study population

From January 2020 to May 2023, clinical and pathological data were gathered from HER2-negative breast cancer patients at Renmin Hospital of Wuhan University who had undergone NAT. Chemotherapy regimens containing taxanes and anthracyclines were used as NAT regimens, and none of the patients received anti-HER2 treatment, and all patients received and completed chemotherapy cycles to undergo surgery and postoperative pathological examination. Inclusion criteria were as follows: (1) female patients, (2) invasive breast cancer diagnosed by core needle biopsy before treatment, and HER2 negative, and (3) Clinical and pathologic characteristics were recorded for all patients. Exclusion criteria comprised patients with bilateral or inflammatory breast cancer, pregnant or lactating patients with breast cancer, those

with concurrent other malignancies, individuals lacking the RCB score, and patients with distant metastasis during NAT. Data on clinicopathological information was retrieved from clinical medical records and pathology databases. The study received approval from the Ethics Committee of Renmin Hospital of Wuhan University (WDRY2023-K070).

Histopathologic evaluation

The assessment of pCR involves determining the absence of invasive cancer in the primary tumor and negative regional lymph nodes, denoted as ypT0/Tis ypN0. The evaluation of the RCB score and class is carried out by specialized breast pathologists who have undergone appropriate training in standard methods [10]. The RCB score is calculated as a continuous variable and is graded into four categories: RCB-0 (indicating pCR, with an RCB score of 0), RCB-I ($0<RCB\leq1.36$), RCB-II($1.36<RCB\leq3.28$), and RCB-III ($RCB>3.28$) [10]. The interpretation of HER2 IHC (clone 4B5, Ventana) results is conducted by two trained specialized breast pathologists, with involvement of a third senior-level specialized breast pathologist in cases of inconsistent results. The criteria for interpreting HER2 IHC conform to the latest guidelines of the ASCO/CAP [14]. Moreover, HER2-low is defined as HER2 IHC results of 1+or 2+with negative FISH test. Additionally, HER2 0 is further divided into HER2-ultralow and HER2-null. HER2-ultralow is characterized by a HER2 IHC score of 0 and ≤10% of tumor cells displaying faint/weak and incomplete membrane staining, while HER2-null is defined as a HER2 IHC score of 0 with a total absence of membrane staining.

Statistical analysis

Statistical analyses for this study utilized the Statistical Package for the Social Sciences software (SPSS) version 25.0. Quantitative data was presented as the number of patients (percentage). To compare categorical variables, both the Chi-square test and Fisher's exact test were employed. In defining disease-free survival (DFS) as the progression from surgery to relapse or death from any cause, survival analysis was conducted using the Kaplan-Meier curve, and log-rank test was used to test the differences between groups. A p value below 0.05 indicated a significant difference for all statistical analysis.

Results

Clinicopathological characteristics of HER2-low and HER2-ultralow subgroups

Baseline characteristics of the cohort

A total of 302 female patients with HER2-negative breast cancer who underwent NAT between January 2020 and May 2023 were included in this study. The selection process for the cases is illustrated in Fig. 1. Out of the 302 patients, 28 patients (9.27%) were identified as HER2-null, while 59 patients (19.54%) were categorized as HER2-ultralow and 215 patients (71.19%) were HER2-low. The histological morphology and IHC manifestations of HER2 in groups with different HER2 expression levels were presented in Fig. 2. The majority of patients (87.71%, $n=264$) were early clinical stage (cT1-2) breast cancer patients, with a median age of 48 years (range: 22–76 years). Furthermore, a substantial proportion of patients (67.55%, $n=204$) were hormone receptor (ER/PR) positive. Table 1 provides a summary of the baseline clinicopathological characteristics of groups with varying levels of HER2 expression before NAT. Statistical differences were noted among the groups with respect to histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, androgen receptor (AR) status, lymph node metastasis, and immunohistochemistry-based alternative molecular typing. Conversely, similar distributions were found in age, number of lesions, clinical T stage, histological type, and Ki67 proliferation index.

HER2-low and HER2-ultralow displayed distinctive clinicopathological characteristics

Our study found significant differences in clinicopathologic characteristics between the HER2-low and HER2-0 groups, summarized as follows in Table 1. Firstly, the HER2-low group demonstrated a higher prevalence of lower histological grade (G1-2) compared to the HER2-0 group (46.98% vs. 32.18%, $p=0.019$). Additionally, the expression of ER (73.49% vs. 42.53%, $p<0.001$), PR (65.58% vs. 34.48%, $p<0.001$), and AR (83.15% vs. 59.26%, $p<0.001$) was found to be more frequent in the HER2-low group compared to the HER2-0 group. Moreover, the HER2-low group mainly consisted of Luminal type breast cancer, accounting for 76.28% of cases, with a smaller proportion being triple-negative breast cancer (23.72%). This proportion of Luminal type breast cancer in the HER2-low group was significantly higher than in the HER2-0 group (76.28% vs. 45.98%, $p<0.001$). There were no significant differences in age, number of lesions, clinical T stage, lymph node metastasis, histological type, and Ki67 proliferation index between the HER2-low group and the HER2-0 group.

Upon further division of the HER2-0 group into HER2-null and HER2-ultralow subgroups, we observed that the HER2-low group showed a higher proportion of positive cases for ER(73.49% vs. 32.14%, 73.49% vs. 47.46%, $p < 0.001$)(Fig. 3A), PR (65.58% vs. 28.57%, 65.58% vs. 37.29%, $p < 0.001$) (Fig. 3B), AR (83.15% vs. 48.15%, $p < 0.001$; 83.15% vs. 64.81%, $p=0.004$) (Fig. 3C), along with a greater prevalence of the luminal subtype (76.28% vs. 39.29%, 76.28% vs. 49.15%, $p < 0.001$) (Fig. 3D), when compared to both the HER2-null and HER2-ultralow

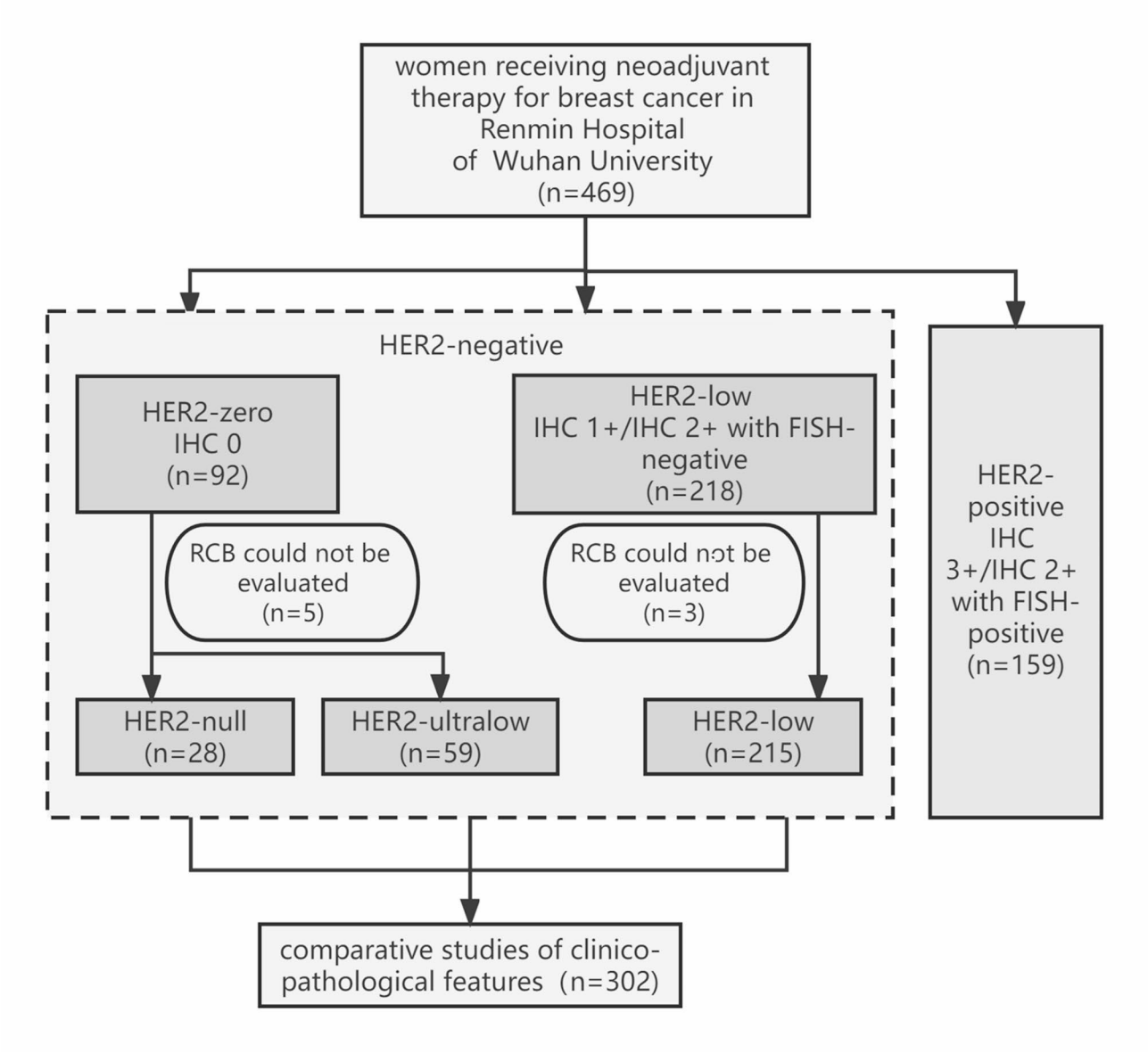

Fig. 1 Flow diagram of case selection

groups. Furthermore, the HER2-low group showed a higher probability of lymph node metastasis compared to the HER2-null group (75.52% vs.47.62%, $p=0.006$) (Fig. 3E). Conversely, no notable difference in lymph node metastasis was observed between the HER2-low group and the HER2-ultralow group. Notably, the HER2-ultralow group exhibited a higher prevalence of lymph node metastasis in comparison to the HER2-null group (75.00% vs. 47.62%, $p=0.026$) (Fig. 3E). In addition, no significant differences were found between the HER2-ultralow group and the HER2-null group in terms of age, number of lesions, clinical T stage, histological grade, histological type, expression of ER, PR, AR, Ki67 proliferation index, and molecular typing.

Analysis of NAT efficacy and clinical outcomes in HER2-low and HER2-ultralow groups

Differences in NAT efficacy among varying levels of HER2 expression subgroups

Upon analysis of our findings, we observed notable variations in the rates of pCR and RCB among different HER2 expression level groups. Notably, our research data unveiled diminished rates of pCR and proportions of patients achieving RCB-0/I class in the HER2-low cohort in comparison to the HER2-0 cohort (12.56% vs. 26.44%, $p=0.003$; 21.86% vs. 36.78%, $p=0.008$) (Fig. 4A and B). Upon further subgrouping of the HER2-0 category into HER2-null and HER2-ultralow, we observed varying pCR rates among the three subcategories, with rates of 35.71%, 22.03%, and 12.56% for HER2-null,

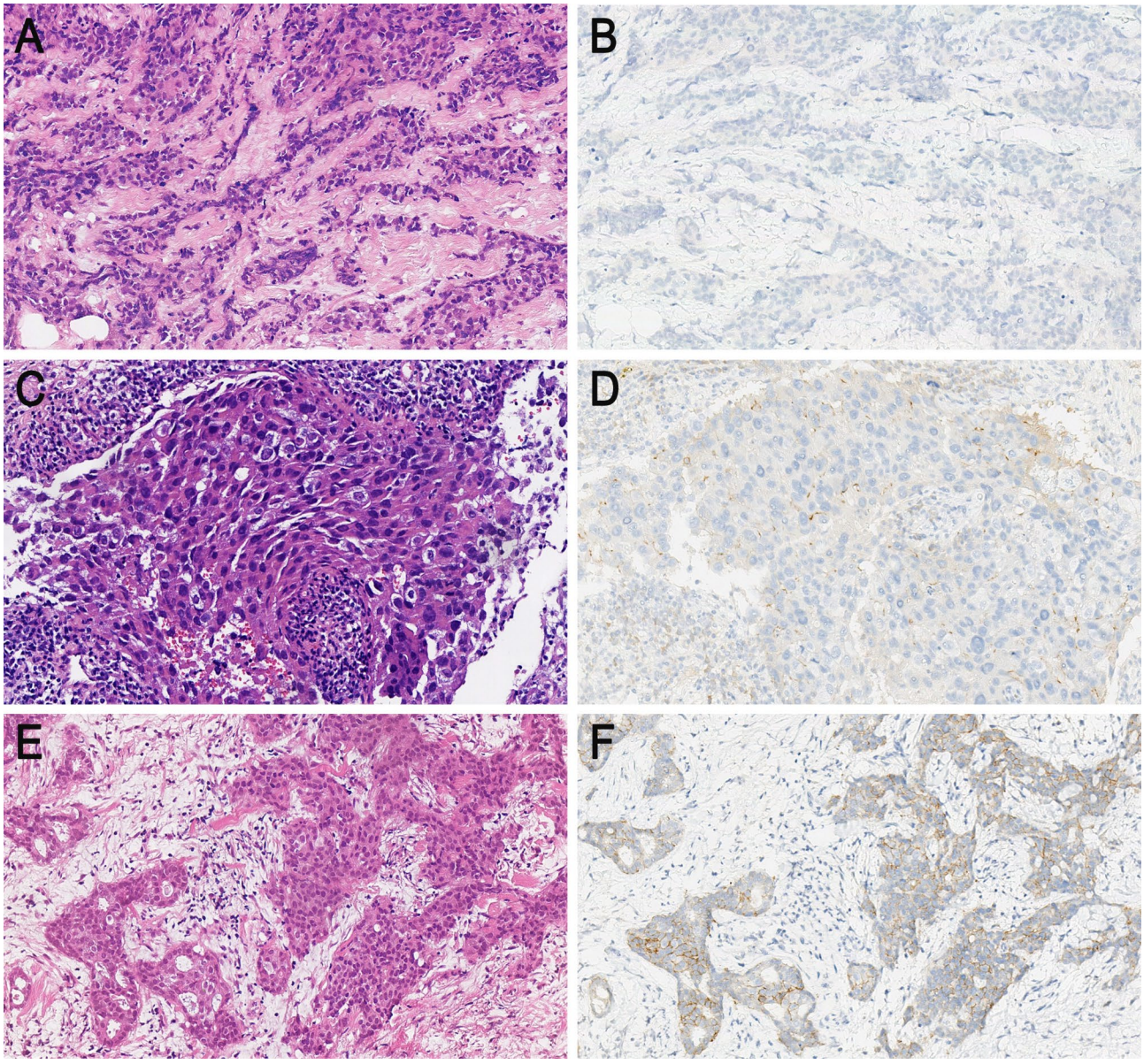

Fig. 2 H&E stained biopsy sections and HER2 IHC sections of representative cases (magnification x200). (**A**, **B**) H&E staining and HER2 IHC of the HER2-null group. (C, **D**)H&E staining and HER2 IHC of the HER2-ultralow group. (**E**, **F**)H&E staining and HER2 IHC of the HER2-low group

HER2-ultralow, and HER2-low, respectively, along with corresponding proportions achieving RCB-0/I of 50.00%, 30.51%, and 21.86% (Fig. 4C and D). However, significant differences in pCR rates were only discernible between the HER2-low and HER2-null subgroups ($p=0.001$). Subsequent stratified comparisons based on the pre-treatment cT staging unveiled significant differences in pCR rates between the HER2-low and HER2-0 groups within early (cT1-T2) and late (cT3-T4) stage cases (14.21% vs. 27.03%, $p=0.014$; 0% vs. 23.08%, $p=0.037$) (Fig. 4A). Additionally, within early stage (cT1-T2) cases, a statistically significant difference in the proportion achieving RCB-0/I was evident between the HER2-ultralow and HER2-null subgroups (30.61% vs. 56.00%, $p=0.034$) (Fig. 4D). However, our analysis did not yield statistically significant differences when comparing the pCR rates and proportion achieving RCB-0/I among different HER2 expression level subgroups within the Luminal and Triple-Negative cohorts. To identify independent predictors of pCR, we further included various clinical and pathological parameters in a multivariate logistic regression analysis. The analysis revealed that age, PR status, and AR status are significant independent predictors of pCR in the overall population. However, HER2 status was not found to be an independent predictor (Table S1).

Table 1 Baseline (**pre-NAT**) clinicopathological features of different HER2 expression groups

Characteristics	IHC/FISH-based HER2 status subgroup						
	HER2-0	HER2-low	*P*	HER2- null	HER2-ultralow	HER2-low	*P*
	(*n* = 87); (28.81%)	(*n* = 215); (71.19%)		(*n* = 28); (9.27%)	(*n* = 59); (19.54%)	(*n* = 215); (71.19%)	
Age							
≤ 50	55(63.22%)	113(52.56%)	0.091	18(64.29%)	37(62.71%)	113(52.56%)	0.238
> 50	32(36.78%)	102(47.44%)		10(35.71%)	22(37.29%)	102(47.44%)	
Number of lesions on imaging[a]							
Single	82(94.25%)	189(88.32%)	0.119	26(92.86%)	56(94.92%)	189(88.32%)	0.284
Multiple	5(5.75%)	25(11.68%)		2(7.14%)	3(5.08%)	25(11.68%)	
Clinical Tumor stage[b]							
cT1-2	74(85.06%)	190(88.79%)	0.372	25(89.29%)	49(83.05%)	190(88.79%)	0.477
cT3-4	13(14.94%)	24(11.21%)		3(10.71%)	10(16.95%)	24(11.21%)	
Lymph node metastasis[c]							
Yes	46(66.67%)	145(75.52%)	0.154	10(47.62%)	36(75.00%)	145(75.52%)	0.022
No	23(33.33%)	47(24.48%)		11(52.38%)	12(25.00%)	47(24.48%)	
Histological grade							
1 ~ 2	28(32.18%)	101(46.98%)	0.019	9(32.14%)	19(32.20%)	101(46.98%)	0.063
3	59(67.82%)	114(53.02%)		19(67.86%)	40(67.80%)	114(53.02%)	
Histological type							
NST	80(91.95%)	180(83.72%)	0.061	25(89.29%)	55(93.22%)	180(83.72%)	0.153
Others	7(8.05%)	35(16.28%)		3(10.71%)	4(6.78%)	35(16.28%)	
ER							
Negative	50(57.47%)	57(26.51%)	< 0.001	19(67.86%)	31(52.54%)	57(26.51%)	< 0.001
Positive	37(42.53%)	158(73.49%)		9(32.14%)	28(47.46%)	158(73.49%)	
PR							
Negative	57(65.52%)	74(34.42%)	< 0.001	20(71.43%)	37(62.71%)	74(34.42%)	< 0.001
Positive	30(34.48%)	141(65.58%)		8(28.57%)	22(37.29%)	141(65.58%)	
AR[d]							
Negative	33(40.74%)	31(16.85%)	< 0.001	14(51.85%)	19(35.19%)	31(16.85%)	< 0.001
Positive	48(59.26%)	153(83.15%)		13(48.15%)	35(64.81%)	153(83.15%)	
Ki-67							
< 30%	16(18.39%)	62(28.84%)	0.060	3(10.71%)	13(22.03%)	62(28.84%)	0.091
≥ 30%	71(81.61%)	153(71.16%)		25(89.29%)	46(77.97%)	153(71.16%)	
Molecular type[e]							
Luminal(ER and/or PR +, HER2-)	40(45.98%)	164(76.28%)	< 0.001	11(39.29%)	29(49.15%)	164(76.28%)	< 0.001
Triple-negative(ER-, PR-, HER2-)	47(54.02%)	51(23.72%)		17(60.71%)	30(50.85%)	51(23.72%)	

[a] Missing data for 1 HER2-low patient

[b] Missing data for 1 HER2-low patient

[c] Missing data for 7 HER2-null patients, 11 HER2-ultralow patients and 23 HER2-low patients

[d] Missing data for 1 HER2-null patient, 5 HER2-ultralow patients and 31 HER2-low patients

[e]Using immunohistochemistry and in situ hybridisation as surrogates for gene expression analyses, all cases were reclassified into Luminal subtype and Triple-negative subtype

Abbreviations: HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; NST, Invasive carcinoma of no special type; ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor

Exploratory survival analysis

The median follow-up time for 302 patients was 25.23 months (range: 1- 54.6 months), with 14 patients lost to follow-up. Subsequently, we conducted an analysis of DFS for all cases. The results revealed no significant difference in DFS between the HER2-low and HER2-0 groups ($p=0.871$) (Fig. 5A), as well as among the HER2-null, HER2-ultralow, and HER2-low groups ($p=0.571$) (Fig. 5B). Furthermore, the DFS between the HER2-low and HER2-0 groups was not influenced by pCR, molecular subtype, histological grade, or histological type (Figure S1).

We further analyzed the differences in DFS among 215 cases of HER2-low and 59 cases of HER2-ultralow in different NAT efficacy groups. Overall, the proportions of different RCB class in the HER2-low group were as follows: 27 patients (12.56%) achieved RCB-0, 20 patients (9.30%) achieved RCB-I, 96 patients (44.65%) achieved

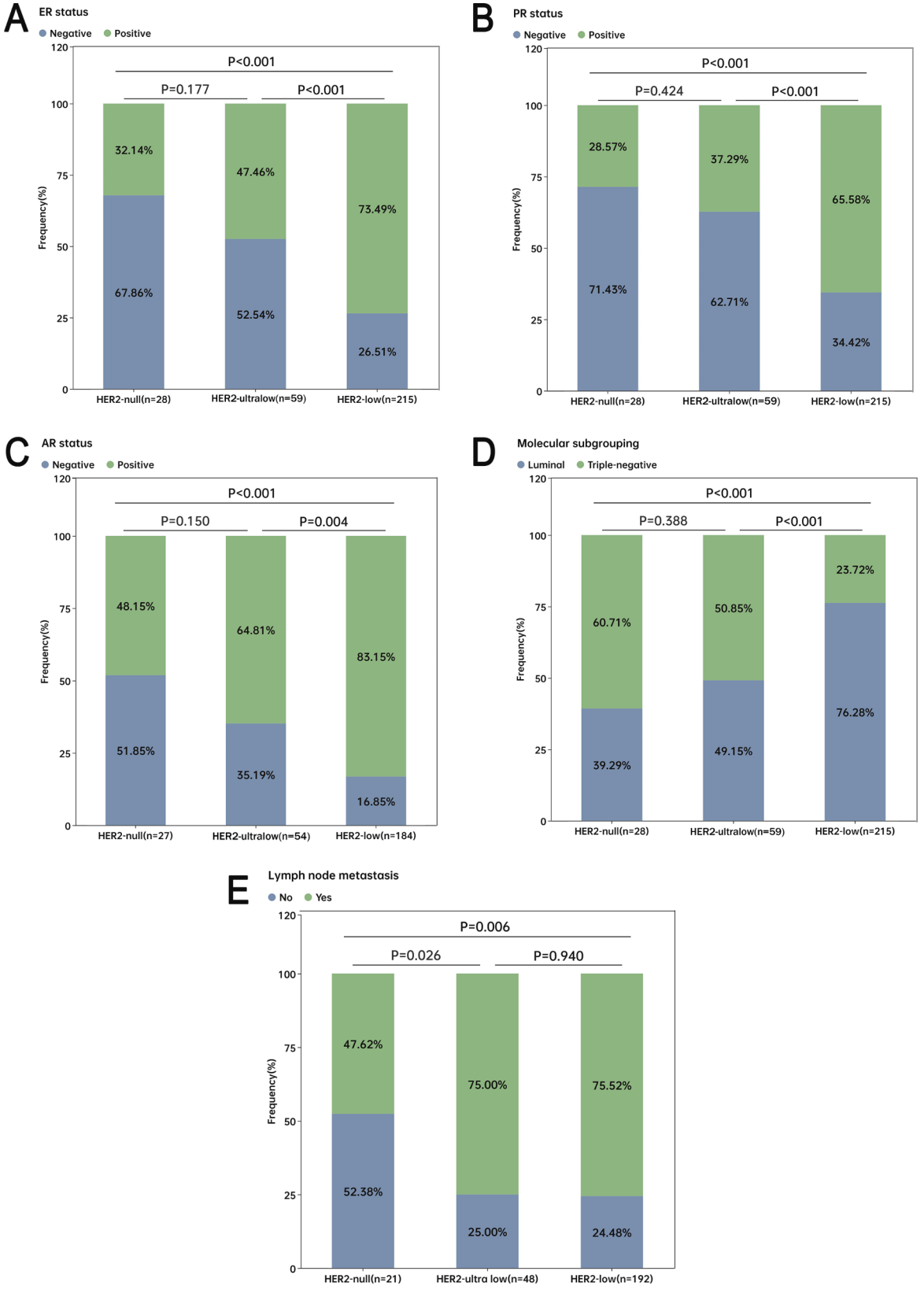

Fig. 3 HER2-null, HER2-ultralow and HER2-low subgroups had different clinical features, including (**A**)estrogen receptor(ER) status, (**B**)progesterone receptor(PR) status, (**C**)androgen receptor(AR) status, (**D**)IHC-based molecular subtype distribution, (**E**)lymph node metastasis

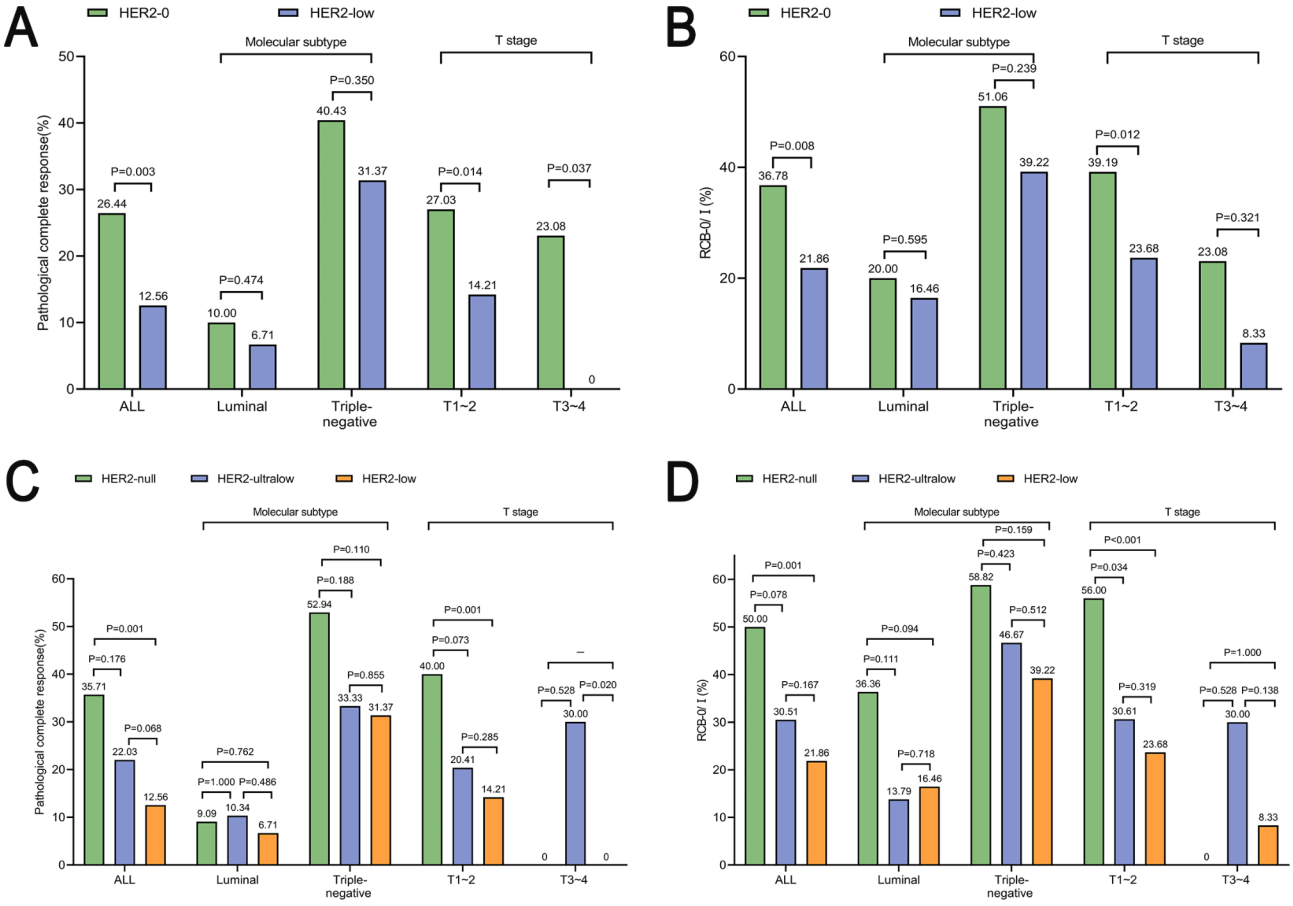

Fig. 4 The efficacy of NAT in different HER2 expression subgroups. (**A**) differences in pCR rates between HER2-0 and HER2-low breast cancer, (**B**) differences in RCB-0/I rates between HER2-0 and HER2-low breast cancer, (**C**) differences in pCR rates between HER2-null, HER2-ultralow and HER2-low breast cancer, (**D**) differences in RCB-0/I rates between HER2-null, HER2-ultralow and HER2-low breast cancer

RCB-II, and 72 patients (33.49%) achieved RCB-III. Our study revealed significant differences in DFS among HER2-low patients in different RCB grades (RCB-0, I, II, III) ($p=0.001$)(Fig. 5C). Specifically, patients with better treatment response (RCB-0/I) exhibited significantly better DFS than those with significant residual disease (RCB-II/III) ($p=0.036$)(Fig. 5D). Notably, we observed a significant difference in DFS between the RCB-0/I and RCB-II/III groups within the triple-negative, HER2-low subtype ($p=0.038$)(Fig. 5E). However, in the luminal, HER2-low subtype, no significant difference in DFS between the RCB-0/I and RCB-II/III groups was observed ($p=0.102$)(Fig. 5F). In the multivariable analysis, the association between the RCB score and DFS in the HER2-low population remained significant even after adjusting for age, clinical tumor stage, lymph node metastasis, histological grade, and ER status at baseline (HR 2.980 [1.610–5.517], $p<0.001$). Furthermore, ER-positive status was associated with a significantly decreased risk of DFS in the multivariable model(Fig. 6). Conversely, for HER2-ultralow, no significant differences were observed in DFS between different RCB class and between RCB-0/I and RCB-II/III ($p=0.582$ and $p=0.172$, respectively)(Figure S2).

The HER2 expression levels show instability in pre-treatment biopsies and residual lesions after NAT

The HER2 immunohistochemical scores of pre- and post-neoadjuvant therapy specimens displayed instability, resulting in an overall discordance rate of 45.24% (114/252) among non-pCR patients. The proportions of baseline HER2-0, HER2-1+, and HER2-2+scores that remained consistent after NAT were 41.38% (36/87), 42.07% (61/145), and 58.57% (41/70) respectively (Fig. 7A). It is noteworthy that HER2-low and HER2-ultralow also showed instability before and after treatment. Specifically, 32.19% ($n=28$) of patients with HER2-0 in the pre-treatment biopsy specimens experienced a transition to HER2-low, while 10.23% ($n=22$) of HER2-low breast cancer patients transitioned to HER2-0 (Fig. 7B). Additionally, it is important to highlight that among patients who converted from HER2-0 to HER2-low, 22 cases (78.57%) had pre-treatment biopsy samples in a HER2-ultralow state.

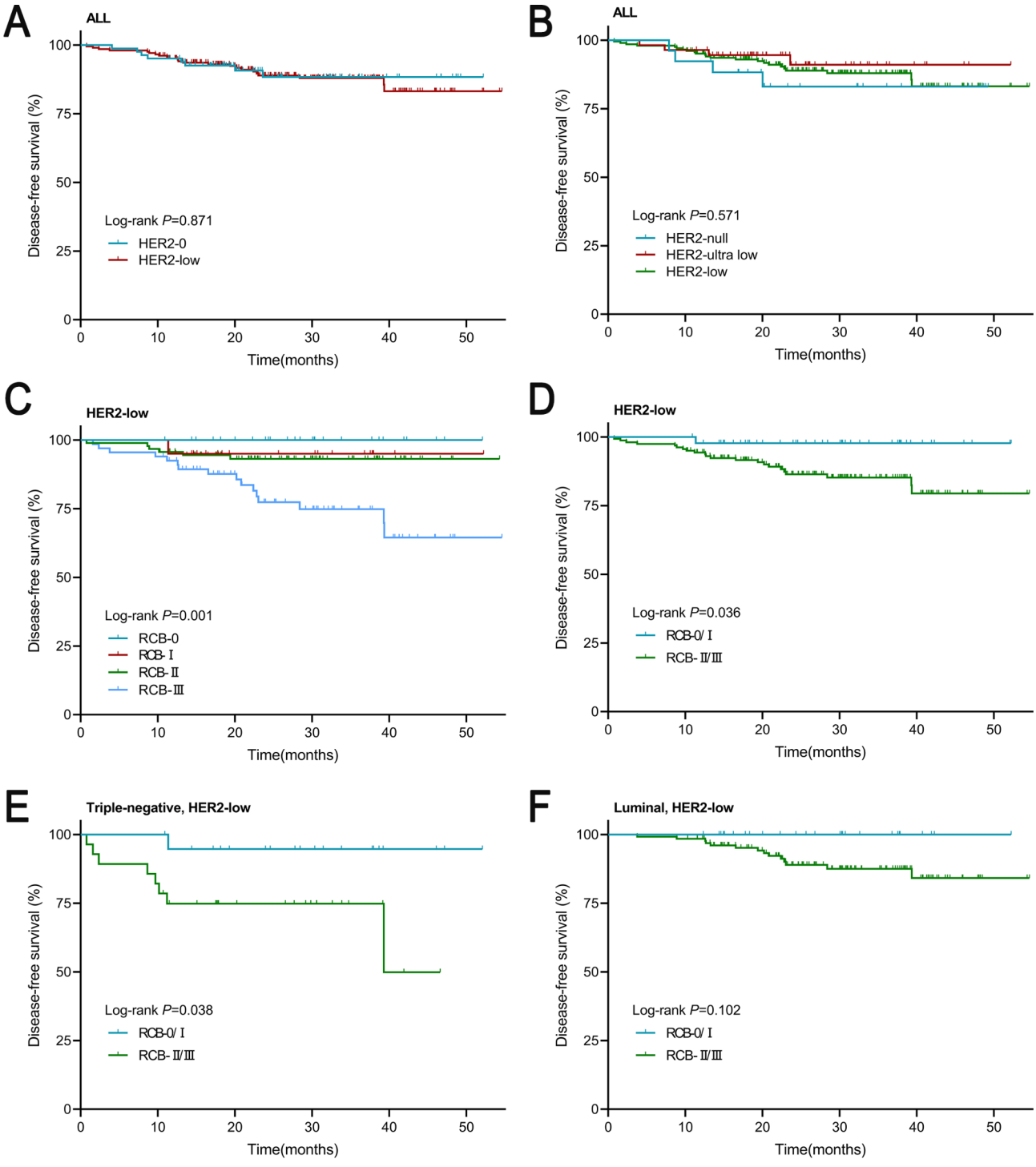

Fig. 5 Kaplan-Meier survival analysis for disease-free survival. (**A**, **B**) The DFS for different HER2 subgroups; (**C**, **D**) The DFS of HER2-low expression subgroup in different RCB class; (**E**) The DFS of Triple-negative, HER2-low within the RCB-0/I group and RCB-II/III group; (**F**) The DFS of Luminal, HER2-low within the RCB-0/I group and RCB-II/III group. The p value from the log-rank test was shown in each box

In the luminal subtype group, 50.00% ($n=20$) of patients with HER2-0 status in pre-treatment biopsy specimens experienced a transformation to HER2-low status, with 16 cases being categorized as HER2-ultralow. Additionally, 9.15% ($n=15$) of the HER2-low patients transformed to HER2-0 status, while 3.05% ($n=5$) acquired HER2-positive status post-treatment (Fig. 7C). Among patients in the triple-negative group with HER2-0 status in pre-treatment biopsy samples, 17.02% ($n=8$) underwent a transformation to HER2-low status, including 6 cases

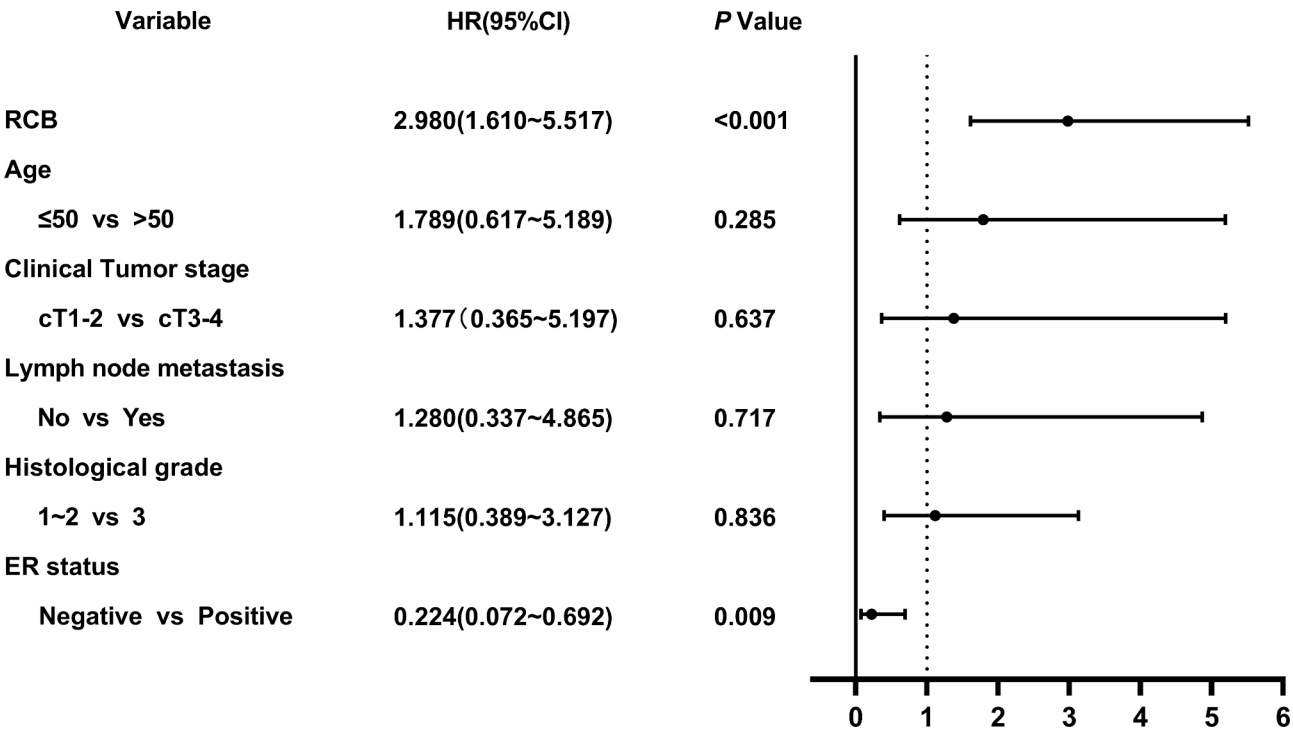

Fig. 6 Multivariable Cox regression analysis for disease-free survival

classified as HER2-ultralow, while 13.73% ($n=7$) of the HER2-low patients transformed to HER2-0 status. Furthermore, 1.96% ($n=1$) acquired HER2-positive status post-treatment (Fig. 7D).

Discussion

This study analyzed 302 consecutive cases of HER2-negative breast cancer that received NAT. Subgroup analysis was performed based on the HER2 expression level. Our results suggested that both HER2-low and HER2-ultralow groups exhibited distinct clinicopathological features. When HER2-0 is subdivided into HER2-ultralow and HER2-null, the pCR rate gradually decreased across the HER2-null, HER2-ultralow, and HER2-low groups, with only HER2-low and HER2-null displaying significant differences. Specifically, the pCR rate of HER2-low was significantly lower than that of HER2-0. It is notable that there was no significant difference in DFS among different HER2 expression levels. However, our validation demonstrated that RCB was a reliable predictor of prognosis in HER2-low populations for the first time. Additionally, we observed the high instability of HER2-low and HER2-ultralow states in pre-treatment core-needle biopsy specimens and post-treatment surgical specimens, indicating their dynamic evolution. This study is the first to investigate the clinical and pathological characteristics, as well as the effectiveness of NAT, in HER2-negative breast cancers, with a specific focus on HER2-ultralow subtype.

Our results indicated that HER2-low expression exhibited distinct clinicopathological characteristics, typified by lower histological grade, higher rates of ER, PR, and AR expression, and a greater prevalence of Luminal types compared to HER2-0, consistent with previous research [15]. Furthermore, the subset of HER2 ultralow expression, derived from HER2 0, has received limited research attention. Our study revealed that the HER2-ultralow expression group displayed lower rates of ER, PR, and AR positivity, as well as fewer Luminal types compared to the HER2-low group. Additionally, the HER2-ultralow group exhibited a higher likelihood of lymph node metastasis than the HER2-null group, while no significant difference in lymph node metastasis was observed between the HER2 low expression and HER2-ultralow groups. A recently published study supported these findings and indicated significant differences between HER2-low and HER2-ultralow patients in N stage, HR status and Ki-67 [16]. Moreover, the study highlighted significant disparities between the two groups in terms of histologic type and postoperative endocrine therapy [16]. Collectively, these differences in clinicopathological characteristics across HER2 expression levels may stem from variations in the enrolled populations. Notably, our cohort consisted of neoadjuvant consecutive HER2-negative breast cancer patients, while the study referred to was conducted on a non-neoadjuvant population [16].

In our study, we compared the pCR rate and RCB class differences between the groups separately and found that

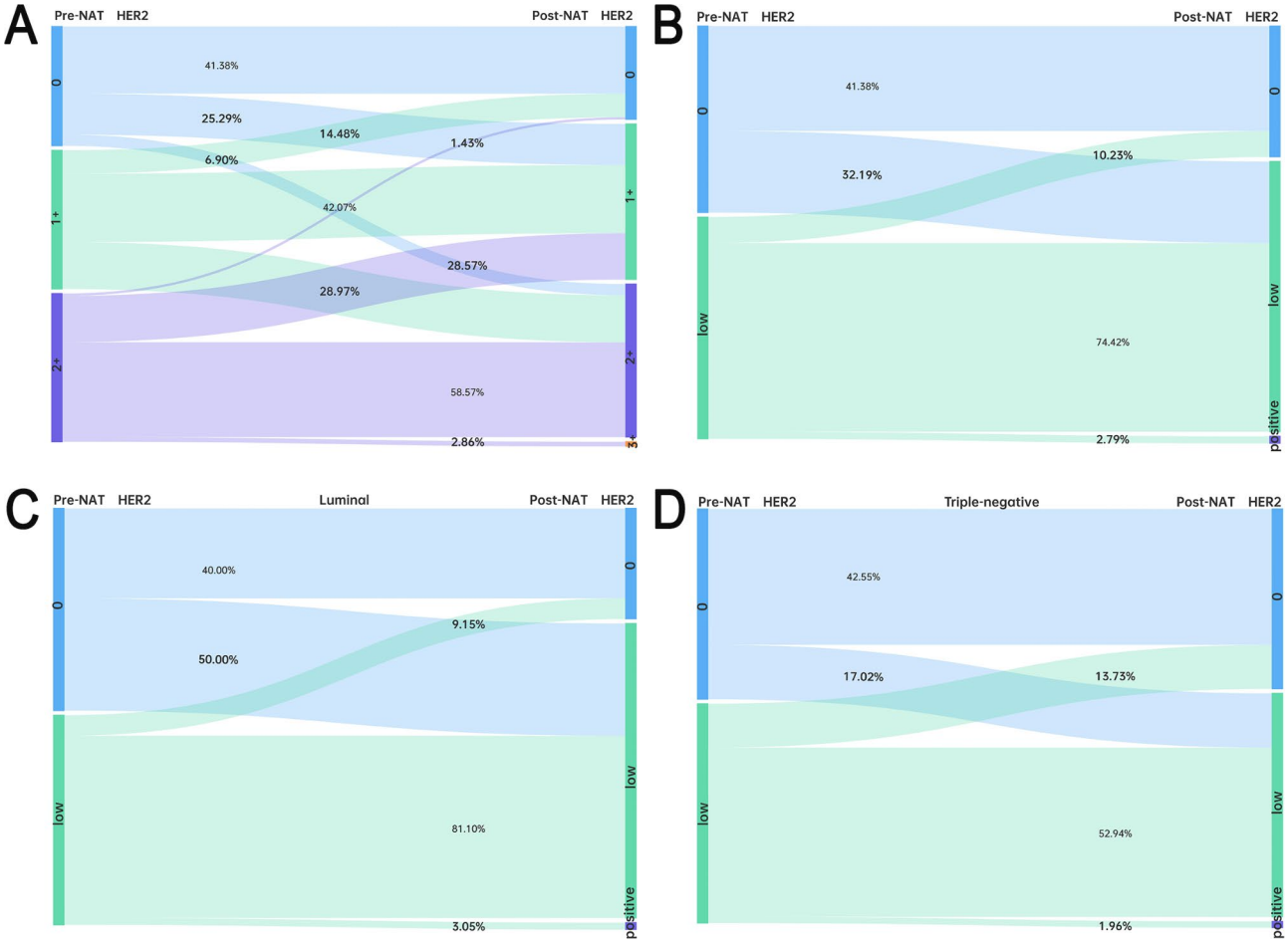

Fig. 7 Evolution of HER2 expression. (**A**, **B**) This Sankey diagram shows the evolution of HER2 expression from baseline biopsy to residual disease after NAT in patients failing to achieve pCR; (**C**) Evolution of HER2 expression in Luminal subtype; (**D**) Evolution of HER2 expression in triple-negative subtype

the pCR rate and RCB efficacy significance rate (RCB-0 to I) were significantly lower in HER2-low than in HER2-0. Additionally, the pCR rate was not influenced by pretreatment cT staging. Furthermore, upon stratifying by molecular staging, we observed that in the luminal and triple-negative subgroups, the pCR rates and efficacy significance rates between the two subgroups no longer exhibited statistical differences. This suggested that the primary determinant of chemosensitivity in HER2-negative breast cancer in our cohort was the HR status, rather than the HER2 expression level, consistent with data from a study conducted in China [4]. However, a recent pooled analysis of individual data from four prospective trials revealed a significantly lower pCR rate for HER2-low than for HER2-0 in the HR-positive group [15], possibly due to the distinct characteristics of the enrolled population. The pCR rates of the three subgroups in our study—HER2-null, HER2-ultralow, and HER2-low—were 35.71%, 22.03%, and 12.56%, respectively. Notably, only HER2-low exhibited a significant difference when compared to HER2-null, while the HER2-ultralow group did not show statistically significant differences with either HER2-null or HER2-low. Additionally, we found a statistically significant difference in the efficacy significance rate between HER2-ultralow and HER2-null in early (cT1-T2) cases.

The proposed subtype of HER2-ultralow holds potential as another eligible subclass for ADC-targeted therapy [17]. Within this "ultralow" phenotype, activating mutations, independent of IHC status, have been documented, providing an additional mechanism for HER2 pathway activation. Notably, the V777LERBB2 mutation is an activating mutation, as evidenced by its significant augmentation of phosphorylation of signaling proteins and enhancement of tyrosine kinase activity [18].

Our findings indicated that there was no significant variation in DFS across different levels of HER2 expression, and this remained unaffected by stratification factors such as molecular typing and pretreatment clinical stage. Correspondingly, most previous research has similarly failed to identify survival disparities based on low HER2 expression status [6, 19–21]. Some studies

suggested that breast cancer patients with low HER2 expression may have better survival outcomes [22–24]. Notably, a comprehensive cohort study comprising over 1 million breast cancer patients diagnosed with low expression of HER2 or negative HER2 revealed a marginal enhancement in overall survival (OS) among breast cancer with low expression of HER2, particularly in advanced TNBC. Specifically, a correlation between HER2-low expression and OS was observed in stage II-IV TNBC and stage III-IV hormone receptor positive breast cancer. Despite the modest nature of these differences, the 5-year OS rate in stage III TNBC patients with low expression of HER2 was 2.0% higher compared to HER2-negative breast cancer, and in stage IV TNBC, the 5-year OS rate increased by 0.4% [25].

Additionally, retrospective studies involving 5161 primary stage I-III breast cancer patients who underwent neoadjuvant chemotherapy and surgical treatment have indicated that RCB can be a predictor of prognosis for various molecular subtypes of breast cancer [12]. The research found that an increased RCB score was significantly associated with worse event-free survival across all four molecular subtypes of breast cancer. Notably, the association between RCB and survival rates was weakest in patients with HR-positive, HER2-negative tumors. Our research further confirmed the association of RCB-II/III with worse DFS in the overall HER2-low population, as compared to RCB-0/I, and similar results were observed in the triple-negative, HER2-low subgroups. Moreover, no significant difference in DFS was observed between patients who achieved RCB-0/I and those who achieved RCB-II/III in the luminal, HER2-low subgroup. However, it is crucial to note that the similarity in the DFS curves of patients with different RCB class in the HER2-ultralow group may be limited by the smaller number of cases in this population.

The evaluation of HER2 IHC scores revealed an overall incidence of inconsistency of 45.24% from pre-treatment baseline biopsies to post-treatment residual tumor bed samples. The major inconsistency was observed in the conversion between HER2-low and HER2-0. Notably, 32.19% of cases initially classified as HER2-0 phenotype on baseline biopsy were later reclassified as HER2-low after NAT, and it is noteworthy that 22 out of these cases (78.57%) originally had an HER2-ultralow status in the pretreatment biopsy sample. It has been reported in previous studies that quantitative testing for HER2 expression in breast cancer patients identified detectable low expression in 67% of patients, suggesting a deficiency in the accuracy of IHC testing [26]. False-negative results may be attributed to human error due to inadequate formalin fixation or insufficient sensitivity of the IHC analysis [27]. This high degree of instability in the evolution of HER2-low and HER2-ultralow status between pre-treatment biopsy specimens and post-treatment surgical specimens reflects the limitations and deficiencies in the detection of HER2 expression. The instability of HER2 low-expression status between primary and metastatic foci, as well as between biopsy tissues and surgical specimens, has been widely documented in the literature [28–31]. Such instability and the limitations of biopsy procedures may hinder the accurate identification of patients who could benefit from effective anti-HER2 ADC therapy.

There are also some limitations of this study. Firstly, it was a single-center retrospective study, which may have led to biased case selection. Additionally, the limited duration of follow-up introduces uncertainty in the findings. Also, few studies have investigated breast cancer with ultralow expression of HER2. Therefore, additional prospective studies are needed to validate our research.

Conclusions

Our study provides a comprehensive analysis of clinicopathologic characteristics, treatment response, and outcomes of patients with HER2-low and HER2-ultralow breast cancers. Both subtypes present distinct clinicopathologic features, and there are notable differences in pCR rates in comparison to HER2-null and HER2-0 breast cancers. Despite these variations, there is no observed prognostic disparity among subgroups characterized by different levels of HER2 expression. Importantly, we found a significant correlation between RCB and DFS following NAT in the HER2-low population. Furthermore, both HER2-low and ultralow expression exhibit high instability before and after neoadjuvant treatment. Therefore, these results can enhance our understanding of HER2-low and HER2-ultralow, and provide a research foundation for further refining the lower limit definition of HER2 low expression.

Supplementary Information

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Supplementary Material 1

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Author contributions
Conceptualization, F.G. and J.Y.; methodology, F.G.; software, X.J. and J.R.; validation, L.C., J.R. and X.K.; formal analysis, X.J.; investigation, X.J. and A.H.; resources, J.Y.; data curation, F.G. and X.J.; writing—original draft preparation, F.G. and X.J.; writing—review and editing, J.Y. and L.C.; visualization, B.L.; supervision, J.Y.; project administration, F.G.; funding acquisition, F.G. All authors have read and agreed to the published version of 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
The study was approved by the Ethics Committee of Renmin Hospital of Wuhan University (Approval code: WDRY2023-K070, date of approval: 16 May 2023). All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments.

Consent for publication
Informed consent was obtained from all individual participants included in the study.

Competing interests
The authors declare no competing interests.

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