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HER2 status results in an unstable switch from primary to recurrent breast cancer

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Accurately distinguishing HER2-2+ tumors from HER2-0/1+ tumors via immunohistochemistry (IHC) is still very challenging. HER2 IHC 2+ is considered to indicate moderate expression and is easier to distinguish, with more reliable results in previous and current clinical practice. We focused on HER2-2+ patients and evaluated the switch in HER2 status between primary and paired recurrent disease patients to evaluate the discordance of HER2-2+ expression. We included patients who were HER2-2+ of primary or rebiopsy tumor samples, to evaluate the evolution of HER2-2+ expression. In the cohort with a total of 159 patients with HER2-2+ expression in either primary tumor or locoregional/distant metastasis samples, 44.0% had HER2-2+ in primary tumor and 88.8% in recurrent disease. Among patients with primary and recurrent HER2-2+ breast cancers, 18.5% and 15.2% of the patients, respectively, had HER2 gene amplification via ISH. The overall rate of discordance in HER2 IHC results was 67.1%. Among primary HER2-2+ patients, 74.6% were maintained in the HER2-2+ cohort at the recurrence. The discordance was mostly driven by patients switching from HER2-2+ to HER2-1+ (64.7%). Among HER2-2+ recurrent patients, discordance in the IHC results was mostly driven by switching from HER2-0 to HER2-2+ (47.1%). When HER2-low was added to the analysis, the overall rate of HER2 discordance was 40.4%. The proportion of patients with discordant HER2 expression was significantly greater among HR-positive patients than negative patients (44.1% vs. 21.7%, p=0.062). HER2 expression in primary and recurrent breast cancer samples was highly unstable. Discordance was more frequently observed in the HR-positive population.

Key words: lbreast cancer; HER2 evolution; HER2-2+; IHC

Breast cancer is the most commonly diagnosed malignancy in women worldwide [1]. It has heterogeneous characteristics with distinct clinicopathologic features, prognoses, oncogenic drivers, and sensitivities to treatments [2]. Gene expression studies on breast cancer have revealed different subgroups [3, 4].

However, treatment decisions are based mainly on conventional histopathological factor data obtained via immunohistochemistry (IHC) and in situ hybridization (ISH), which reveal four clinical subtypes: luminal A-like, luminal B-like, human epidermal growth factor receptor 2 (HER2)-negative, and triple-negative breast cancer (TNBC) [5, 6]. The different subtypes exhibit different clinical behaviors, prognoses, and treatment sensitivities.

According to the 2018 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) HER2 Testing Guideline update [7], HER2-positive breast cancer was defined by HER2 protein overexpression according to IHC analysis, with a score of 3+ or an IHC score of 2+ and HER2 gene amplification identified via ISH. To date, HER2-positive diseases are eligible for anti-HER2 targeted therapy. However, as there is an emerging exploration in patients with low levels of HER2 expression [8], a large cohort of patients with HER2-low breast cancer, defined by IHC 1-2+ without amplification via ISH, represents an attractive area of research [9].

To explore the specific data of HER2-negative breast cancer patients, 13 independent datasets were collected from more than 3,000 patients. Overall, 40.3% of patients presented with HER2-0 tumors, 40.4% presented with HER2-1+ tumors, and 19.3% presented with HER2-2+ tumors [10]. The hormone receptor (HR) or HER2 expression pattern can occur during

the switch from primary to recurrent or metastatic breast cancer [11–13]. Therefore, international guidelines emphasize biopsy of recurrent/metastatic sites to guide further treatment [14, 15].

Recent studies including the HER2-low subgroup found significant differences in HER2 expression between primary and recurrent breast cancer [16, 17]. The evolution, in most cases, was related to HER2-0 tumors converting to HER2-low expression in the metastatic lesion or an increasing HER2 score.

Although ASCO/CAP 2023 still uses the previously recommended scoring criteria, the IHC 0 and IHC 1+ groups are often combined for practical purposes [18]. Recent surveys from the CAP [19] showed that 19% of cases read by laboratories generated results with less than or equal to 70% concordance for IHC 0 vs. 1+. There was only 26% concordance between HER2-0 and 1+ compared with 58% concordance between HER2-2+ and 3+. For this reason, moderate HER2 IHC 2+ expression is easier to distinguish than moderate HER2 expression, and more reliable results have been obtained in previous and current clinical practice.

In this study, we focused on HER2-2+ patients and evaluated the difference in HER2-2+ status between primary and paired recurrent/metastatic disease patients to evaluate the discordance of HER2-2+ expression. Another aim was to evaluate the differences in patient characteristics between HR+ and HR- breast cancer patients with HER2-2+ expression. Moreover, we included the HER2-low category for further analysis.

Patients and methods

Population. The current study retrospectively reviewed the data of breast cancer patients who had both primary breast tumor and matched recurrent/metastatic rebiopsy tumor samples at Beijing Cancer Hospital between January 2017 and June 2022. Patients who were HER2-2+ according to either the primary tumor sample or recurrent/metastatic tumor sample were included. Both primary tumor and matched relapse samples were available for HER2 status in all included patients. Patient clinicopathologic characteristics, including age, HR status, HER2 status, breast cancer phenotype, and locoregional treatment and/or distant relapse, were recorded. Patients were excluded from the study if they were male or had a second primary malignancy.

Ethics approval. This study was approved by the Ethical Committees of Peking University Cancer Hospital and Institute (Beijing, China).

Pathology. HER2 results were reassessed by the pathology team of our institute according to the latest standard of HER2 diagnosis by ASCO/CAP Guidelines Update. HER2 IHC was performed on 4 μm-thick tissue sections using a Ventana BenchMark Ultra autostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) with Ventana anti-HER2 (4B5) rabbit antibody according to the manufacturer's instructions. HER2-positivity was considered a score of 3+ according to IHC and/or HER2 amplification determined via ISH, while HER2 negativity was classified as a score of 0/1+/2+ according to IHC in the absence of HER2 amplification identified via ISH. HER2-negative patients were further subclassified as HER2 0 in patients with an IHC score = 0 and as HER2-low in patients with an IHC score = 1+/2+ in the absence of HER2 gene amplification by ISH.

The estrogen receptor (ER) and progestogen receptor (PR) levels were determined via IHC. At least 1% of the cancer cells were positive for ER or PR. HR-positivity was defined as positivity for either the ER or the PR, while HR-negativity was defined as negative if both the ER and the PR were negative. Triple-negative breast cancer was defined as ER-negative, PR-negative, or HER2-negative.

Statistical analysis. All statistical analyses were conducted with SPSS 22.0 statistical software (SPSS, Chicago, IL, USA). All reported p-values are two-sided, with $p<0.05$ being considered to indicate statistical significance. Student's t-test and the Mann-Whitney and Kolmogorov-Smirnov nonparametric tests were used to evaluate the distribution of continuous variables across groups according to clinicopathologic characteristics. The chi-square test (χ^2) was used to compare categorical variables across subgroups. Sankey diagrams were generated to visualize the switch in HER2 expression from primary to recurrent breast cancer.

Post-recurrence survival (PRS) was defined as the time from the date of diagnosis of locoregional relapse/distant metastasis to the date of death or last follow-up. Overall survival (OS) was defined as the time from the date of primary breast cancer diagnosis to the date of death or the last follow-up. The Kaplan-Meier method was adopted to estimate survival curves, and the log-rank test was used to test for differences across groups.

Results

Patient cohorts and clinicopathologic characteristics. A total of 159 patients who had both primary breast tumor and matched recurrent/metastatic rebiopsy tumor samples between January 2017 and June 2022 were diagnosed in our institute. Patients who were HER2-2+ according to either the primary tumor sample or recurrent/metastatic tumor sample were included. All patients had tissue confirmation of breast cancer recurrence or metastasis, as summarized in Table 1.

The distributions according to the primary tumor phenotypes were as follows: HR-positive/HER2-negative, 66.6% (106/159); HR-negative/HER2-negative, 10.7% (17/159); and HER2-positive, 12.6% (20/159). The distribution of recurrent tumor phenotypes was as follows: HR-positive/HER2-negative, 66.6% (106/159); HR-negative/HER2-negative, 15.1% (24/159); and HER2-positive, 14.5% (23/159). The ISH results of certain HER2-2+ primary tumor patients were unavailable because of economic problems in most areas of China between 2005 and 2015.

Table 1. Clinicopathological characteristics in overall patients at diagnosis and metastasis/metastasis.

Characteristics		n (%)/median		n (%)/median
Age at primary BC diagnosis (yy)			48 (23–83)	
Age (years)				
	≤ 50	94 (59.1)		
	> 50	65 (40.9)		
Primary BC			Metastatic/Recurrent tumor	
Histotype				
	Ductal	126 (79.2)		
	Non-ductal	33 (20.8)		
Hormone receptor				
	Negative	25 (15.7)	Negative	35 (22.0)
	Positive	134 (84.3)	Positive	121 (76.1)
			Discordant	3 (1.9)
HER2				
	0	43 (27.0)	0	3 (1.9)
	1+	36 (22.6)	1+	11 (6.9)
	2+	70 (44.0)	2+	135 (84.9)
	3+	10 (6.3)	3+	3 (1.9)
			Discordant	7 (4.4)
HER2-2+				
	HER2-2+ with ISH	54	HER2-2+ with ISH	132
	ISH+	10 (18.5)	ISH+	20 (15.2)
	ISH–	44 (81.5)	ISH–	112 (84.8)
Primary BC histologic grade				
	I	5 (3.0)		
	II	99 (62.3)		
	III	44 (27.7)		
	Unknown	11 (7.0)		
Phenotype				
	HR+/HER2–	106 (66.6)	HR+/HER2–	106 (66.6)
	Triple-negative	17 (10.7)	Triple-negative	24 (15.1)
	HER2-positive	20 (12.6)	HER2-positive	23 (14.5)
	HER2-2+ without ISH	16 (10.1)	HER2-2+ without ISH	3 (1.9)
			HR Discordant	3 (1.9)
Treatment				
Adjuvant			Advanced	
	Chemotherapy	135 (84.9)	Chemotherapy	120 (75.5)
	Hormonal therapy	123 (77.4)	Hormonal therapy	118 (74.2)
	Anti-HER2	17 (10.7)	Anti-HER2	29 (18.2)
Neoadjuvant			PARP-inhibitor	9 (5.7)
	Chemotherapy	36 (22.6)	antiangiogenics	31 (19.5)
	Hormonal therapy	3 (1.9)	immunotherapy	4 (2.5)
Disease-free survival				
	≤ 24 months	41 (25.8)		
	> 24 months	118 (74.2)		
Time from primary BC diagnosis and relapse (mo), median				47.8 (3.0–243.5)
Additional biopsy during the course of disease (n)				18
HER2 score concordant with first biopsy (n)				10
HER2 score discordant with first biopsy (n)				8

Evolution of HER2 expression according to IHC results. Among the 159 patients included, 44.0% (70/159) of the primary breast cancer samples were HER2-2+. Among recurrent/metastatic breast cancer patients, 18 patients underwent a second tissue sampling when the disease progressed. Among these patients, 8 had different HER2 expression levels. Among them, 7 patients exhibited HER2 expression switching after recurrence/metastasis (Table 1). Among the remaining 152 patients with tissue confirmation of recurrence or metastasis, 88.8% (135/152) had HER2-2+ tumors.

Among HER2-2+ primary breast cancer patients who had ISH results (n=54), 18.5% (10/54) had ISH amplification (ISH+); these patients were HER2-positive. However, 81.5% (44/54) of the patients had HER2-2+/ISH nonamplification (ISH–) and were HER2-low.

Among HER2-2+ recurrent/stage IV breast cancer patients who had ISH results (n=132), 15.2% (20/132) were HER2-positive, with ISH amplification. 84.8% (112/132) of patients were HER2-2+/ISH–.

For the HER2-negative patients, the HER2-2+ distribution according to breast cancer subtype is summarized in Supplementary Table S1. Particularly, HER2-2+ expression represented 51.5% (34/66) of primary HR-positive/HER2-negative vs. 71.4% (10/14) of triple-negative cohort, respectively (p=0.240) and 92.0% (92/100) vs. 85.7% (18/21) of recurrent/stage IV HR-positive/HER2-negative vs. triple-negative cohort, respectively (p=0.402).

Among patients with HER2-2+ primary breast cancer (Table 2), the proportion of ISH+ patients was numerically greater in the HR-positive cohort (ISH+ patients in the HR-positive vs. HR-negative cohort: 20.9% (9/43) vs. 9.1% (1/11), respectively; p=0.667). A positive correlation was observed between the HER2 ISH result and the HR in recurrent/metastatic breast cancer patients. In particular, ISH+ represented 9.8% (10/102) of the recurrent/metastatic HR-positive cohort and 33.3% (9/27) of the HR-negative cohort (p=0.005).

The overall rate of HER2 IHC discordance was 67.1% (102/152). In particular, in 26.3% (40/152) and 23.0% (35/152) and 6.6% (10/152) of the patients, a conversion from HER2-0/HER2-1+/HER2-3+ to HER2-2+ was observed, and in 2.0% (3/152) and 7.2% (11/152) and 2.0% (3/152) of the patients, a change from HER2-2+ primary breast cancer to HER2-0/HER2-1+/HER2-3+ recurrent/stage IV breast cancer was found.

Among primary HER2-2+ patients, 74.6% (50/67) were maintained in the HER2-2+ cohort at recurrence/metastasis. The discordance was mostly driven by patients switching from HER2-2+ to HER2-1+ (64.7%, 11/17). Among the HER2-2+ recurrent/metastatic patients, discordance was mostly driven by these patients switching from HER2-0 to HER2-2+ (47.1%, 40/85). The results of IHC staining for HER2 expression in the primary and recurrent breast cancer cohorts are summarized in Table 3 and shown in Figure 1.

When patients without ISH results were excluded, despite at least one additional biopsy after the first biopsy in the advanced setting, for which HER2 was differentially expressed (n=136), 36/136 (26.5%), 33/136 (24.3%), and 30/136 (22.1%) patients with HER2-2+/ISH– were identified in recurrent/metastatic samples after switching from HER2-0, HER2-1+ and HER2-2+/ISH–, respectively (Table 4). However, most of the HER2-2+/ISH– (30/43, 69.8%) primary patients still exhibited the same pattern of expression. The results of the evolution of HER2 status by IHC and ISH results between primary and recurrent/metastatic breast cancer are shown in Figure 2.

Figure 3 shows examples of typical IHC staining of HER2 changing in six cases.

Among the HR-positive primary breast cancer patients (Supplementary Table S2), 32/35 of the HER2-0 patients and 30/31 of the HER2-1+ patients had a conversion to HER2-2+/ISH– status, while 3/35 of the HER2-0 patients and 1/31 of the HER2-1+ patients had a conversion to HER2-2+/ISH+ status. However, 2/33 and 7/33 of the HER2-2+/ISH– patients achieved conversion to HER2-0 and HER2-1+, respectively. With the maintenance of 23/33 maintaining HER2-2+/ISH– status, 1/33 patients achieved ISH+ status while maintaining HER2-2+ expression. Among HER2-2+/ISH+ patients,

Table 2. ISH results in HER2-2 expression of primary and recurrent/metastatic breast cancer samples.

	HER2-2+		
	ISH-neg	ISH-pos	p-value
Primary breast cancer n (%)			
HR-positive	34 (79.1)	9 (20.9)	0.667
HR-negative	10 (90.9)	1 (9.1)	
Recurrent breast cancer n (%)			
HR-positive	92 (90.2)	10 (9.8)	0.005
HR-negative	18 (66.7)	9 (33.3)	

Table 3. The discordance of HER2 expression by IHC in the primary and recurrent BC.

		HER2 recurrence/metastasis			
		0	1+	2+	3+
HER2 primary	0			40 (26.3%)	
	1+			35 (23.0%)	
	2+	3 (2.0%)	11 (7.2%)	50 (32.9%)	3 (2.0%)
	3+			10 (6.6%)	

Table 4. The discordance of HER2 expression by IHC and ISH results in the primary and recurrent BC.

		HER2 recurrence/metastasis				
		0	1+	2+, ISH–	2+, ISH+	3+
HER2 primary	0			36 (26.5)	4 (2.9)	
	1+			33 (24.3)	1 (0.7)	
	2+, ISH–	2 (1.5)	9 (6.6)	30 (22.1)	2 (1.5)	
	2+, ISH+	1 (0.7)	1 (0.7)	4 (2.9)	3 (2.2)	1 (0.7)
	3+			2 (1.5)	7 (5.1)	

Figure 1. Evolution of HER2 expression between primary and recurrent/metastatic breast cancer.

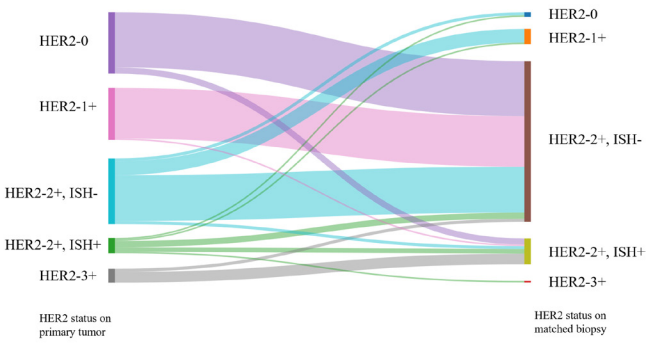

Figure 2. Evolution of HER2 status by IHC and ISH results between primary and recurrent/metastatic breast cancer.

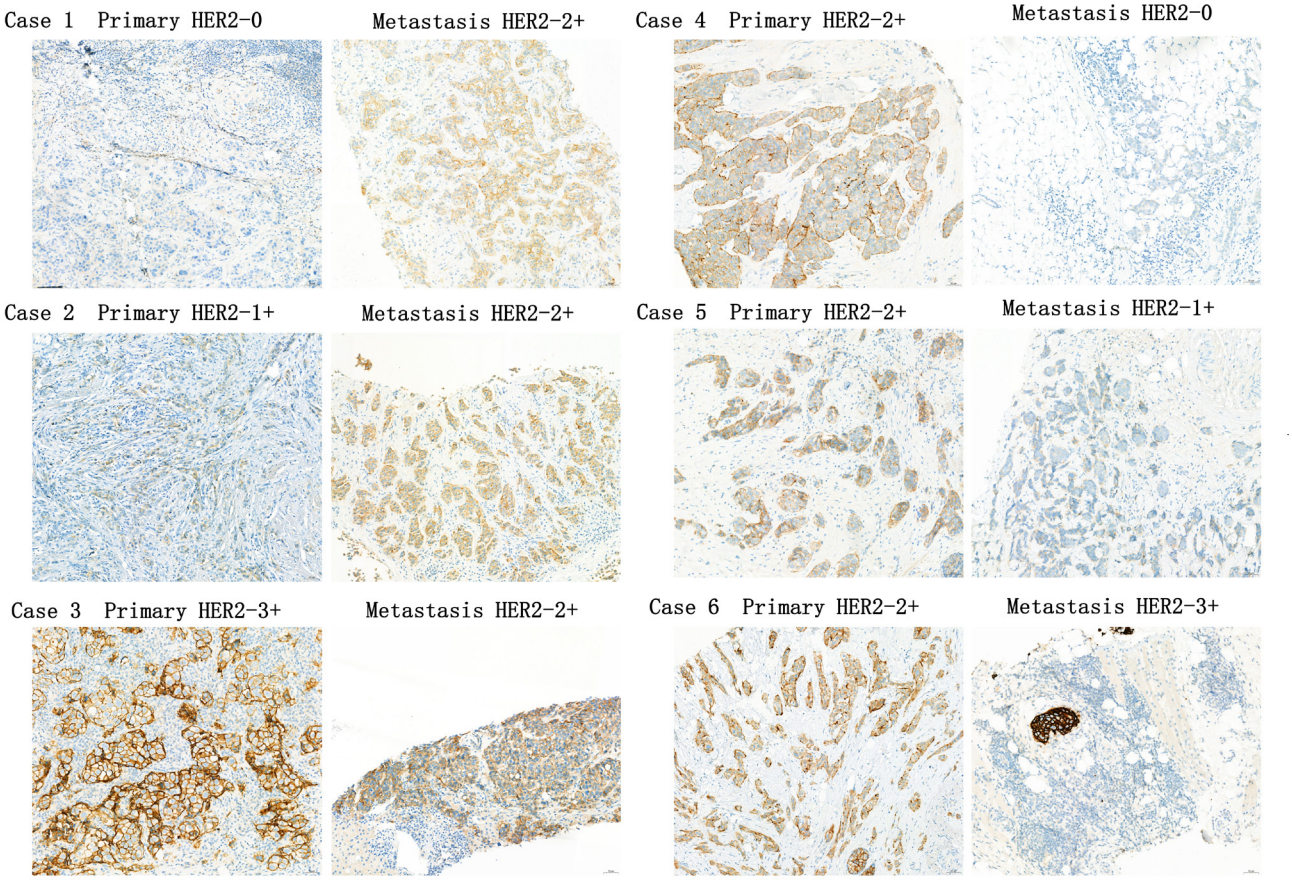

Figure 3. Six examples of HER2 status in IHC evolution from primary breast cancer to distant metastases. Case 1: A HER2-0 primary tumor with a HER2-2+ metastasis. Case 2: A HER2-1+ primary tumor with a HER2-2+ metastasis. Case 3: A HER2-3+ primary tumor with a HER2-2+ metastasis. Case 4: A HER2-2+ primary tumor with a HER2-0 metastasis. Case 5: A HER2-2+ primary tumor with a HER2-1+ metastasis. Case 6: A HER2-2+ primary tumor with a HER2-3+ metastasis.

1/8 and 3/8 patients achieved conversion to HER2-1+ and HER2-2+/ISH+ status, respectively. However, half of the (4/8) HER2-2+/ISH+ patients maintained HER2-positive expression. Among patients with HER2-3+ expression, half (2/4) lost HER2 ISH positivity with HER2-2+/ISH– conversion.

Among the HR-negative primary breast cancer patients (Supplementary Table S2), 2/3 of the HER2-0 patients and 3/3 of the HER2-1+ patients had a conversion to HER2-2+/ISH– status, while 1/3 of the HER2-0 patients had a conversion to HER2-2+/ISH+ status. While 7/10 patients

maintained HER2-2+/ISH– status, 1/10 patients achieved ISH+ status while maintaining HER2-2+ expression. While one HER2-2+/ISH+ patient lost ISH positivity, all patients with HER2-3+ expression still maintained HER2 positivity (5/5) after switching to HER2-2+/ISH+ status.

HER2 expression before and after surgery according to HER2 IHC results. A total of 48 patients had biopsies of primary breast tumors before surgery. All patients who received neoadjuvant therapy (n=36) had biopsies taken before treatment, and 11.1% (4/36) of these patients underwent a switch in HR or HER2 expression. Specifically, 2 patients had higher HER2 expression-from HER2-0 to HER2-1+ and from HER2-1+ to HER2-2+/ISH– while maintaining the same HR expression after neoadjuvant treatment. One patient lost HR positivity but had the same HER2-positive expression, while another patient lost HR positivity but gained HER2-1+ expression from HER2-0. All patients above received chemotherapy as a neoadjuvant therapy. However, all HER2-positive patients did not receive anti-HER2 therapy because of economic problems.

Among patients who were not treated before surgery (n=12), 3 had a switch in HER2 expression but a stable HR expression. Specifically, 2 patients had higher HER2 expression-from HER2-0 to HER2-1+ and from HER2-1+ to HER2-2+/ISH–. One patient maintained HER2 positivity from HER2-3+ to HER2-2+/ISH+.

Evolution of HER2 expression according to HER2-low status. Among the HER2-low patients, 55.9% (80/143) had a primary tumor and 83.6% (127/152) had a recurrent tumor; these patients accounted for 65.0% (80/123) and 97.7% (127/130) of the entire HER2-negative primary breast cancer and recurrent cohort, respectively. In particular, patients with low HER2 expression represented 62.3% (66/106) of the primary HR-positive/HER2-negative cohort and 82.4% (14/17) of the triple-negative cohort (p=0.169), and 99.0% (103/104) and 95.7% (22/23) of the recurrent/stage IV HR-positive/HER2-negative and triple-negative cohorts, respectively (p=0.331).

The distribution of HER2-negative patients according to breast cancer subtype is summarized in Supplementary Table S3.

The overall rate of HER2 discordance was 40.4% (57/141), mostly represented by HER2-0 switching to HER2-low (39/57, 68.4%). A total of 73/141 (51.8%) patients maintained HER2-low expression, and 11/141 (7.8%) maintained HER2-positive expression from primary to recurrent/metastatic breast cancer.

Among patients with HER2-negative primary tumors (Supplementary Table S4), the rate of HER2 discordance was 40.2% (49/122). The proportion of HER2 discordant cases among HER2-negative primary breast cancer patients was numerically greater in the HR-positive/HER2-negative cohort (HER2 discordant cases in HR-positive/HER2-negative vs. triple-negative: 42.9% (45/105) vs. 23.5% (4/17), respectively; p=0.184), and this difference was mainly driven by the proportion of HER2-0 breast cancer patients switching to HER2-low expression (79.6%, 39/49).

Among the total population (Supplementary Table S4), the proportion of HER2 discordant patients was significantly greater among HR-positive patients than among HR-negative patients (HR-positive vs. HR-negative: 44.1% (52/118) vs. 21.7% (5/23), respectively; p=0.062).

Specifically, among primary HR-positive/HER2-negative patients, 35.0% (36/103) switched from HER2-0 to HER2-low expression, while 1.9% (2/103) converted from HER2-low to HER2-0 breast cancer. Among HR-negative/HER2-negative primary breast cancer patients, 11.8% (2/17) had a conversion from the HER2-0 to the HER2-low phenotype, while none of the patients had a conversion from the HER2-low to the HER2-0 phenotype.

The conversion of HER2 expression according to the tumor phenotype of the secondary lesion is shown in Supplementary Table S5. In detail, 38.0% (38/100) of the HR-positive patients showed a conversion from HER2-0/HER2-positive to HER2-low expression or an opposite change while maintaining HR positivity. However, 17.6% (3/17) of the HR-negative patients showed a conversion from HER2-0/HER2-positive to HER2-low expression or an opposite change while maintaining HR-negative status.

Among HR-positive/HER2-negative patients, 36.0% (31/86) of patients underwent conversion from HER2-0 to HER2-low or the opposite change while maintaining the same breast cancer phenotype. However, among triple-negative patients, only a conversion from HER2-0 to HER2-low could be found (18.2%, 2/11) in patients maintaining the same breast cancer phenotype.

Evolution of HER2 expression according to tumor sample HER2-low status. The percentage of patients with discordant HER2-low status was significantly different across the different metastatic sites (p=0.026; Supplementary Table S6). In detail, all contralateral breast and skin metastases presented discordant HER2 results. However, all central nervous system metastases maintained the same low HER2 expression pattern. Higher rates of HER2 discordance were observed for bone (50.0%) and other sites (50.0%), followed by the lymph nodes (41.2%), chest wall/ipsilateral breast (40.7%), lung/pleura (38.5%), and liver (24.3%). The specific direction of discordance in the contralateral breast was considered to be conversion from HER2-0 to HER2-2+/ISH–. The direction of discordance in the skin was defined as the conversion from HER2-0 to HER2-2+/ISH– or from HER2-0 to HER2-2+/ISH+.

Exploratory survival analysis. According to the exploratory survival analyses of the entire population (Supplementary Figure S1), no significant differences in PRS or OS were observed between patients with or without discordance in HER2 expression (p=0.298 for PRS, p=0.104 for OS). When focusing on HR-positive primary tumors, the presence or absence of HER2 during evolution did not influence the PRS (p=0.166). However, a significant association with

overall survival was observed. In particular, among patients with HR-positive breast cancer in the primary tumor, those with discordant HER2 expression experienced a slight trend toward longer OS than patients with concordant HER2 expression (discordant vs. concordant: median OS 198.6 vs. 122.7 months, respectively; p=0.070).

Switching of HER2 positivity. The HER2-positive phenotype exhibited a proportion of 10.7% (15/140) in primary or recurrent breast cancer samples when HER2 was lost or increased. Specifically, the HER2-positive phenotype in primary breast cancer samples was not detected in 42.1% (8/19) of the patients, while 11 patients were positive. A total of 7/121 (5.8%) recurrent breast cancer samples were positive for HER2. Among the patients with HER2 positivity (n=15), 53.3% (8/15) lacked HER2 overexpression or amplification, and 46.7% (7/15) had HER2-positive tumors.

Among HER2-positive breast cancer patients who exhibited HER2 loss from primary to recurrent breast cancer (n=8), the great majority (87.5%, n=7) exhibited low HER2 expression. Among the HER2-negative breast cancer patients who gained HER2 positivity from primary to recurrent breast cancer (n=7), 3 had HER2-low tumors (42.9%, n=3), while 4 had HER2-0 tumors.

Discussion

Breast cancer patients with HER2-2+ expression exhibited heterogeneous characteristics. We explored patients with HER2-2+ expression in matched primary or recurrent/metastatic tumor samples to evaluate the evolution of HER2-2 expression and its association with HR status. Moreover, with the emergence of a novel entity, we included the HER2-low-expression breast cancer category in the analysis.

Among the cohort with a total of 159 breast cancer patients with HER2-2+ expression in either primary tumor or locoregional/distant metastasis samples, 44.0% of these patients had HER2-2+ breast cancer in the primary tumor, and 88.8% had recurrent disease. Among the primary and recurrent/metastatic HER2-2+ breast cancer patients, 18.5% and 15.2%, respectively, had ISH amplification. We found a numerically greater proportion of HER2-low patients with recurrent disease than primary tumor patients, which was similar to the findings of previous studies [15].We found a numerically enriched population of both HER2-2+ and HER2-low tumor patients among recurrent/metastatic HR-positive/HER2-negative patients compared with that among TNBC patients, which was consistent with the findings of previous studies showing a greater incidence of HR-positive/HER2-negative tumors rather than TNBC among HER2-low breast tumors [10, 16, 18, 20]. However, the opposite results were obtained for primary TNBC lesions enriched in HER2-2+ or HER2-low tumors, possibly due to the relatively small sample size of TNBC patients.

According to the ISH results of HER2-2+ patients, there was a numerically enriched population of HER2-2+/ISH+ tumors in primary HR-positive patients (20.9%) compared with that in HR-negative patients (9.1%). This result might be consistent with our findings and real status in a larger database, in which a greater percentage of the HR-negative patients were HER2-positive. This phenomenon could be explained by the much greater incidence of HR+/HER2– than of the other subtypes, which accounts for a much lower percentage of HR+ patients who were HER2-positive. As in our study, there were more HER2-2+/ISH+ HR-positive patients than HR-negative patients, which was similar to the findings of a previous study in which the absolute percentage of HR+/HER2+ patients was greater (10.3%) than that of HR–/HER2+ patients (4.6%) in the whole population [21]. Interestingly, for recurrent/metastatic breast cancer samples, we found a significantly greater proportion of HR-negative HER2-2+/ISH+ patients than HR-positive patients.

Our aim was to investigate the evolution of HER2 expression from primary breast cancer to matched locoregional recurrent or distant metastatic tumor samples from HER2-2+ patients and HER2-low patients.

The overall rate of HER2 IHC discordance was 67.1%. According to our study of HER2-2+ expression in either primary or recurrent/metastatic breast cancer, more than 70.0% of patients maintained HER2-2+ expression when the disease progressed. For primary breast cancer patients, almost two-thirds (64.7%) of the changes in HER2-2+ expression were driven by patients switching from HER2-2+ to HER2-1+ expression in the metastatic lesion. This result was consistent with a recent report showing a decreased rate of HER2-2+ conversion to HER2-0 or HER2-3+ [22]. Almost half (47.1%) of the patients who expressed HER2-2+ in metastatic lesions exhibited conversion from HER2-0 in the primary tumor.

When combined with the ISH results, the proportions of patients whose metastatic lesions were HER2-0, HER2-1+, or HER2-2+/ISH– were converted to HER2-2+/ISH– were similar, as were the proportions of patients with HER2-2+/ISH+ or HER2-3+ (22.1–26.5%). However, most of the HER2-2+/ISH– (69.8%) primary patients still maintained the same HER2 expression pattern in advanced lesions.

When combining the HER2-1+ and HER2-2+/ISH– results as HER2-low status, the overall rate of HER2 discordance was 40.4%, with more than two-thirds (68.4%) converting from HER2-0 to HER2-low expression, especially in HER2-negative breast cancer (79.6%). More than half of the patients maintained HER2-low expression or HER2-positive expression. Recent studies have also investigated the evolution of HER2 expression from primary breast cancer to matched samples of locoregional recurrences or distant metastases and have shown that HER2-low expression is mostly driven by patients switching to or from HER2-low expression, especially from HER2-0 to HER2-low [16]. Although the population was not the same, we still found a similar proportion of patients with HER2-0 and HER2-low disease. Overall, our results suggest

that HER2 expression may be highly unstable when the disease progresses. This switch may open new therapeutic opportunities in a relevant proportion of patients.

It is also worth mentioning that HR-positive/HER2-negative patients had numerically greater HER2 discordance rates than triple-negative patients (p=0.184). When we divided the whole population according to HR status, our results showed a similar trend that discordance was more frequent in the HR-positive population (p=0.062). This phenomenon has also been observed in a study concerning HER2-low expression discordance, in which higher conversion rates were observed in HR-positive tumors than in triple-negative tumors [16]. This might mean that HR-positive primary breast cancer patients are more likely to experience changes in treatment strategies when disease recurrence/metastasis occurs.

A total of 35.0% of the primary HR-positive/HER2-negative patients switched from HER2-0 to HER2-low expression when they progressed, while only 11.8% of the HR-negative/HER2-negative patients exhibited a change from HER2-0 to HER2-low expression. This observation was consistent with that of a previous study concerning the evolution of HER2 status, which revealed that 13.9% of triple-negative primary breast cancer patients gained HER2-low expression from HER2-0, whereas 21.4% of HR-positive/HER2-negative patients had HER2-low expression [16]. This finding may lead to the identification of patients with primary HER2-0 expression who exhibit low HER2 expression after disease progression and when treatment options are exhausted, especially for primary HR-positive patients.

With the emergence of anti-HER2 antibody-drug conjugates (ADCs), HER2-low patients gained an established benefit from anti-HER2 ADCs, as shown by the findings of trastuzumab deruxtecan in both HR-positive and HR-negative populations [23], although HER2-low evolution was less frequently observed in triple-negative patients than in HR-positive patients in our study.

Notably, more than one-third of the HR-positive patients and approximately 18.0% of the triple-negative patients were converted to or from the HER2-low subgroup while maintaining the same breast cancer phenotype.

For primary HR-positive patients, most HER2-0 and HER2-1+ patients had HER2-2+/ISH– results, whereas HER2-2+/ISH– patients were less likely to have HER2-2+/ISH-positive results. Most patients with HR-positive HER2-2+/ISH– expression maintained a low HER2 expression status when the disease progressed, with 27.3% (9/33) having lower HER2 expression. Half of the HR-positive patients lost HER2 positivity when the disease progressed. However, for primary HR-negative patients, we found that almost all HER2-positive patients (83.3%, 5/6) maintained their positivity after disease progression, which was different from what was observed in HR-positive patients. This phenomenon was similar to the findings of a large-scale study in which phenotypic profiles were compared between matched primary and metastatic breast cancer patients in the European Society of Medicine database; the findings demonstrated that primary HR+/HER2+ tumors exhibited the highest rate of change [13], which could completely change the treatment strategy.

Among the HER2-positive phenotypes of primary breast cancer, 42.1% of the patients in our study switched to the HER2-negative subtype. However, a lower proportion of HER2-negative patients acquired HER2 positivity, which was consistent with the findings of previous studies [11]. Moreover, in our study, as expected, and consistent with available research [11, 13, 16, 24–26], the HER2-positive phenotype in both primary tumors and recurrent lesions was relatively stable, with 10.7% of the total cases showing HER2 positivity gain or loss. However, this proportion was slightly greater than that in some available evidence, with less than 10% of the total patients showing HER2 positivity changes [11, 13, 16]. This difference may be explained by the small sample size as well as the inclusion of only HER2-2+ patients with matched primary or recurrent/metastatic samples in our study, which may exclude some HER2-3+ patients.

Among patients who had biopsies taken before neoadjuvant therapy or surgery, we found a 14.6% change in HR or HER2 expression. Changes from HR-positive to HR-negative were observed only in patients who had received treatment before surgery. This phenomenon was also observed in a previous study concerning the receptor from Tru-cut biopsy and residual tumor with or without neoadjuvant therapy, which revealed that a change in receptor status from positive to negative was mainly associated with the effect of neoadjuvant chemotherapy [27]. Most of the changes occurred in HER2 expression. For HER2-negative breast cancer patients, changes in HER2 expression are presented as HER2-0 to HER2-1+ or HER2-1+ to HER2-2+/ISH–; these phenomena are not influenced by whether patients received neoadjuvant therapy. A previous study concluded that although neoadjuvant treatment plays a role in receptor discordance, the shift from negative to positive is thought to be primarily related to intratumoral heterogeneity. Although the higher but not positive expression of HER2 after surgery, that we observed, did not influence the current treatment strategy, therefore this trend could still be explained by heterogeneity holding clinical importance due to adjuvant therapy requirements.

According to our observations, HER2 expression discordance appeared to be affected by the type of sample at the relapse site, with all contralateral breast and skin metastases presenting discordant HER2 results and all central nervous system (CNS) metastases maintained HER2-low expression without discordance compared to those at other metastatic sites. Specifically, all contralateral breast metastases were converted from HER2-0 to HER2-2+/ISH–, and HR expression was maintained. Thus, this switch would not change the molecular category of breast cancer, but a reconsideration of the HER2-low treatment strategy might be needed. In our

study, and in previous studies, stable HER2 expression was found in CNS metastatic sites compared with that in live and bone sites [16], while other studies presented similar discordance rates across different biopsy sites [13, 17]. However, different results showing a higher discordance rate in bone have been obtained [16, 22, 28]. Despite the limitation of sample size in our observation, we still found clues demonstrating that rebiopsy at different metastatic sites could lead to treatment with novel anti-HER2 drugs.

We did not find any difference in PRS or OS between patients with or without discordance in HER2 expression when focusing on patients with HER2-low expression, while there existed evidence that HER2-low tumors had specific biology and showed differences in response to therapy and prognosis [18]. Interestingly, in HR-positive patients, we found a trend toward longer OS when patients had different HER2 expression. This slight difference had not been demonstrated in previous studies, suggesting that changing from a HER2-low status to a HER2-low status in HR+ BC should be considered more often, although this would be verified in larger populations. While, in a latest large-scale work concerning HER2-low heterogeneity between primary and paired recurrent/metastatic breast cancer established the first prediction tool to estimate the probability of HER2-0 tumors converting to HER2-low/positive tumors, by combining ER status, Ki-67 index, biopsy site, and disease-free interval as predictors [29].

Our study investigated the evolution of HER2-2+ patients with either primary tumors or relapsed tumors. This analysis has several limitations. The retrospective nature of this study could contribute to a potential bias in the enrichment of patients with unusual clinical courses. Moreover, selection bias may occur when selecting HER2-2+ patients who have either a primary tumor or a recurrent tumor. Though we reassessed the results with a unified standard, some slides that have been stored for a long time may fade. Meanwhile, HER2 expression may also be affected by formalin-fixations variables and artifacts, as well as intratumoral heterogeneity [30]. At the same time, the possible role of systemic treatments after disease progression in affecting HER2 expression on secondary lesions cannot be excluded. Finally, given the relatively small sample size, some of the findings in our study, such as discordance rates in different biopsy sites, should be considered in further research.

In conclusion, in the present study, we explored patients with HER2-2+ expression in matched primary or recurrent/metastatic tumor samples to evaluate the evolution from or to HER2-2+ expression and the association with HR status. We showed that HER2 expression is highly unstable during disease progression and is more frequent in the HR-positive population. Rebiopsy at different metastatic sites could lead to treatment with novel anti-HER2 drugs.

Supplementary information is available in the online version of the paper.

References

[1] SUNG H, FERLAY J, SIEGEL RL, LAVERSANNE M, SOERJOMATARAM I et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209–249. https://doi.org/10.3322/caac.21660

[2] HEALTH COMMISSION OF THE PEOPLE'S REPUBLIC OF CHINA N. National guidelines for diagnosis and treatment of breast cancer 2022 in China (English version). Chin J Cancer Res 2022; 34: 151–175. https://doi.org/10.21147/j.issn.1000-9604.2022.03.02

[3] SØRLIE T, PEROU CM, TIBSHIRANI R, AAS T, GEISLER S et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001; 98: 10869–10874. https://doi.org/10.1073/pnas.191367098

[4] CANCER GENOME ATLAS NETWORK: KOBOLDT DC, RS, MCLELLAN MD, SCHMIDT H, KALICKI-VEIZER J et al. Comprehensive molecular portraits of human breast tumours. Nature 2012; 490: 61–70. https://doi.org/10.1038/nature11412

[5] CURIGLIANO G, BURSTEIN HJ, WINER EP, GNANT M, DUBSKY P et al. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol 2017; 28: 1700–1712. https://doi.org/10.1093/annonc/mdx308

[6] GRADISHAR WJ, MORAN MS, ABRAHAM J, ABRAMSON V, AFT R et al. NCCN Guidelines® Insights: Breast Cancer, Version 4.2023. J Natl Compr Canc Netw 2023; 21: 594–608. https://doi.org/10.6004/jnccn.2023.0031

[7] WOLFF AC, HAMMOND MEH, ALLISON KH, HARVEY BE, MCSHANE LM et al. HER2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update summary. J Oncol Pract 2018; 14: 437–441. https://doi.org/10.1200/JOP.18.00206

[8] PEIFFER DS, ZHAO F, CHEN N, HAHN OM, NANDA R et al. Clinicopathologic Characteristics and Prognosis of ERBB2-Low Breast Cancer Among Patients in the National Cancer Database. JAMA Oncol 2023; 9: 500–510. https://doi.org/10.1001/jamaoncol.2022.7476

[9] TARANTINO P, HAMILTON E, TOLANEY SM, CORTES J, MORGANTI S et al. HER2-Low Breast Cancer: Pathological and Clinical Landscape. J Clin Oncol 2020; 38: 1951–1962. https://doi.org/10.1200/jco.19.02488

[10] SCHETTINI F, CHIC N, BRASÓ-MARISTANY F, PARÉ L, PASCUAL T et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. NPJ breast cancer 2021; 7: 1. https://doi.org/10.1038/s41523-020-00208-2

[11] DIECI MV, BARBIERI E, PIACENTINI F, FICARRA G, BETTELLI S et al. Discordance in receptor status between primary and recurrent breast cancer has a prognostic impact: a single-institution analysis. Ann Oncol 2013; 24: 101–108. https://doi.org/10.1093/annonc/mds248

[12] GUARNERI V, GIOVANNELLI S, FICARRA G, BETTELLI S, MAIORANA A et al. Comparison of HER-2 and hormone receptor expression in primary breast cancers and asynchronous paired metastases: impact on patient management. Oncologist 2008; 13: 838–844. https://doi.org/10.1634/theoncologist.2008-0048

[13] GRINDA T, JOYON N, LUSQUE A, LEFÈVRE S, ARNOULD L et al. Phenotypic discordance between primary and metastatic breast cancer in the large-scale real-life multicenter French ESME cohort. NPJ breast cancer 2021; 7: 41. https://doi.org/10.1038/s41523-021-00252-6

[14] CARDOSO F, PALUCH-SHIMON S, SENKUS E, CURIGLIANO G, AAPRO MS et al. 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). Ann Oncol 2020; 31: 1623–1649. https://doi.org/10.1016/j.annonc.2020.09.010

[15] VAN POZNAK C, SOMERFIELD MR, BAST RC, CRISTOFANILLI M, GOETZ MP et al. Use of Biomarkers to Guide Decisions on Systemic Therapy for Women With Metastatic Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol 2015; 33: 2695–2704. https://doi.org/10.1200/jco.2015.61.1459

[16] MIGLIETTA F, GRIGUOLO G, BOTTOSSO M, GIARRATANO T, LO MELE M et al. Evolution of HER2-low expression from primary to recurrent breast cancer. NPJ Breast Cancer 2021; 7: 137. https://doi.org/10.1038/s41523-021-00343-4

[17] TARANTINO P, GANDINI S, NICOLÒ E, TRILLO P, GIUGLIANO F et al. Evolution of low HER2 expression between early and advanced-stage breast cancer. Eur J Cancer 2022; 163: 35–43. https://doi.org/10.1016/j.ejca.2021.12.022

[18] DENKERT C, SEITHER F, SCHNEEWEISS A, LINK T, BLOHMER JU et al. Clinical and molecular characteristics of HER2-low-positive breast cancer: pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. Lancet Oncol 2021; 22: 1151–1161. https://doi.org/10.1016/S1470-2045(21)00301-6

[19] FERNANDEZ AI, LIU M, BELLIZZI A, BROCK J, FADARE O et al. Examination of Low ERBB2 Protein Expression in Breast Cancer Tissue. JAMA Oncol 2022; 8: 607–610. https://doi.org/10.1001/jamaoncol.2021.7239

[20] BALLARD M, JALIKIS F, KRINGS G, SCHMIDT RA, CHEN YY et al. 'Non-classical' HER2 FISH results in breast cancer: a multi-institutional study. Mod Pathol 2017; 30: 227–235. https://doi.org/10.1038/modpathol.2016.175

[21] HOWLADER N, ALTEKRUSE SF, LI CI, CHEN VW, CLARKE CA et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst 2014; 106: dju055. https://doi.org/10.1093/jnci/dju055

[22] CAI M, LI M, LV H, ZHOU S, XU X et al. HER2-low breast cancer: evolution of HER2 expression from primary tumor to distant metastases. BMC Cancer 2023; 23: 656. https://doi.org/10.1186/s12885-023-11134-4

[23] MODI S, JACOT W, YAMASHITA T, SOHN J, VIDAL M et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med 2022; 387: 9–20. https://doi.org/10.1056/NEJMoa2203690

[24] HOEFNAGEL LD, VAN DE VIJVER MJ, VAN SLOOTEN HJ, WESSELING P, WESSELING J et al. Receptor conversion in distant breast cancer metastases. Breast Cancer Res 2010; 12: 1–9. https://doi.org/10.1186/bcr2645

[25] SCHRIJVER WA, SUIJKERBUIJK KP, VAN GILS CH, VAN DER WALL E, MOELANS CB et al. Receptor conversion in distant breast cancer metastases: a systematic review and meta-analysis. J Natl Cancer Inst 2018; 110: 568–580. https://doi.org/10.1093/jnci/djx273

[26] CHEN R, QARMALI M, SIEGAL GP, WEI S. Receptor conversion in metastatic breast cancer: analysis of 390 cases from a single institution. Mod Pathol 2020; 33: 2499–2506. https://doi.org/10.1038/s41379-020-0615-z

[27] YILMAZ C, CAVDAR DK. Biomarker Discordances and Alterations Observed in Breast Cancer Treated with Neoadjuvant Chemotherapy: Causes, Frequencies, and Clinical Significances. Curr Oncol 2022; 29: 9695–9710. https://doi.org/10.3390/curroncol29120761

[28] YEUNG C, HILTON J, CLEMONS M, MAZZARELLO S, HUTTON B et al. Estrogen, progesterone, and HER2/neu receptor discordance between primary and metastatic breast tumours-a review. Cancer Metastasis Rev 2016; 35: 427–437. https://doi.org/10.1007/s10555-016-9631-3

[29] LIN M, LUO T, JIN Y, ZHONG X, ZHENG D et al. HER2-low heterogeneity between primary and paired recurrent/metastatic breast cancer: Implications in treatment and prognosis. Cancer 2024; 130: 851–862. https://doi.org/10.1002/cncr.35101

[30] MARCHIÒ C, ANNARATONE L, MARQUES A, CASORZO L, BERRINO E et al. Evolving concepts in HER2 evaluation in breast cancer: Heterogeneity, HER2-low carcinomas and beyond. Semin Cancer Biol 2021; 72: 123–135. https://doi.org/10.1016/j.semcancer.2020.02.016

https://doi.org/10.4149/neo_2024_240229N89

HER2 status results in an unstable switch from primary to recurrent breast cancer

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Supplementary Information

Supplementary Table S1. HER2 expression distribution according to breast cancer subtype in the HER2-negative primary and recurrent breast cancer cohort.

	HER2 Expression		
	1+	2+	p-value
Primary breast cancer n (%)			
HR-positive/HER2-negative	32 (48.5)	34 (51.5)	0.240
Triple-negative	4 (28.6)	10 (71.4)	
Recurrent breast cancer n (%)			
HR-positive/HER2-negative	8 (8.0)	92 (92.0)	0.402
Triple-negative	3 (14.3)	18 (85.7)	

Supplementary Table S2. Details of HER2 conversion according to HR status and HER2 expression.

			HER2 Recurrence/metastasis									
			HR+					HR–				
			0	1+	2+, ISH–	2+, ISH+	3+	0	1+	2+, ISH–	2+, ISH+	3+
HER2 Primary	HR+	0			28	2				4	1	
		1+			27	1				3		
		2+, ISH–	1	7	21	1		1		2		
		2+, ISH+		1	2	2	1			1	1	
		3			2	2						
	HR–	0								2	1	
		1+			1					2		
		2+, ISH–			3				2	4	1	
		2+, ISH+			1							
		3+				1					4	

Supplementary Table S3. HER2-low expression distribution according to breast cancer subtype in the HER2-negative primary and recurrent breast cancer cohort.

	HER2 Expression		
	0	Low	p-value
Primary breast cancer n (%)			
HR-positive/HER2-negative	40 (37.7)	66 (62.3)	0.169
Triple-negative	3 (17.6)	14 (82.4)	
Recurrent breast cancer n (%)			
HR-positive/HER2-negative	1 (1.0)	103 (99.0)	0.331
Triple-negative	1 (4.3)	22 (95.7)	

Supplementary Table S4. HER2 discordance status in different subtypes of primary breast cancer.

	HER2 discordance statues		
	Concordance	Discordance	p-value
Primary breast cancer n (%)			
HR-positive/HER2-negative	60 (57.1)	45 (42.9)	0.184
Triple-negative	13 (76.5)	4 (23.5)	
HR-positive	66 (55.9)	52 (44.1)	0.062
HR-negative	18 (78.3)	5 (21.7)	

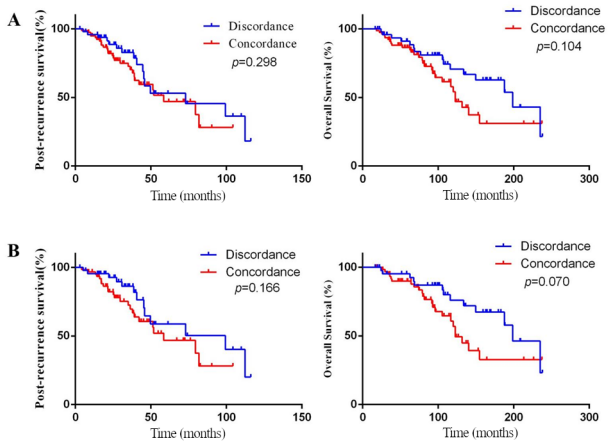
Supplementary Table S5. Conversion of HER2 expression according to the tumor phenotype of the secondary lesion.

			HER2 Recurrence/metastasis					
			HR+			HR–		
			0	Low	Positive	0	Low	Positive
HER2 Primary	HR+	0		30	2		4	1
		Low	1	55	2	1	5	
		Positive		5	5		1	1
	HR–	0					2	1
		Low		4			9	1
		Positive		1	1			4

Supplementary Table S6. Evolution of HER2 expression according to relapsed tumor sample.

	n	HER2 status		
Biopsy sites		HER2 Concordant	HER2 Discordant	p-value
Chest wall/Ipsilateral breast	27	16 (59.3)	11 (40.7)	0.026
Lymph nodes	34	20 (58.8)	14 (41.2)	
Liver	37	28 (75.7)	9 (24.3)	
Lung/pleura	13	8 (61.5)	5 (38.5)	
CNS	2	2 (100.0)	0 (0.0)	
Bone	6	3 (50.0)	3 (50.0)	
Contralateral breast	4	0 (0.0)	4 (100.0)	
Skin	2	0 (0.0)	2 (100.0)	
Other sites*	4	2 (50.0)	2 (50.0)	

Note: *other sites including eye, retina, pelvic cavity and oral



Supplementary Figure S1. Survival analysis according to HER2 discordance. Survival analysis was compared among the population of patients with or without discordance in HER2 expression, with separate analyses for the overall population (A) and HR-positive tumors (B).