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Original Research

Prognostic implications of HER2 changes after neoadjuvant chemotherapy in patients with HER2-zero and HER2-low breast cancer

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Temporal heterogeneity;
Prognosis

Abstract ***Background:*** The transition of human epidermal growth factor receptor 2 (HER2) status after neoadjuvant chemotherapy (NAC) in HER2-low breast cancer has not been thoroughly evaluated. Here, we evaluated the HER2 transition among HER2-zero and HER2-low breast cancer cases post-NAC and its impact on clinical outcomes.
Methods: We included 1288 patients with HER2-low or zero breast cancer who underwent NAC and surgery between 2014 and 2018 and had paired pre- and post-therapeutic HER2 status results.
Results: Among patients who were HER2-zero pre-NAC (n = 650), 68% and 29% were HER2-zero and HER2-low, respectively, post-NAC. Among patients who were HER2-low pre-NAC (n = 638), 32% of patients showed HER2 changes (low to zero), and 59% of patients had a constant HER2-low status post-NAC. Patients with constant HER2-low or transitions from HER2-low to zero had a higher proportion of hormone receptor positivity (84% and

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79%) than those with changes from HER2-zero to low (77%) or with constant HER2-zero (56%), respectively. Multivariable logistic regression analysis revealed that patients with oestrogen receptor positivity had a higher probability of gaining HER2-low expression than those with oestrogen receptor negativity (odds ratio 2.48). No significant differences were observed in terms of overall survival or disease-free survival between patients with and without HER2-changes according to their hormone receptor status, except in the post-therapeutic HER2-low, hormone receptor-negativity subset.

Conclusion: Temporal heterogeneity of HER2-low expression is observed in substantial numbers of post-NAC breast cancer patients. Clinical outcomes show no significant associations, except in the post-therapeutic HER2-low, hormone receptor negativity subset. The prognostic implications of HER2 transition in HER2-low breast cancer require further investigation.

1. Introduction

Breast cancer is a heterogeneous disease with distinct biology and treatment outcomes, and the hormone receptor and human epidermal growth factor receptor 2 (HER2) statuses of tumours are essential characteristics guiding treatment strategies [1]. Newly developed anti-HER2 antibody-drug conjugates (ADC; trastuzumab deruxtecan [T-DXd] and trastuzumab duocarmazine) have shown promising efficacy in patients with HER2-low breast cancer (tumours with immunohistochemistry [IHC] 1+ or IHC 2+ and in situ hybridisation [ISH] negativity), and these observations have prompted ongoing research into the biology and clinical implications of HER2-low breast cancer [2,3]. However, it remains debatable whether HER2-low breast cancer is a separate entity, and there is conflicting evidence about the prognostic role of HER2-low expression in breast cancer [4–9].

Neoadjuvant chemotherapy (NAC) before surgery is a standard treatment option for locally advanced breast cancer to induce tumour shrinkage and increase the possibility of breast-conserving surgery [10]. In fact, HER2 status changes (positive to negative, negative to positive) of residual tumours after NAC have been frequently reported, and the association between HER2 changes and prognostic implications has been evaluated [11–14]. However, HER2 status changes, including the concept of HER2-low and its possible transitions (HER2-zero to low or positive, or vice versa), have not been well delineated. Anti-HER2 ADCs have shown promising efficacy for previously treated metastatic HER2-low breast cancer [2]; therefore, re-testing of HER2 status in residual disease may assist in the identification of potential candidates for anti-HER2 targeting agents. Additionally, investigations into HER2 status changes between pre-therapeutic tissue and post-therapeutic residual tumours may help clarify the treatment-resistant mechanisms. Therefore, we aimed to evaluate the changes in HER2 status after NAC and the implications of HER2 changes for clinical outcomes in patients with HER2-negative (HER2-zero and HER2-low) breast cancer as a follow-up on our initial report on pathologic complete response, long-term outcomes, and recurrence patterns between these two groups post-NAC [7].

2. Methods

2.1. Patients

This retrospective study was performed at Asan Medical Centre, Seoul, Republic of Korea. Fig. 1 shows the Consolidated Standards of Reporting Trials (CONSORT) diagram of this study. We identified 1572 patients who were treated with NAC and underwent curative resection between 2014 and 2018; the details of these patients have been published previously [7]. Among the 1572 patients, we excluded patients who showed a pathological complete response (n = 195) and those with bilateral breast cancer categorised post-operatively (n = 64), multifocal or multicentric breast cancer (n = 4), and unavailable HER2 status (n = 21). Finally, 1288 patients with available HER2 status in paired pre-and post-operative samples were included in this analysis. We collected the patients' data from their medical records, including their baseline characteristics, tumour characteristics, and survival outcomes.

The Allred scoring system was used to assess the oestrogen receptor (ER) and progesterone receptor (PgR) statuses of the tumours. The cut-off value for defining ER or PgR positivity was an Allred score of three or above. We defined a tumour as hormone receptor-positive when it was either ER or PgR-positive, or both.

HER2 status was determined following the 2018 American Society of Clinical Oncology and College of American Pathologists (ASCO-CAP) guidelines [15]. HER2-low tumour was defined as a tumour with HER2 IHC scores 1+ or 2+ accompanied by ISH negativity, and a HER2-zero tumour was defined as a tumour with HER2 IHC score zero. The ER, PgR, and HER2 statuses were assessed using pre-treatment core biopsy tissue and post-therapeutic samples after resection.

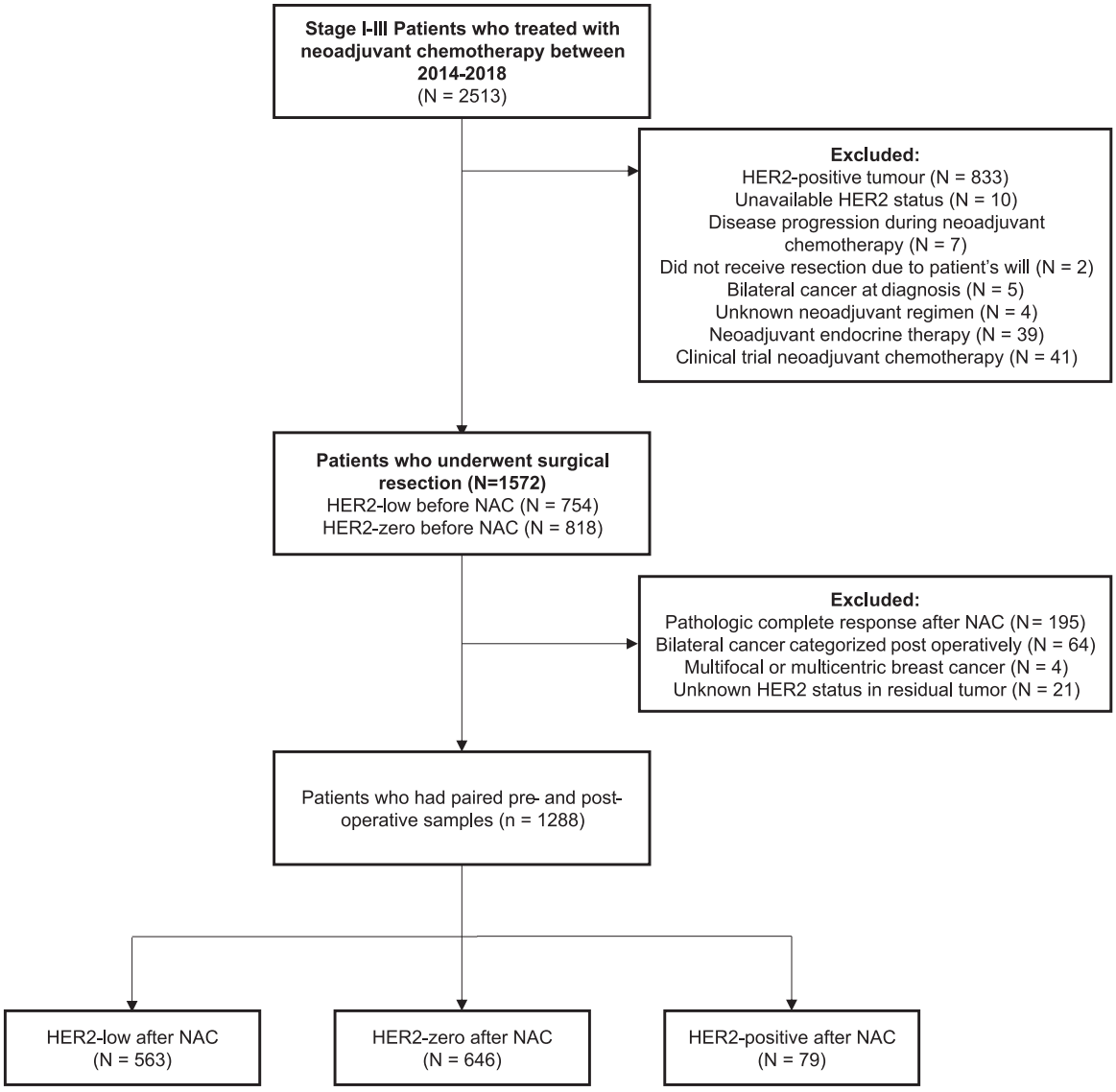

Fig. 1. CONSORT diagram of the included patients who were treated. HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy. CONSORT, Consolidated Standards of Reporting Trials.

2.2. Outcomes

The study outcomes were disease-free survival (DFS) and overall survival (OS) according to HER2 changes post-NAC. DFS was calculated from the date of surgery to the date of recurrence or occurrence of a secondary malignancy or death from any cause (whichever occurred first). OS was calculated from the date of surgery to death from any cause.

2.3. Statistical analysis

We used descriptive statistics to show the patients' baseline and tumour characteristics. Chi-square and Fisher's exact tests were used to analyse the categorical variables, as appropriate. Additionally, a Cohen's Kappa analysis was used to assess the level of concordance of HER2 expression between the primary tumour and residual disease. The agreement between the two lesions was defined as follows: poor, ≤0.2; mild, 0.2–0.4; moderate, 0.4–0.6; substantial, 0.6–0.8; and almost perfect, > 0.8 [16]. We also used Kaplan–Meier methods and log-rank tests to estimate the DFS and OS and compare the subgroups. Bivariable logistic regression analysis was used to evaluate the association between the HER2 transition and other variables, while univariate and multivariate analyses using a Cox proportional hazard model were used to estimate the prognostic implications of the HER2 status and other variables. For the multivariate analysis, age, histologic grade, Ki-67 expression before NAC, ER status before NAC, clinical T stage, clinical N stage, and HER2 transition were included as prognostic factors. The ypT stage, ypN stage, and ER status after NAC were not included in the multivariate analysis because of the interactions between other variables (clinical T stage,

clinical N stage, and ER status before NAC). All statistical analyses were performed using statistical software R, version 4.2.2 (R Core Development Team, Vienna, Austria), and statistical significance was set at $p < 0.05$.

2.4. Ethical approval and consent to participate

This study was reviewed and approved by the Institutional Review Board of Asan Medical Centre (Approval number 2022-0541). The requirement for informed consent was waived owing to this study's retrospective nature.

3. Results

3.1. Changes in HER2 status after NAC

Among the 650 patients who were HER2-zero before NAC, 68% (n = 445) and 29% (n = 189) of patients showed HER2-zero and HER2-low after NAC, respectively. Among the 638 patients who were HER2-low before NAC, 32% (n = 201) of patients showed HER2 changes (low to zero) and 59% (n = 374) of patients showed constant HER2-low status after NAC. Additionally, 16 (2.5%) and 63 (9.9%) patients developed HER2 positivity after NAC in the HER2-zero and HER2-low groups, respectively (Fig. 2A). The discordance rate was 36.5%, and Cohen's kappa value was 0.27.

HER2-low status is strongly associated with hormone receptor status; therefore, we analysed the HER2 transition after NAC separately by hormone receptor status. In the hormone receptor-positive (HR+) groups, 36% (n = 145) of HER2-zero before NAC and 30% (n = 158) of HER2-low before NAC showed a HER2 transition (zero to low, or vice versa) after NAC (Fig. 2B). The discordance rate was 33.8%, and Cohen's kappa value was 0.31. In contrast, in the hormone receptor-negative (HR−) group, 40% (n = 43) of patients with initial HER2-low tumours before NAC developed HER2-zero tumours after NAC, whereas 18% (n = 44) of patients showed a HER2 transition (zero to low) in the initial HER2-zero group (Fig. 2C). The discordance rate was 25.6%, and Cohen's kappa value was 0.40.

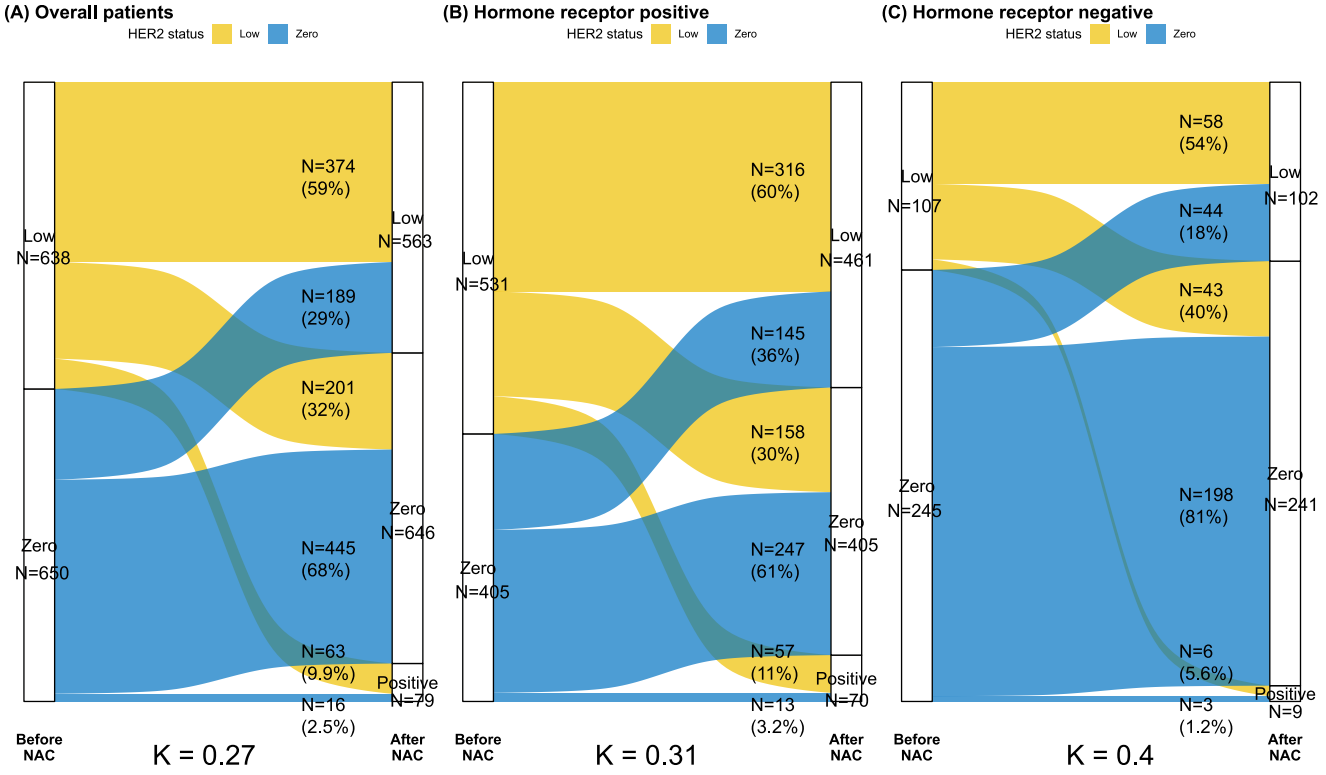

Fig. 2. Changes in HER2 status from the primary tumour to residual disease after neoadjuvant chemotherapy. (A) Overall cohort. (B) Patients with hormone receptor-positivity. (C) Patients with hormone receptor-negativity. HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy.

3.2. Baseline patient characteristics

Table 1 shows the baseline characteristics of the patients with HER2-zero or HER2-low after NAC according to their pre- and post-therapeutic HER2 statuses. Among the patients with HER2-zero after NAC, those with constant HER2-zero tumours showed a higher proportion of nuclear and histologic grades, less HR+ (pre-therapeutic samples, 56% versus 79%; post-therapeutic samples, 51% versus 73%), and higher Ki-67 expression compared to those with HER2 transition (low to zero). Among the patients with HER2-low after NAC, those with constant HER2-low tumours showed more HR+ (pre-therapeutic samples, 84% versus 77%; post-therapeutic samples, 83% versus 77%) and less Ki-67 expression than those with HER2 changes after NAC (zero to low). However, no differences were observed in terms of nuclear and histologic grades among these patients. Furthermore, no differences were found in the distribution of residual cancer burden categories between patients with and without a HER2-change after NAC (HER2-zero post-NAC group, $p = 0.6$; HER2-low post-NAC group, $p = 0.4$).

Supplementary Table 1 presents the baseline characteristics of the patients with HER2-positive tumours after NAC according to their HER2 status before NAC. No significant differences were observed among these patients according to their HER2 status before NAC.

3.3. Association between HER2 changes and other variables

We conducted bivariable and multivariable logistic regression analyses to evaluate the association between a HER2 transition and other variables. We included the variables that showed significant differences between patients who did and did not show HER2 status changes. Among the patients with HER2-zero before NAC, patients with HR+ showed a higher probability of HER2 transition (zero to low) than those with HR− (odds ratio [OR], 2.48; 95% confidence interval [CI], 1.62–3.87) upon multivariable analysis. However, histologic grade and Ki-67 expression were not associated with a HER2 transition (Table 2A). Among the patients with HER2-low before NAC, none of the variables showed a significant association with HER2 changes (low to zero), including ER status (OR, 0.7; 95% CI, 0.42–1.15; $p = 0.2$, Table 2B).

3.4. Prognostic implications of HER2 changes for clinical outcomes

Supplementary Fig. 1 shows the OS and DFS according to the post-therapeutic HER2 status. The 5-year OS rates were 82.4% (95% CI, 79.4–85.5), 90.2% (95% CI, 87.6–92.8), and 92.9% (95% CI, 87.0–99.1) in the HER2-zero, HER2-low, and HER2-positive groups, respectively. Furthermore, the 5-year DFS rates were 71.2% (95% CI, 67.4–75.2), 71.7% (95% CI, 67.7–75.9), and 77.4% (95% CI, 68.5–87.5) in the HER2-zero, HER2-low, and HER2-positive groups, respectively.

To investigate the prognostic implications of HER2 changes, we compared OS and DFS according to pre- and post-therapeutic HER2 statuses, as well as hormone receptor status (Figs. 3 and 4). Among the patients with post-therapeutic HER2-zero tumours, those who showed a HER2 transition (low to zero) had better OS and DFS compared to those with constant HER2-zero tumours (OS, $p = 0.0009$, Fig. 3A; DFS, $p = 0.00028$, Fig. 3B). However, when analysing hormone receptor status separately, no differences were observed in terms of OS and DFS according to HER2 changes among the HR+ subsets (OS, $p = 0.064$, Fig. 3C; DFS, $p = 0.053$, Fig. 3D) and HR− subsets (OS, $p = 0.29$, Fig. 3E; DFS, $p = 0.12$, Fig. 3F).

Furthermore, among the patients with HER2-low in the residual tumours, no significant differences were observed in terms of OS and DFS according to HER2 transition in the overall cohort (OS, $p = 0.067$, Fig. 4A; DFS, $p = 0.51$, Fig. 4B) and HR+ subsets (OS, $p = 0.4$, Fig. 4C; DFS, $p = 0.17$, Fig. 4D). However, among patients with HR− tumours, those with a HER2 transition (zero to low) showed significantly worse OS ($p = 0.0054$) and DFS ($p = 0.0041$) than those with constant HER2-low statuses (Fig. 4E and F).

3.5. Factors associated with clinical outcomes

Tables 3A and 3B show the results of the univariate and multivariate analyses in terms of OS and DFS among patients with HER2-zero and HER2-low after NAC, respectively. Among the patients with HER2-zero in the residual tumours, a HER2 transition (low to zero) was significantly associated with OS (hazard ratio [HR] 0.61; 95% CI, 0.38–0.99; $p = 0.047$) and DFS (HR 0.64; 95% CI, 0.44–0.93; $p = 0.02$, Table 3A). However, among the patients with post-therapeutic HER2-low, a HER2 transition (zero to low) was not associated with OS (HR 1.16; 95% CI, 0.70–1.91; $p = 0.6$) or DFS (HR 1.02; 95% CI, 0.72–1.44; $p > 0.9$) (Table 3B).

4. Discussion

In this study, we evaluated the changes in HER2-low expression among patients who underwent NAC and curative surgical resection. The discordance rate between the primary tumour and residual disease was 36.5%, indicating the temporal heterogeneity of HER2-low expression. Additionally, we found a higher proportion of HR+ among patients with constant HER2-low (84%) or a transition from HER2-low to zero (79%) than in those with changes from HER2-zero to low (77%) or with constant HER2-zero (56%), respectively,

Table 1
Baseline characteristics of patients according to their pre-therapeutic and post-therapeutic HER2 status.

Characteristic	HER2-zero after NAC (n = 646)			HER2-low after NAC (n = 563)		
	Constantly HER2-zero, N = 445 (%)	HER2-low to HER2-zero, N = 201 (%)	*p*-value	HER2-zero to HER2-low, N = 189 (%)	Constantly HER2-low, N = 374 (%)	*p*-value
Age, median (range)	46 (24–74)	46 (24–77)	0.2	46 (27–77)	47 (24–78)	0.7
Histology			0.4			0.8
Invasive ductal carcinoma	409 (92%)	185 (92%)		174 (92%)	350 (94%)	
Invasive lobular carcinoma	13 (2.9%)	9 (4.5%)		7 (3.7%)	11 (2.9%)	
Others	23 (5.2%)	7 (3.5%)		8 (4.2%)	13 (3.5%)	
Nuclear grade before NAC			0.011			0.2
1	5 (1.1%)	3 (1.5%)		3 (1.6%)	3 (0.8%)	
2	301 (68%)	157 (78%)		141 (75%)	305 (82%)	
3	139 (31%)	41 (20%)		43 (23%)	64 (17%)	
Missing	0	0		2	2	
Histologic grade before NAC			0.010			0.3
1	7 (1.6%)	3 (1.5%)		3 (1.6%)	4 (1.1%)	
2	298 (67%)	157 (78%)		141 (75%)	304 (81%)	
3	140 (31%)	41 (20%)		43 (23%)	64 (17%)	
Missing	0	0		2	2	
Clinical stage before NAC			0.7			0.7
Stage IA	3 (0.7%)	2 (1.0%)		1 (0.5%)	1 (0.3%)	
Stage IIA	130 (29%)	54 (27%)		57 (30%)	110 (29%)	
Stage IIB	140 (31%)	75 (37%)		69 (37%)	123 (33%)	
Stage IIIA	71 (16%)	31 (15%)		24 (13%)	58 (16%)	
Stage IIIB	14 (3.1%)	8 (4.0%)		8 (4.2%)	15 (4.0%)	
Stage IIIC	86 (19%)	31 (15%)		29 (15%)	67 (18%)	
Unknown	1 (0.2%)	0 (0%)		1 (0.5%)	0 (0%)	
ER positivity before NAC	247 (56%)	158 (79%)	< 0.001	145 (77%)	316 (84%)	0.024
Ki-67 status before NAC			< 0.001			0.025
≤15.0%	63 (14%)	43 (21%)		38 (20%)	94 (25%)	
15.1–35.0	91 (20%)	70 (35%)		54 (29%)	133 (36%)	
> 35.0	291 (65%)	88 (44%)		97 (51%)	147 (39%)	
Tumour-infiltrating lymphocytes before NAC			0.14			0.14
≤10.0%	314 (71%)	156 (78%)		137 (72%)	300 (80%)	
10.0–59.0	76 (17%)	28 (14%)		33 (17%)	47 (13%)	
> 59.0%	12 (2.7%)	1 (0.5%)		2 (1.1%)	6 (1.6%)	
Unknown	43 (9.7%)	16 (8.0%)		17 (9.0%)	21 (5.6%)	
Pathologic T stage			0.2			0.4
T0 or Tis	20 (4.5%)	4 (2.0%)		2 (1.1%)	10 (2.7%)	
T1 or T1mi	199 (45%)	99 (49%)		74 (39%)	149 (40%)	
T2	169 (38%)	67 (33%)		72 (38%)	154 (41%)	
T3	47 (11%)	28 (14%)		37 (20%)	55 (15%)	
T4	10 (2.2%)	3 (1.5%)		4 (2.1%)	6 (1.6%)	
Pathologic N stage			0.11			0.2
N0	175 (39%)	66 (33%)		64 (34%)	121 (32%)	
N1 or N1mi	165 (37%)	92 (46%)		75 (40%)	175 (47%)	
N2 or N3	105 (24%)	43 (21%)		50 (26%)	78 (21%)	
Neoadjuvant chemotherapy regimen			0.9			0.3
AC based	415 (93%)	188 (94%)		178 (94%)	359 (96%)	
AC	106 (24%)	55 (27%)		58 (31%)	109 (29%)	
AC → docetaxel, carboplatin 4	7 (1.6%)	2 (1.0%)		2 (1.1%)	2 (0.5%)	
AC → weekly paclitaxel 12	10 (2.2%)	1 (0.5%)		3 (1.6%)	3 (0.8%)	
AC → docetaxel 4	285 (64%)	130 (65%)		113 (60%)	241 (64%)	
Non-AC based						
AD 4	5 (1.1%)	0 (0%)		2 (1.1%)	4 (1.1%)	
FAC 6	2 (0.4%)	0 (0%)		0 (0%)	0 (0%)	
FEC → docetaxel 3	30 (6.7%)	13 (6.5%)		11 (5.8%)	15 (4.0%)	
ER status after NAC			< 0.001			0.076
Negative	218 (49%)	54 (27%)		43 (23%)	62 (17%)	
Positive	226 (51%)	147 (73%)		146 (77%)	312 (83%)	
Unknown	1	0		0	0	

(*continued on next page*)

Table 1 (*continued*)

Characteristic	HER2-zero after NAC (n = 646)			HER2-low after NAC (n = 563)		
	Constantly HER2-zero, N = 445 (%)	HER2-low to HER2-zero, N = 201 (%)	*p*-value	HER2-zero to HER2-low, N = 189 (%)	Constantly HER2-low, N = 374 (%)	*p*-value
ER Allred score after NAC	N = 226	N = 147	0.12	N = 146	N = 312	0.5
Low (3–4)	10 (4.4%)	7 (4.8%)		8 (5.5%)	11 (3.5%)	
Moderate (5–6)	22 (9.7%)	6 (4.1%)		5 (3.4%)	8 (2.6%)	
High (7–8)	194 (86%)	134 (91%)		133 (91%)	293 (94%)	
PgR status after NAC			0.002			0.4
Negative	310 (70%)	115 (57%)		87 (46%)	188 (50.2%)	
Positive	133 (30%)	86 (43%)		102 (54%)	186 (49.7%)	
Unknown	2	0		0	0	
PgR Allred score after NAC	N = 133	N = 86	< 0.001	N = 102	N = 186	0.7
Low (3–4)	47 (35%)	23 (27%)		34 (33%)	69 (37%)	
Moderate (5–6)	53 (40%)	28 (33%)		42 (41%)	68 (37%)	
High (7–8)	33 (25%)	35 (41%)		26 (25%)	49 (26%)	
Residual cancer burden			0.6			0.4
Class I	39 (8.8%)	15 (7.5%)		9 (4.8%)	22 (5.9%)	
Class II	260 (59%)	129 (64%)		111 (59%)	236 (63%)	
Class III	128 (29%)	50 (25%)		65 (34%)	105 (28%)	
Unknown	18	7		4	11	

AC, doxorubicin plus cyclophosphamide; AD, doxorubicin plus docetaxel; ER, oestrogen receptor; FAC, 5-fluorouracil, doxorubicin, cyclophosphamide; FEC, 5-fluorouracil, epirubicin, cyclophosphamide; HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy; PgR, progesterone receptor status.

suggesting that HER2-low expression has a strong relationship with HR+. These results are consistent with previous reports that showed high instability of HER2-low expression in residual disease after NAC [17,18] and an association between HER2-low and HR+ [19].

Previously, two studies evaluated the evolution of HER2-low expression among patients with relapsed or metastatic breast cancer and reported a significant discordance rate between primary and recurred or metastatic tumours (38% and 66%, respectively) [20,21]. Notably, these studies observed that HER2-expression was more unstable among the HR+ subgroups compared to the HR− subgroups [22]. For example, in Miglietta et al.'s study [20], in the HR+ subgroup, 47.3% and 53.8% were HER2-low in the primary and matched relapsed tumours, respectively. In contrast, in the HR− subgroup, 35.4% and 36.2% were HER2-low. These results are similar to those of Tarantino et al. [21], which showed a greater degree of instability in HER2-low expression among patients with HR+ (57% versus 64% in primary and metastatic tumours, respectively) than in those with HR− (36% versus 38%, respectively). Our results are similar to their findings, as follows: in the HR+ subgroup, 56.7% (531/936) were HER2-low before NAC, and 49.2% (461/936) were HER2-low after NAC, whereas 30.3% (107/352) were HER2-low before NAC and 28.9% (102/352) were HER2-low after NAC in the HR− subgroup. Additionally, as shown in the multivariable logistic regression analysis, patients with ER-positivity had a higher probability of gaining HER2-low expression than those with ER-negativity (OR, 2.48; $p < 0.001$). Therefore, we hypothesise that HER2-low expression appears to be highly unstable both in residual disease and advanced breast cancer, and HR+ may affect changes in HER2-low after anti-cancer treatment.

The mechanisms underlying the dynamic changes in HER2 expression after NAC remain unclear; however,

Table 2A
Bivariable logistic regression analysis of HER2 status after NAC (HER2-zero versus HER2-low) among the patients with HER2-zero before NAC by hormone receptor status and other variables.

Characteristic	Bivariable			Multivariable		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Histologic grade G3 (versus G1 or G2)	0.67	0.45–0.97	0.036	1.13	0.71–1.78	0.6
ER positivity before NAC (versus negative)	2.72	1.88–3.99	< 0.001	2.48	1.62–3.87	< 0.001
Ki-67 expression (%)						
15.1–35.0 (versus ≤15)	1.05	0.63–1.76	0.86	1.08	0.64–1.84	0.8
> 35.0 (versus ≤15)	0.59	0.38–0.94	0.025	0.8	0.48–1.34	0.4

ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy; OR, odds ratio; CI, confidence interval.

Table 2B
Bivariable logistic regression analysis of HER2 status after NAC (HER2-low versus HER2-zero) among the patients with HER2-low before NAC by hormone receptor status and other variables.

	Bivariable			Multivariable		
Characteristic	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Histologic grade G3 (versus G1 or G2)	1.25	0.82–1.91	0.30	0.97	0.58–1.61	> 0.9
ER positivity before NAC (versus negative)	0.67	0.43–1.03	0.066	0.7	0.42–1.15	0.2
Ki-67 expression (%)						
15.1–35.0 (versus ≤15)	1.16	0.74–1.83	0.53	1.14	0.72–1.82	0.6
> 35.0 (versus ≤15)	1.33	0.86–2.07	0.21	1.19	0.73–1.95	0.5

ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy; OR, odds ratio; CI, confidence interval.

one possible explanation is that therapeutic selective pressure due to chemotherapy results in the up- or down-regulation of HER2 expression as resistance mechanisms [21,23]. Furthermore, inter-observer variations of HER2-low expression may be related to the discordance rate of HER2 status. In fact, distinguishing HER2-low, especially IHC0 versus IHC1+, is highly variable among pathologists [24] because current ASCO-CAP 2018 guidelines for HER2 measurements have not been developed for distinguishing lower levels of HER2 expression [15]. The results of the Destiny-Breast 06 trial (NCT04494425), which includes a mandatory central assessment of HER2 status, is expected to provide a better understanding of this limitation [25]. Additionally, developing reliable quantitative HER2 testing methods are required for accurately identifying lower levels of HER2 expression in the future.

Currently, only a few studies have reported the prognostic implications of a HER2-change (including HER2-low) following NAC [19,26]. According to Miglietta et al., there was no significant association between changes in HER2 status and DFS when compared to patients who showed concordant HER2 status (constant HER2-low versus loss of HER2-low [low to zero], $p = 0.23$; constant HER2-zero versus gaining HER2-low [zero to low], $p = 0.77$) [19]. In contrast, at the 2022 San-Antonio Breast Cancer Symposium, the German Breast Group (GBG) presented new findings suggesting that a shift from HER2-zero to HER2-low was significantly associated with reduced invasive DFS (iDFS) relative to the constant HER2-low group (constant HER2-low versus gaining HER2-low [zero to low], HR 1.43; 95% CI, 1.01–2.01; $p = 0.04$) in patients with HR+ tumours after NAC [26].

These inconsistent results might be attributable to the differences in the included patients' characteristics. For example, Miglietta et al. showed the results of the entire population without separately analysing the clinical outcomes based on hormone receptor status. In contrast, the GBG study focused exclusively on patients with HR+ tumours. Additionally, Miglietta et al. compared within the patients who showed initial HER2-zero or HER2-low, whereas the GBG study compared among the patients with post-therapeutic HER2-low. Considering the differences in the included patients' characteristics and the inconsistent results between the two aforementioned studies [19,26], it is challenging to directly compare these findings and reach definitive conclusions regarding the prognostic role of dynamic changes of HER2-low expression after NAC.

In our study, we observed that the transition from HER2-low to HER2-zero was associated with better OS and DFS in the overall cohort compared to those with constant HER2-zero tumours (Fig. 3A and B). These results are also supported by the multivariate analyses. However, when we analysed the data separately according to the hormone receptor status, no significant differences were observed in terms of OS and DFS, as depicted in Fig. 3C–F. Notably, in the HR+ subset, the shift from HER2-zero to HER2-low was not significantly associated with a decreased DFS or OS compared to constant HER2-low (Fig. 4C and D), which contrasts with the findings of the GBG study. An intriguing finding from our study is that tumours gaining HER2-low expression after NAC exhibited significantly worse OS and DFS compared to the constant HER2-low group in the HR− subset (Fig. 4E and F).

To the best of our knowledge, this is the first study that analysed the clinical outcomes separately according to hormone receptor status and the transition of HER2-low expression after NAC. Based on our findings, we assume that gaining HER2-low from HER2-zero tumour may not be a significant prognostic factor in HR + tumours, but it might be related to DFS and OS in HR− tumours. While our study provides valuable insights, it is important to note that definitive conclusions cannot be drawn at this time due to the limited evidence available. Further investigation is warranted to understand the prognostic role of the transition of HER2-low after NAC. We believe that our study's findings will contribute to designing future studies in this field.

Lastly, HER2-low is now considered a predictive biomarker of the efficacy of T-DXd in metastatic breast

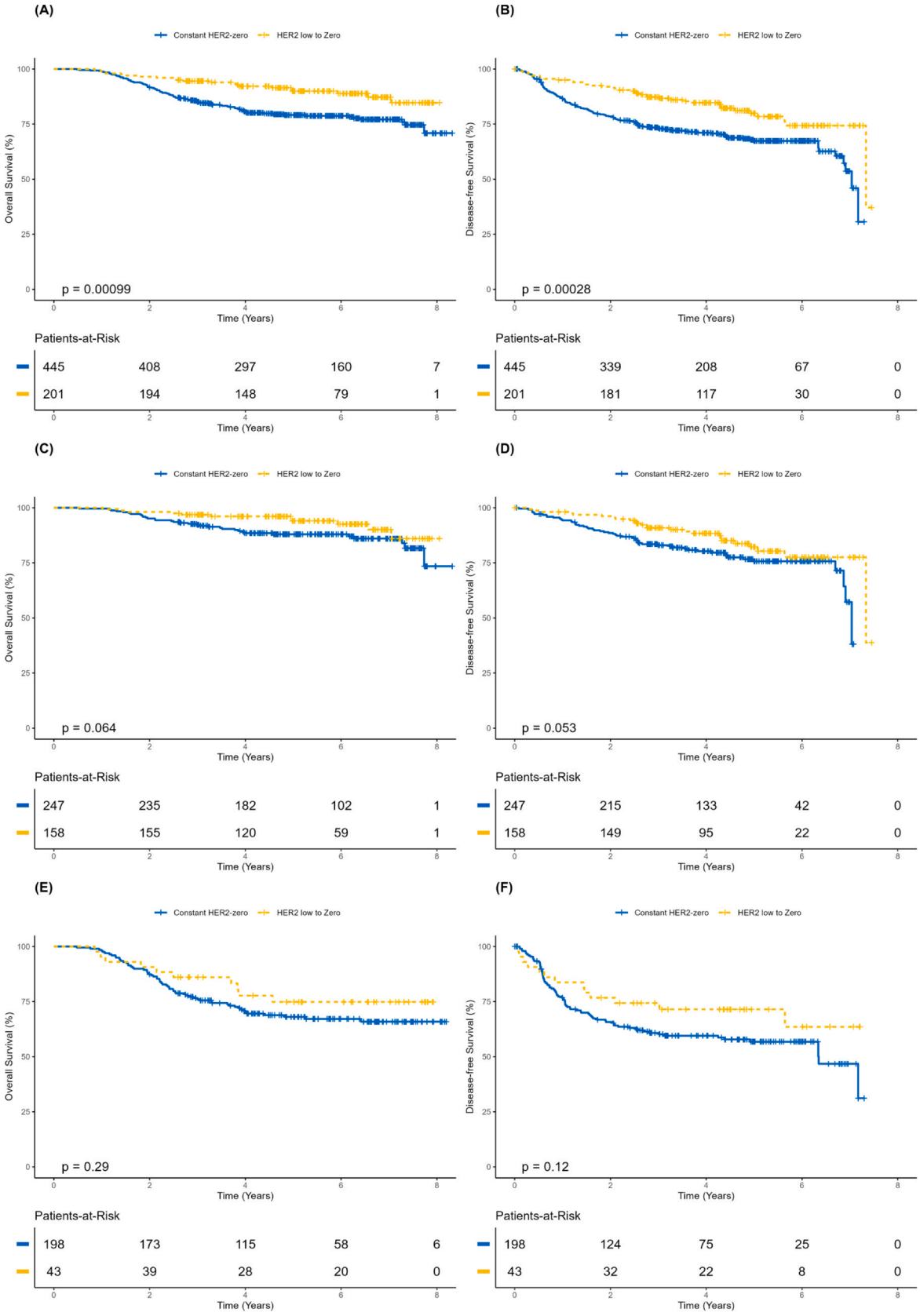

Fig. 3. Clinical outcomes of patients with HER2-zero after NAC according to HER2 status before NAC. (A) OS of all patients. (B) DFS of all patients. (C) OS of patients with hormone receptor positivity. (D) DFS of patients with hormone receptor positivity. (E) OS of patients with hormone receptor negativity. (F) DFS of patients with hormone receptor negativity. DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy; OS, overall survival.

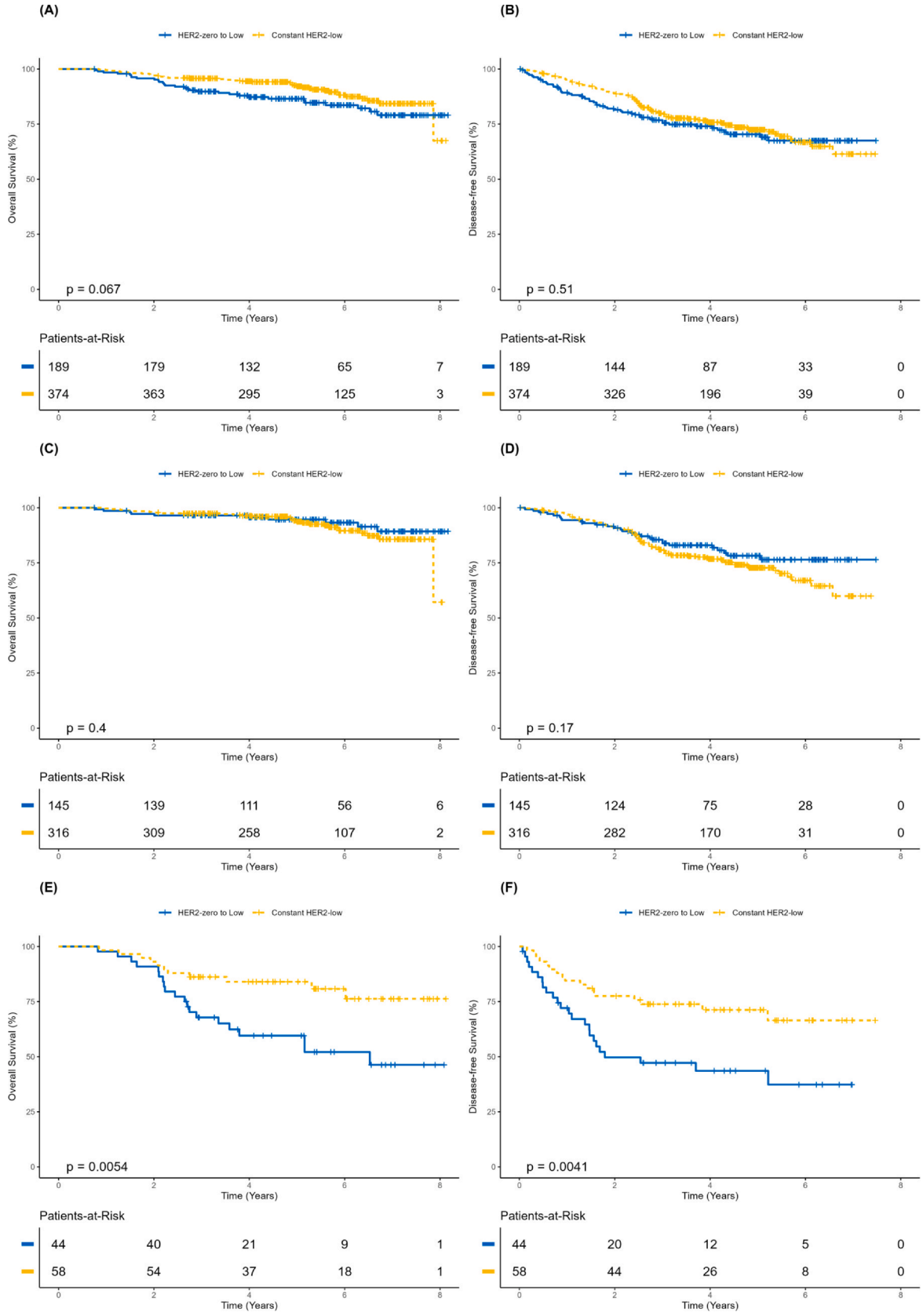

Fig. 4. Clinical outcomes of patients with HER2-low after NAC according to HER2 status before NAC. (A) OS of all patients. (B) DFS of all patients. (C) OS of patients with hormone receptor positivity. (D) DFS of patients with hormone receptor positivity. (E) OS of patients with hormone receptor negativity. (F) DFS of patients with hormone receptor negativity. DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy; OS, overall survival.

Table 3A
Univariable and multivariable analyses of patients with HER2-zero after NAC.

	Overall survival						Disease-free survival					
	Univariable			Multivariable			Univariable			Multivariable		
Characteristic	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age < 40 (versus ≥40)	1.42	0.97–2.09	0.075	1.47	0.99–2.17	0.057	1.33	0.97–1.83	0.076	1.38	1.00–1.92	0.052
Clinical T stage–T3, T4 (versus T1, T2)	1.75	1.21–2.55	0.003	1.57	1.07–2.29	0.021	1.33	0.97–1.83	0.078	1.25	0.91–1.73	0.2
Clinical N stage–N+ (versus N0)	2.21	1.35–3.62	0.002	2.55	1.54–4.22	< 0.001	1.77	1.22–2.55	0.002	2.07	1.42–3.02	< 0.001
Histologic grade G3 (versus G1, G2)	1.76	1.21–2.55	0.003	1.02	0.68–1.52	> 0.9	1.66	1.22–2.25	0.001	1.15	0.82–1.61	0.4
Ki-67 expression (%)												
15.1–35.0 (versus ≤15.0)	5.99	1.38–25.9	0.017	5.88	1.35–25.5	0.018	2.16	1.09–4.27	0.026	1.91	0.96–3.81	0.065
> 35.0 (versus ≤15.0)	15.2	3.76–61.8	< 0.001	9.76	2.35–40.5	0.002	3.91	2.12–7.24	< 0.001	2.43	1.26–4.66	0.008
ER negative before NAC (versus positive)	3.07	2.11–4.47	< 0.001	2.12	1.40–3.22	< 0.001	2.51	1.86–3.37	< 0.001	2.04	1.46–2.85	< 0.001
Pathologic T stage												
T1mi, T1, T2 (versus T0, Tis)	1.02	0.38–2.78	0.97				1.03	0.45–2.33	0.95			
T3, T4 (versus T0, Tis)	1.75	0.61–5.07	0.30				1.69	0.71–4.04	0.23			
Pathologic N stage												
N1, N1mi (versus N0)	1.24	0.78–1.97	0.37				0.96	0.66–1.40	0.85			
N2, N3 (versus N0)	2.63	1.66–4.17	< 0.001				2.30	1.61–3.29	< 0.001			
ER negative after NAC (versus positive)	5.26	3.42–8.10	< 0.001				3.27	2.40–4.46	< 0.001			
HER2 transition from low to zero (versus constantly zero)	0.46	0.29–0.74	0.001	0.61	0.38–0.99	0.047	0.52	0.36–0.74	< 0.001	0.64	0.44–0.93	0.02

CI, confidence interval; ER, oestrogen receptor; HR, hazard ratio; HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy.

Table 3B
Univariable and multivariable analyses of patients with HER2-low after NAC.

	Overall survival						Disease-free survival					
	Univariable			Multivariable			Univariable			Multivariable		
Characteristic	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age < 40 (versus ≥40)	1.63	0.98–2.69	0.057	1.14	0.68–1.92	0.6	1.68	1.20–2.37	0.003	1.49	1.06–2.10	0.023
Clinical T stage–T3, T4 (versus T1, T2)	2.17	1.34–3.50	0.002	2.09	1.27–3.43	0.004	1.64	1.17–2.28	0.004	1.56	1.12–2.19	0.010
Clinical N stage–N+ (versus N0)	4.27	1.95–9.35	< 0.001	5.63	2.52–12.5	< 0.001	2.78	1.79–4.30	< 0.001	3.07	1.96–4.80	< 0.001
Histologic grade G3 (versus G1, G2)	3.93	2.44–6.34	< 0.001	1.95	1.04–3.64	0.036	2.16	1.52–3.06	< 0.001	1.62	1.03–2.56	0.037
Ki67 expression (%)												
15.1–35.0 (versus ≤15.0)	1.19	0.46–3.08	0.72	0.88	0.34–2.30	0.8	1.15	0.70–1.87	0.59	0.96	0.59–1.58	0.9
> 35.0 (versus ≤15.0)	4.44	2.01–9.79	< 0.001	1.99	0.83–4.73	0.12	2.07	1.33–3.21	0.001	1.29	0.79–2.10	0.3
ER negative before NAC (versus positive)	4.46	2.77–7.20	< 0.001	2.89	1.58–5.31	< 0.001	2.15	1.50–3.09	< 0.001	1.71	1.09–2.68	0.019
Pathologic T stage												
T1mi, T1, T2 (versus T0, Tis)	0.52	0.12–2.13	0.36				0.86	0.27–2.71	0.79			
T3, T4 (versus T0, Tis)	1.42	0.33–6.01	0.64				2.21	0.69–7.12	0.18			
Pathologic N stage												
N1, N1mi (versus N0)	1.65	0.81–3.36	0.17				1.81	1.16–2.82	0.008			
N2, N3 (versus N0)	5.13	2.59–10.2	< 0.001				4.01	2.55–6.32	< 0.001			
ER negative after NAC (versus positive)	4.58	2.84–7.38	< 0.001				2.32	1.63–3.29	< 0.001			
HER2 transition from zero to low (versus constantly low)	1.56	0.97–2.53	0.069	1.16	0.70–1.91	0.6	1.12	0.80–1.57	0.51	1.02	0.72–1.44	> 0.9

CI, confidence interval; ER, oestrogen receptor; HR, hazard ratio; HER2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy.

cancer [2], and the application of T-DXd is now moving from an advanced setting to an earlier setting, such as neoadjuvant chemotherapy [27,28]. As of today, no anti-HER2-targeting antibodies have been approved for adjuvant therapy of HER2-low tumours, since trastuzumab did not show clinically significant benefits among these patients [29]. Owing to the poorer outcomes of patients who failed to achieve pathologically complete responses after NAC, there are still unmet needs for better adjuvant chemotherapy for HER2-low breast cancer, and a future clinical trial is required to investigate the efficacy of anti-HER2 ADCs as adjuvant chemotherapy for HER2-low breast cancer. However, as shown in our study, HER2-low expression is highly discordant between the primary tumour and residual disease. Therefore, it is largely unknown which patients would benefit most from anti-HER2 ADC adjuvant chemotherapy: those expressing HER2-low continuously or those gaining or losing HER2-low expression after NAC, or both. In future clinical trials, it may be necessary to test the HER2 status of both the primary tumour and residual disease after NAC to answer this question, and this may help with identifying optimal candidates for adjuvant chemotherapy with anti-HER2 ADC.

Our study had several limitations. First, this was a single-centre retrospective study, which is inherently susceptible to selection bias. However, the relatively large number of patients included in this analysis and the similar treatment strategy used for most patients may overcome this limitation. Additionally, while there was no central review of HER2 expression for the present study, experienced pathologists reviewed the HER2 status within the same institution based on current ASCO-CAP guidelines; therefore, our findings are considered reliable. Second, we did not evaluate the genomic profiles and molecular statuses in our cohort, which may help with understanding the biology of HER2-low tumours. Third, we only included Korean patients. Considering the ethnic disparities in the characteristics and clinical outcomes of breast cancer, our results may be insufficient to generalise to all patients.

In conclusion, temporal heterogeneity of HER2-low expression is observed in substantial numbers of post-NAC breast cancer patients. The clinical outcomes showed no significant associations with changes in HER2 status, except in the post-therapeutic HER2-low, HR− subset. Therefore, the prognostic implications of a HER2 transition in HER2-low breast cancer require further investigation.

Data statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Sora Kang: Data curation, Formal analysis, Writing – review and editing. **So Heun Lee:** Data curation, Writing – review and editing. **Hee Jin Lee:** Review and editing. **Hyehyun Jeong:** Data curation, Review and editing. **Jae Ho Jeong:** Data curation, Review and editing. **Jeong Eun Kim:** Data curation, Review and editing. **Jin-Hee Ahn**: Data curation, Review and editing. **Kyung Hae Jung:** Data curation, Review and editing. **Gyungyub Gong:** Review and editing. **Hak Hee Kim:** Review and editing. **Saebyeol Lee:** Review and editing. **Jongwon Lee:** Review and editing. **Sung-Bae Kim:** Conceptualization, Methodology, Project administration, Supervision, Writing – review and editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kyung Hae Jung has advisory roles at Astra-Zeneca, BIXINK, MSD, Novartis, Pfizer, Roche, Takeda and Everest Medicine. Sung-Bae Kim is a consultant on the advisory boards of Novartis, AstraZeneca, Lilly, Dae Hwa Pharmaceutical Co. Ltd, ISU Abxis, and Daiichi-Sankyo, and has received research funding from Novartis, Sanofi-Aventis, and DongKook Pharm Co., and owns stock in Genopeaks and NeogeneTC. None of the other authors has any conflicts of interest to declare in relation to this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.112956.

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