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Pathologic complete response to neoadjuvant anti-HER2 therapy is associated with HER2 immunohistochemistry score in HER2-positive early breast cancer

Hai-long Chen, MD, Qiang Chen, MS, Yong-chuan Deng, MD*

Abstract

To evaluate whether pathologic complete response (pCR) to neoadjuvant anti-human epidermal growth factor receptor 2 (HER2) therapy is dependent on the HER2 immunohistochemistry (IHC) score.

A total of 181 HER2-positive early breast cancer patients who had received neoadjuvant anti-HER2 therapy were included in this study. Associations were examined between IHC score and tumor pCR status (commonly defined by ypT0+ypN0, ypT0/is+ypN0, or ypT0/is).

In trastuzumab-based neoadjuvant-treated patients, ypT0+ypN0 was achieved in 46.0% of patients with HER2 IHC 3+ tumors but only 25.0% of patients with HER2 IHC 2+/fluorescence in situ hybridization (FISH)-positive tumors ($P = .016$). When pCR was defined as ypT0/is+ypN0 or ypT0/is, 54.7% and 61.3% of patients with HER2 IHC 3+ tumors had a pCR, whereas only 29.5% and 38.6% with HER2 IHC 2+/FISH-positive tumors achieved pCR ($P = .004$ and $P = .008$, respectively). The association between dual HER2 blockade and pCR was almost exclusively confined to HER2 IHC 3+ tumors (ypT0+ypN0: 61.9% vs 38.9%, $P = .013$; ypT0/is+ypN0: 71.4% vs 47.4%, $P = .009$; and ypT0/is: 81.0% vs 52.6%, $P = .002$) and was absent in HER2 IHC 2+/FISH-positive tumors. Multivariate logistic regression revealed that HER2 IHC 3+ tumors had a significantly higher probability of achieving ypT0+ypN0 (odds ratio [OR], 0.265; 95% confidence interval [CI], 0.109–0.645; $P = .003$), ypT0/is+ypN0 (OR, 0.221; 95% CI, 0.094–0.521; $P = .001$), and ypT0/is (OR, 0.254; 95% CI, 0.111–0.583; $P = .001$) than HER2 IHC 2+/FISH-positive tumors. A significantly better pCR rate was also found in patients with T1 tumors and patients with dual HER2 blockade.

The pCR rate was highly correlated with the HER2 IHC score in neoadjuvant anti-HER2 treatment. The addition of pertuzumab to a neoadjuvant trastuzumab-based regimen improved pCR rates, but there was no significant difference in pCR rates in the IHC 2+/FISH-positive group. This suggests that HER2 IHC scores can predict the effectiveness of treatment.

Abbreviations: CEP17 = centromere 17, CI = confidence interval, ER = estrogen receptor, FISH = fluorescence in situ hybridization, HER2 = human epidermal growth factor receptor 2, HR = hormone receptor, IHC = immunohistochemistry, ISH = in situ hybridization, OR = odds ratio, pCR = pathologic complete response, PR = progesterone receptor.

Keywords: breast cancer, HER2-positive, immunohistochemistry, neoadjuvant therapy, pathologic complete response

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1. Introduction

Human epidermal growth factor receptor 2 (HER2) gene overexpression and amplification occur in approximately 20% of primary breast cancers.[1,2] It is associated with a poor clinical prognosis.[3–5] Nevertheless, with the development of monoclonal antibodies against HER2, many studies have demonstrated that HER2-targeted therapies improve survival in HER2-positive patients with early and advanced breast cancer.[6–9] The NOAH and NEOSPHERE studies also confirmed the improvement of pathologic complete response (pCR) when HER2 block therapy was added in neoadjuvant treatment, and NOAH study showed a sustained benefit in event-free survival from trastuzumab-containing neoadjuvant therapy followed by adjuvant trastuzumab in patients.[10,11]

However, HER2 blockade treatment is not effective in all HER2-positive breast cancer patients. Twenty-five percent to 30% of women treated with adjuvant trastuzumab-based chemotherapy experience relapse within the first 10 years of diagnosis,[8,12] and even when using dual HER2 blockade, the recurrence rate still reaches 8% to 12% within 4 years.[9,13] As a

consequence, it is necessary to find an effective method for predicting the response to HER2 blockade-based chemotherapy.

To date, the identification of cancers that are likely to respond to anti-HER2 therapy is based on HER2 status, which is identified by immunohistochemistry (IHC) or in situ hybridization (ISH). IHC assesses the level of HER2 protein expression based on circumferential membrane staining, and scores range from 0 to 3+, with 3+ indicating a positive HER2 status and 0 and 1+ indicating a negative HER2 status. An IHC score of 2+ is considered equivocal, and samples should then be tested using dual-probe ISH. Dual-probe ISH evaluates HER2 gene amplification by identifying the number of copies of the HER2 gene on chromosome 17 and the number of centromere 17 (CEP17) copies per nucleus.[14]

A small number of studies have shown that the therapeutic response to HER2 blockade therapy is correlated with the level of HER2 amplification.[15–17] High HER2 amplification indicated a high response rate. However, IHC is the first-choice method to assess HER2 status in many medical institutions; for patients with tumors with IHC scores of 0, 1+, and 3+, further ISH is not necessary. Among patients with HER2 IHC 2+ tumors, approximately 20% of the tumors were ISH-positive.[18] The relationship between HER2 protein expression levels and response to HER2 blockade therapy is still unclear. Therefore, we investigated the relationship between HER2 expression and pCR in HER2 blockade using IHC analysis.

2. Patients and methods

2.1. Patients

We retrospectively analyzed operable, HER2-positive, stage II/III breast cancer tumors in patients treated with trastuzumab-based neoadjuvant chemotherapy at the Second Affiliated Hospital, Zhejiang University (Hangzhou China), from January 2018 to December 2020. HER2 status was first determined by IHC, and patients with IHC 3+ tumors were diagnosed as positive; for patients with HER2 IHC 2+ tumors, HER2 gene amplification was identified by fluorescence in situ hybridization (FISH) according to the 2013 ASCO/CAP guidelines.[19] All patients completed the neoadjuvant therapy regimen before surgery. Patients previously treated with chemotherapy, radiation therapy, or targeted therapy were excluded. Patients with metastatic disease, bilateral breast cancer, or other malignancies were also excluded.

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine (Hangzhou, China; approval no. 2021-0291). The need for informed consent was waived by the Ethics Committee because the study was an observational, retrospective study, and the patients' identification information had been removed.

2.2. Procedures

All eligible patients were diagnosed with invasive carcinoma by core needle biopsy. Core needle biopsy or fine needle aspiration was performed on axillary lymph nodes suspected to have metastatic involvement. Standard systemic assessment was performed for all patients.

All patients underwent a standard pre-operative therapy regimen according to the NCCN guidelines for invasive breast cancer and classic clinical trials,[20,21] including AC (anthracycline plus cyclophosphamide) followed by T (taxane) + trastuzumab (8 cycles), AC followed by T + trastuzumab +

pertuzumab (8 cycles), TCH (taxane, carboplatin plus trastuzumab, 6 cycles), TCH + pertuzumab (6 cycles), and THP (taxane + trastuzumab + pertuzumab, 4 cycles). The pre-operative therapy regimens were decided in 1 week when all tests were completed. Cardiac function was monitored by electrocardiography and echocardiography throughout the treatment process. There were no cases of conversion to surgery without completing the scheduled chemotherapy. Surgery was performed after the pre-operative therapy was completed. The types of surgery included total mastectomy, breast-conserving surgery, and breast reconstructions depending on the patient's condition.

2.3. HER2 IHC scores

HER2 immunoreactivity was assessed using the following scoring approach: 0, no immunoreactivity or weak incomplete membrane staining within <10% of tumor cells; 1+, weak incomplete membrane staining within >10% of tumor cells; 2+, weak to moderate incomplete membrane staining within >10% of tumor cells or complete and circumferential membrane staining within ≤10% of tumor cells; and 3+, strong complete membrane staining within >10% of tumor cells.

2.4. Fluorescence in situ hybridization analysis

When the IHC score was considered equivocal (2+), the assessment of the HER2 gene and chromosome 17 status was performed in paraffin-embedded tissue from core needle biopsies of the primary tumor. An average HER2 copy number ≥6.0 signals/cell or a HER2/CEP17 ratio ≥2.0 was considered positive.

2.5. Pathologic complete response assessment

We used the 3 most commonly used pCR definitions: ypT0 ypN0 (the absence of invasive cancer and in situ cancer [DCIS] in breast and axillary lymph nodes), ypT0/is ypN0 (the absence of invasive cancer in breast and in axillary lymph nodes, irrespective of remaining DCIS in the primary tumor), and ypT0/is (the absence of invasive cancer in the breast irrespective of the presence of DCIS or nodal involvement). The assessments were performed locally by experienced pathologists.

2.6. Statistics

We used the Mann–Whitney U test to compare continuous values, and chi-square and Fisher exact (for smaller sample size) tests to compare categorical values between the 2 groups. Univariate logistic regression was conducted for all clinicopathologic parameters with pCR as the outcome, including age, tumor grade, nodal status, hormone receptor status, HER2 status, Ki67, chemotherapy regimen and anti-HER2 therapy regimen. The parameters found to be significant in univariate analysis were assessed with multivariate analysis using an enter logistic regression model to evaluate which parameters were independent. For all analyses, a P value of <.05 was considered statistically significant. All statistical analyses were performed with SPSS version 19.0 (SPSS, Inc.).

3. Results

3.1. Patient characteristics

Overall, 373 patients with early breast cancer who underwent neoadjuvant chemotherapy and surgery in our center were
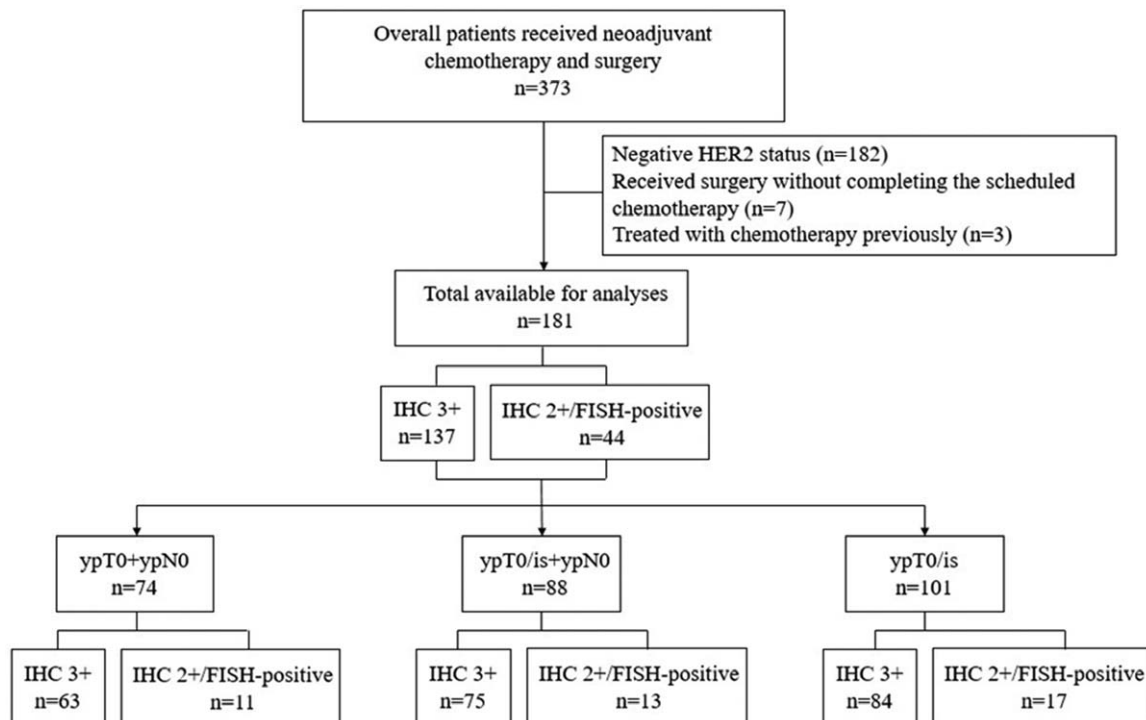
Figure 1. Flow diagram of patient selection. FISH = fluorescence in situ hybridization, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry, ISH = in situ hybridization.

identified. A total of 182 individuals were then excluded from the study because of negative HER2 status. Seven patients converted to surgery without completing the scheduled chemotherapy and 3 patients were previously treated with chemotherapy. They were also excluded, leaving 181 patients eligible for the study. A total of eligible 137 patients had HER2 IHC 3+ tumors, and 44 had IHC 2+/FISH-positive tumors (Fig. 1). Baseline patient characteristics by HER2 status are shown in Table 1. The median age was 53 years (range 25–76). Compared with patients with HER2 IHC 2+/FISH-positive tumors, those with IHC 3+ tumors were more likely to be ER- and PR-negative ($P = .03$). Most patients received anthracycline plus taxane or taxane plus carboplatin neoadjuvant chemotherapy. However, 12 (8.8%) patients in the IHC 3+ tumor group and 4 (9.1%) in the IHC 2+/FISH-positive tumor group underwent other regimens (taxane for 4 cycles). All eligible patients had received trastuzumab neoadjuvant therapy; 42 (30.7%) patients in the IHC 3+ tumor group and 20 (45.5%) in the IHC 2+/FISH-positive tumor group received pertuzumab treatment.

3.2. IHC scores and pathologic complete response

Overall, trastuzumab-based neoadjuvant chemotherapy resulted in pCR rates of 40.9% in ypT0+ypN0, 48.6% in ypT0/is+ypN0, and 55.8% in ypT0/is. When ypT0+ypN0 was selected as an endpoint, tumors with IHC 3+ achieved a pCR in 63 of 137 cases (46.0%), which was significantly higher than the pCR rate in tumors with IHC 2+/FISH-positive (11/44 cases; 25.0%; $P = .016$). Similar results were seen when pCR was defined as ypT0/is+ypN0 (54.7% vs 29.5%, $P = .004$) or ypT0/is (61.3% vs 38.6%, $P = .008$) (Fig. 2).

In addition, we found that patients treated with dual HER2 blockade achieved a significantly better pCR rate than those

Table 1

Patient characteristics.

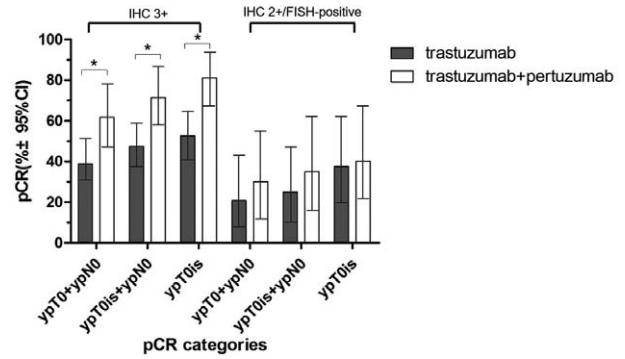|	IHC 3+	IHC 2+/FISH-positive	P
Number	137	44	
Age (yrs)			.613
Median (range)	53 (30–72)	53.5 (25–76)	
Sites of cancer, n (%)			.132
Left	60 (43.8)	25 (56.8)	
Right	77 (56.2)	19 (43.2)	
Tumor stage, n (%)			.194
T1	7 (5.1)	5 (11.4)	
T2	88 (64.2)	30 (68.2)	
T3–T4	42 (30.7)	9 (20.4)	
Nodal stage, n (%)			.395
N0	13 (9.5)	7 (15.9)	
N1	91 (66.4)	25 (56.8)	
N2–N3	33 (24.1)	12 (27.3)	
Hormone receptor status, n (%)			.030[†]
ER- or PR-positive	81 (59.1)	34 (77.3)	
ER- and PR-negative	56 (40.9)	10 (22.7)	
Ki-67 n (%)			.160
≤15%	14 (10.2)	8 (18.2)	
>15%	123 (89.8)	36 (81.8)	
Chemotherapy regimen, n (%)			.914
Anthracycline plus taxane, 8 cycles[*]	64 (46.7)	22 (50.0)	
Taxane plus carboplatin, 6 cycles	61 (44.5)	18 (40.9)	
Taxane, 4 cycles	12 (8.8)	4 (9.1)	
Anti-HER2 therapy regimen			.072
Trastuzumab only, n (%)	95 (69.3)	24 (54.5)	
Trastuzumab+ pertuzumab, n (%)	42 (30.7)	20 (45.5)	

ER = estrogen receptor, FISH = fluorescence in situ hybridization, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry, PR = progesterone receptor.
[*] Including AC followed by T + trastuzumab for 8 cycles and AC followed by T + trastuzumab + pertuzumab for 8 cycles.
[†] $P < .05$.
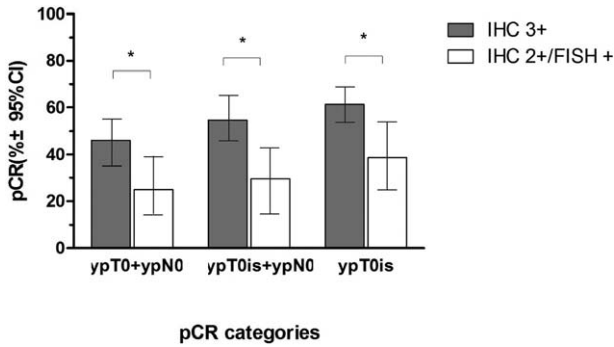
Figure 2. HER2 IHC scores and pCR as defined by ypT0+ypN0, ypT0/is +ypN0, and ypT0/is. CI = confidence interval, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry, pCR = pathologic complete response.

Figure 4. pCR and HER2 blockade in IHC 3+ and IHC 2+/FISH-positive tumors. CI = confidence interval, FISH = fluorescence in situ hybridization, HER2 = human epidermal growth factor receptor 2, IHC = immunohisto-chemistry, pCR = pathologic complete response.

treated with trastuzumab only (ypT0+ypN0: 51.6% vs 35.3%, $P=.034$; ypT0/is+ypN0: 60.0% vs 42.9%, $P=.032$; ypT0/is: 67.7% vs 49.6%, $P=.020$, Fig. 3). The same superiority was shown in the subgroup of IHC 3+ tumors (ypT0+ypN0: 61.9% vs 38.9%, $P=.013$; ypT0/is+ypN0: 71.4% vs 47.4%, $P=.009$; and ypT0/is: 81.0% vs 52.6%, $P=.002$). In IHC 2+/FISH-positive group, patients treated with dual HER2 blockade had higher pCR rate than those with single HER2 blockade, but no significant difference was observed (ypT0+ypN0: 30.0% vs 20.8%, $P=.484$; ypT0/is+ypN0: 35.0% vs 25.0%, $P=0.469$; and ypT0/is: 40.0% vs 37.5%, $P=.865$) (Fig. 4). In addition, pCR was more common in HR-negative tumors, but the HR status (ER- and/or PR-positive cases vs ER- and PR-negative cases) was not significantly correlated with pCR, irrespective of the pCR definition (Fig. 5).

3.3. Analyses of pCR-related factors using univariate and multivariate logistic regression models

The results of the pCR-related factor analyses, which were performed using univariate and multivariate logistic regression models, are shown in Tables 2 and 3. According to the results of the univariate analysis, tumor grade, HER2 status, Ki67, chemotherapy regimen and anti-HER2 therapy regimen were assessed in the multivariate analysis, as shown in Table 3. Patients with HER2 IHC 3+ tumors also exhibited a higher pCR rate than patients with IHC 2+/FISH-positive tumors. In

addition, we found a significantly better pCR rate in patients with T1 tumors and patients with dual HER2 blockade, regardless of the definition of pCR. When pCR was defined as ypT0+ypN0, patients with Ki67 >15% showed a significantly better pCR rate than patients with Ki67 ≤15%. Interestingly, the anthracycline plus taxane chemotherapy regimen was independently associated with a worse pCR rate, according to the results of our univariate and multivariate analyses, when pCR was defined as ypT0/is. No other histopathologic or clinical parameters, such as age, site of cancer, nodal stage, or hormone receptor status, were significantly associated with pCR.

4. Discussion

In this study, we retrospectively compared the pCR rate of operable breast cancer in IHC 3+ and IHC 2+/FISH-positive tumors. The 3 most common definitions of pCR were used in our study: ypT0 ypN0, ypT0/is ypN0, and ypT0/is. The ypT0/is criterion has been widely used as the endpoint in several pivotal trials.[10,20] The total pCR (ypT0/is) rate of HER2-positive breast cancer in our study was 55.8%, which was similar to previous studies.[10,11] However, it was reported that the complete absence of invasive neoplasms in the breast and lymph nodes (ypT0 +ypN0 or ypT0/is+ypN0) has a stronger association with improved event-free survival and overall survival than the
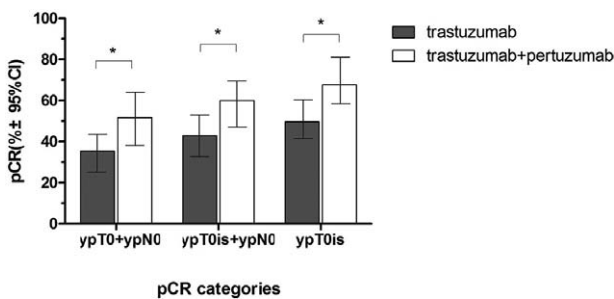
Figure 3. HER2 blockade and pCR as defined by ypT0+ypN0, ypT0/is+ypN0, and ypT0/is. CI = confidence interval, HER2 = human epidermal growth factor receptor 2, pCR = pathologic complete response.
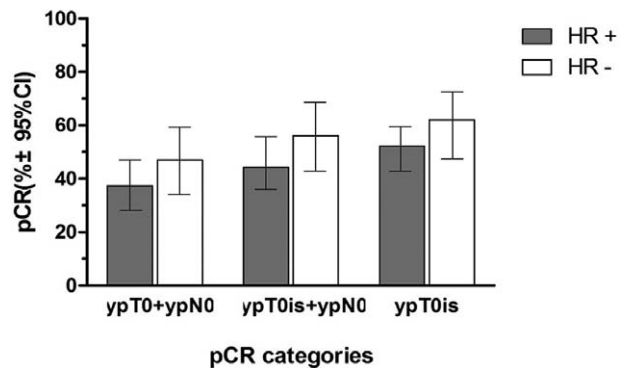
Figure 5. HR status and pCR as defined by ypT0+ypN0, ypT0/is+ypN0 and ypT0/is. CI = confidence interval, HR = hormone receptor, pCR = pathologic complete response.

Table 2

Univariate logistic regression analysis for assessing pCR-related factors.

| | pCR categories | | | | | |
| | ypT0+ypN0 | | ypT0/is+ypN0 | | ypT0/is | |
Variable	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Age	0.997 (0.968–1.027)	.849	1.007 (0.978–1.037)	.639	1.026 (0.995–1.057)	.098
Sites of cancer						
Left	1		1		1	
Right	0.742 (0.409–1.345)	.326	1.029 (0.574–1.846)	.923	1.244 (0.691–2.241)	.466
Tumor stage						
T1	1		1		1	
T2	0.213 (0.055–0.828)	.026[*]	0.187 (0.039–0.890)	.035[*]	0.119 (0.015–0.955)	.045[*]
T3–T4	0.198 (0.048–0.823)	.026[*]	0.140 (0.028–0.706)	.017[*]	0.075 (0.009–0.662)	.016[*]
Nodal stage						
N0	1		1		1	
N1	1.831 (0.657–5.099)	.247	1.035 (0.401–2.674)	.943	1.189 (0.460–3.072)	.721
N2–N3	1.417 (0.457–4.387)	.546	0.731 (0.254–2.104)	.561	1.647 (0.569–4.772)	.358
HER2 status						
IHC 3+	1		1		1	
IHC 2+/FISH-positive	0.392 (0.183–0.838)	.016[*]	0.347 (0.167–0.719)	.004[*]	0.397 (0.198–0.798)	.009[*]
Hormone receptor status						
ER- or PR-positive	1		1		1	
ER- and PR-negative	1.636 (0.885–3.023)	.116	1.764 (0.957–3.253)	.069	1.661 (0.893–3.090)	.109
Ki-67						
≤15%	1		1		1	
>15%	3.539 (1.146–10.932)	.028[*]	1.772 (0.704–4.458)	.224	1.983 (0.801–4.909)	.139
Chemotherapy regimen						
Anthracycline plus taxane, 8 cycles	1		1		1	
Taxane plus carboplatin, 6 cycles	1.362 (0.724–2.562)	.339	1.707 (0.915–3.184)	.093	2.429 (1.285–4.593)	.006[*]
Taxane only, 4 cycles	1.971 (0.723–5.371)	.184	2.498 (0.895–6.973)	.081	2.737 (0.951–7.874)	.062
Anti-HER2 therapy						
Trastuzumab only	1		1		1	
Trastuzumab+pertuzumab	1.956 (1.048–3.650)	.035[*]	1.973 (1.057–3.683)	.033[*]	2.136 (1.123–4.060)	.021[*]

CI = confidence interval, ER = estrogen receptor, FISH = fluorescence in situ hybridization, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry, OR = odds ratio, pCR = pathologic complete response, PR = progesterone receptor.

[*] P < .05.

Table 3

Multivariate logistic regression analysis for assessing pCR-related factors.

| | pCR categories | | | | | |
| | ypT0+ypN0 | | ypT0/is+ypN0 | | ypT0/is | |
Variable	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Tumor stage						
T1	1		1		1	
T2	0.152 (0.034–0.675)	.013[*]	0.122 (0.023–0.654)	.014[*]	0.075 (0.009–0.663)	.020[*]
T3–T4	0.142 (0.029–0.683)	.015[*]	0.089 (0.015–0.515)	.007[*]	0.047 (0.005–0.443)	.008[*]
HER2 status						
IHC 3+	1		1		1	
IHC 2+/FISH-positive	0.265 (0.109–0.645)	.003[*]	0.221 (0.094–0.521)	.001[*]	0.254 (0.111–0.583)	.001[*]
Ki-67						
≤15%	1		1		1	
>15%	4.029 (1.199–13.532)	.024[*]	1.909 (0.681–5.348)	.219	2.354 (0.839–6.608)	.104
Chemotherapy regimen						
Anthracycline plus taxane, 8 cycles	1		1		1	
Taxane plus carboplatin, 6 cycles	1.275 (0.636–2.558)	.493	1.556 (0.784–3.087)	.206	2.379 (1.179–4.800)	.015[*]
Taxane only, 4 cycles	2.569 (0.835–7.907)	.100	3.092 (0.997–9.592)	.051	3.445 (1.074–11.052)	.038[*]
Anti-HER2 therapy						
Trastuzumab only	1		1		1	
Trastuzumab+pertuzumab	2.117 (1.049–4.273)	.036[*]	2.042 (1.009–4.133)	.047[*]	2.092 (1.006–4.350)	.048[*]

CI, confidence interval, FISH = fluorescence in situ hybridization, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry, OR = odds ratio, pCR = pathologic complete response.

[*] P < .05.

eradication of tumors from the breast alone (ypT0/is). The strongest association between pCR and long-term outcome was in patients with aggressive breast cancer subtypes, such as HER2-positive.[22] In our study, the total pCR rate was 40.9% and 48.6% when pCR was defined as ypT0+ypN0 and ypT0/is+ypN0, respectively, similar to the studies that defined pCR as ypT0+ypN0.[23,24]

With the widespread use of trastuzumab, many studies have demonstrated that patients with HER2 gene amplification or HER2 protein expression (IHC 3+) could benefit from trastuzumab therapy.[25–27] However, the NSABP B-47 trial did not find a benefit to using adjuvant trastuzumab for patients whose tumors lack gene amplification and are IHC 1+ or 2+.[28] Therefore, whether the level of HER2 gene amplification or HER2 protein expression is related to the benefit of anti-HER2 therapy is worthy of study. In the HERA trial, the HER2/CEP17 ratio, HER2 copy number, and CEP17 copy number were not associated with disease-free survival.[29] The NSABP B-31 trial also did not find a relationship between HER2 copy number and therapeutic benefits. However, high HER2 protein and high HER2 and HER3 mRNA levels showed a significantly better prognosis ($P < .05$) for MBC in the CLEOPATRA trial.[30] Kim et al[16] reported that HER2/CEP17 ratios and HER2 IHC scores might predict clinical outcome for patients with HER2 FISH-positive MBC; patients with a HER2/CEP17 tumor ratio ≥3.0 had significantly longer progression-free survival, and patients with HER2 IHC 1+ tumors had significantly shorter overall survival. In the neoadjuvant setting, a study by Singer et al also revealed a relationship between the level of HER2 amplification and the pCR rate in trastuzumab-based neoadjuvant treatment.[17] Nevertheless, most of these studies focused on the level of HER2 amplification and its therapeutic effect, and studies focused on the relationship between the level of HER2 protein expression or IHC scores and anti-HER2 neoadjuvant therapy are lacking.

In our study, the pCR rate was significantly better in breast cancer patients with IHC 3+ tumors than in those with IHC 2+/FISH-positive tumors when pCR was defined as ypT0+ypN0 (46.0% vs 25%, $P = .014$), ypT0/is+ypN0 (54.7% vs 29.5%, $P = .004$), and ypT0/is (61.3% vs 38.6%, $P = .008$). The correlation between HER2 IHC score and pCR rate was confirmed, and 1 possible reason is HER2 heterogeneity. HER2 heterogeneity has been demonstrated in some breast cancer patients, and the presence of HER2 heterogeneity has been associated with worse prognosis and response to anti-HER2 treatment in several studies,[31,32] but the frequency of intra-tumoral HER2 heterogeneity is variable.[33,34] Seol et al found that HER2 intra-tumoral heterogeneity was more frequent in tumors with IHC 2+ or 1+ than in tumors with IHC 3+ (76.5% vs 23.5%, $P < .001$). This showed that HER2 heterogeneity was closely associated with equivocal HER2 protein expression. The results of our study were consistent with these studies.

Various studies have demonstrated that dual HER2 blockade will be more effective than single anti-HER2 blockade therapy. In the neoadjuvant setting, the NeoALTTO trial found that the combination of the tyrosine kinase inhibitor lapatinib and trastuzumab had a higher pCR rate than therapy with trastuzumab or lapatinib alone.[35] Similarly, the NeoSphere study showed that the pCR rate was approximately twice as high with dual HER2 blockade with the anti-HER2 antibodies trastuzumab and pertuzumab than with a single blockade.[11] In our study, the pCR rate was significantly better when pertuzumab was added to trastuzumab treatment (ypT0+ypN0,

51.6% vs 35.3%, $P = .034$; ypT0/is+ypN0, 60.0% vs 42.9%, $P = .032$; ypT0/is, 67.7% vs 49.6%, $P = .02$). Multivariate logistic regression analysis also showed that dual HER2 blockade was independently associated with a better pCR rate. Interestingly, within the subgroup of IHC 3+ tumors, patients treated with dual HER2 blockade achieved a significantly higher pCR rate than those treated with a single HER2 blockade regardless of pCR definitions ($P < .05$; Fig. 4). Higher pCR rate was also observed in the IHC 2+/FISH-positive tumor group when pertuzumab was added to trastuzumab treatment, although these results were not statistically significant ($P > .05$; Fig. 4). This implied that low HER2 expression reduces the effect of dual HER2 blockade. However, the number of IHC 2+/FISH-positive tumor group is too small, the effect of pertuzumab in the IHC 2+/FISH-positive group deserve further study.

In addition, when pCR was defined as ypT0/is, the results of our univariate and multivariate analyses indicated that the anthracycline plus taxane chemotherapy regimen was independently associated with a worse pCR. However, it might be caused by the fact that pertuzumab was added to the regimen with taxane or taxane plus carboplatin. There were no significant differences in disease-free or overall survival between the 2 trastuzumab regimens (AC-TH and TCH) in the BCIRG-006 trial.[26] However, data are lacking in the neoadjuvant setting between the 2 trastuzumab regimens (with or without pertuzumab), and further research is needed to determine regimen efficacy.

Although our data showed the HR status (ER- and/or PR-positive cases vs ER- and PR-negative cases) was not significantly correlated with pCR, pCR was more common in HR-negative tumors. Similar results were reported in other previous studies, regardless of trastuzumab alone plus chemotherapy[17,23,36] or pertuzumab and trastuzumab plus chemotherapy.[20,24] The possible reason for this result is bidirectional cross-talk between HER2, estrogen receptor and hormone receptor signaling acting as a mechanism of resistance to HER2 inhibition.[37] In our study, there were more patients with HR-negative tumors in the IHC 3+ tumor group than in the IHC 2+/FISH + tumor group (40.9% vs 22.7%, $P = .030$), which may be another reason for these results. Further study is needed to elucidate the relationship between HR status and pCR.

As a retrospective study, our study has some limitations. First, it was a single-center study with a limited sample size, and more patients were included in the IHC 3+ tumor group than in the IHC 2+/FISH-positive tumor group. Studies conducted on larger sample sizes are required. Second, because this is a retrospective study, pCR assessment was not performed in a pre-specified period, which could potentially lead to biased results.

In conclusion, we demonstrated that the IHC score was associated with pCR in HER2 blockade neoadjuvant treatment in HER2-positive breast cancer patients. Patients with HER2 IHC 3+ tumors have a significantly higher probability of achieving pCR than patients with IHC 2+/FISH-positive tumors. The addition of pertuzumab to a neoadjuvant trastuzumab-based regimen improved pCR rates, particularly in the IHC 3+ group, but the superiority was decreased in the IHC 2+/FISH-positive tumor group. This suggests that HER2 IHC scores will help predict the effectiveness of treatment.

Author contributions

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References

[1] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–82.

[2] Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244:707–12.

[3] Gusterson BA, Gelber RD, Goldhirsch A, et al. Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. J Clin Oncol 1992;10:1049–56.

[4] Press MF, Bernstein L, Thomas PA, et al. HER-2/neu gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. J Clin Oncol 1997;15:2894–904.

[5] Andrulis IL, Bull SB, Blackstein ME, et al. neu/erbB-2 amplification identifies a poor-prognosis group of women with node-negative breast cancer. Toronto Breast Cancer Study Group. J Clin Oncol 1998; 16:1340–9.

[6] Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.

[7] Swain SM, Miles D, Kim SB, et al. Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA): end-of-study results from a double-blind, randomised, placebo-controlled, phase 3 study. Lancet Oncol 2020;21:519–30.

[8] Cameron D, Piccart-Gebhart MJ, Gelber RD, et al. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. Lancet 2017;389:1195–205.

[9] von Minckwitz G, Procter M, de Azambuja E, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. N Engl J Med 2017;377:122–31.

[10] Gianni L, Eiermann W, Semiglazov V, et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet Oncol 2014;15:640–7.

[11] Gianni L, Pienkowski T, Im YH, et al. 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. Lancet Oncol 2016;17:791–800.

[12] Perez EA, Romond EH, Suman VJ, et al. Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2-positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. J Clin Oncol 2014;32:3744–52.

[13] Piccart-Gebhart M, Holmes E, Baselga J, et al. Adjuvant lapatinib and trastuzumab for early human epidermal growth factor receptor 2-positive breast cancer: results from the randomized phase III adjuvant lapatinib and/or trastuzumab treatment optimization trial. J Clin Oncol 2016;34:1034–42.

[14] Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline focused update. J Clin Oncol 2018;36:2105–22.

[15] Arnould L, Arveux P, Couturier J, et al. Pathologic complete response to trastuzumab-based neoadjuvant therapy is related to the level of HER-2 amplification. Clin Cancer Res 2007;13:6404–9.

[16] Kim JW, Kim JH, Im SA, et al. HER2/CEP17 ratio and HER2 immunohistochemistry predict clinical outcome after first-line trastuzumab plus taxane chemotherapy in patients with HER2 fluorescence in situ hybridization-positive metastatic breast cancer. Cancer Chemother Pharmacol 2013;72:109–15.

[17] Singer CF, Tan YY, Fitzal F, et al. Pathological complete response to neoadjuvant trastuzumab is dependent on HER2/CEP17 ratio in HER2-amplified early breast cancer. Clin Cancer Res 2017;23:3676–83.

[18] Hanna WM, Slodkowska E, Lu FI, Nafisi H, Nofech-Mozes S. Comparative analysis of human epidermal growth factor receptor 2 testing in breast cancer according to 2007 and 2013 American Society of Clinical Oncology/College of American Pathologists guideline recommendations. J Clin Oncol 2017;35:3039–45.

[19] Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31:3997–4013.

[20] Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012; 13:25–32.

[21] Shao Z, Pang D, Yang H, et al. Efficacy, safety, and tolerability of pertuzumab, trastuzumab, and docetaxel for patients with early or locally advanced ERBB2-positive breast cancer in Asia: the PEONY phase 3 randomized clinical trial. JAMA Oncol 2020;6:e193692.

[22] Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014;384:164–72.

[23] Untch M, Fasching PA, Konecny GE, et al. Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: results from the TECHNO trial of the AGO and GBG study groups. J Clin Oncol 2011;29:3351–7.

[24] Schneeweiss A, Chia S, Hickish T, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). Ann Oncol 2013;24:2278–84.

[25] Gianni L, Dafni U, Gelber RD, et al. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. Lancet Oncol 2011;12:236–44.

[26] Slamon D, Eiermann W, Robert N, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 2011;365:1273–83.

[27] Perez EA, Suman VJ, Davidson NE, et al. Sequential versus concurrent trastuzumab in adjuvant chemotherapy for breast cancer. J Clin Oncol 2011;29:4491–7.

[28] Fehrenbacher L, Cecchini RS, Geyer CEJr, et al. NSABP B-47/NRG oncology phase III randomized trial comparing adjuvant chemotherapy with or without trastuzumab in high-risk invasive breast cancer negative for HER2 by FISH and with IHC 1+ or 2. J Clin Oncol 2020;38:444–53.

[29] Zabaglo L, Stoss O, Ruschoff J, et al. HER2 staining intensity in HER2-positive disease: relationship with FISH amplification and clinical outcome in the HERA trial of adjuvant trastuzumab. Ann Oncol 2013;24:2761–6.

[30] Baselga J, Cortes J, Im SA, et al. Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2-positive, first-line metastatic breast cancer. J Clin Oncol 2014;32:3753–61.

[31] Ohlschlegel C, Zahel K, Kradolfer D, Hell M, Jochum W. HER2 genetic heterogeneity in breast carcinoma. J Clin Pathol 2011;64:1112–6.

[32] Seol H, Lee HJ, Choi Y, et al. Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance. Mod Pathol 2012;25:938–48.

[33] Andersson J, Linderholm B, Bergh J, Elmberger G. HER-2/neu (c-erbB-2) evaluation in primary breast carcinoma by fluorescent in situ hybridization and immunohistochemistry with special focus on intra-tumor heterogeneity and comparison of invasive and in situ components. Appl Immunohistochem Mol Morphol 2004;12:14–20.

[34] Glockner S, Buurman H, Kleeberger W, Lehmann U, Kreipe H. Marked intratumoral heterogeneity of c-myc and cyclinD1 but not of c-erbB2 amplification in breast cancer. Lab Invest 2002;82:1419–26.

[35] Baselga J, Bradbury I, Eidtmann H, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 2012;379:633–40.

[36] Untch M, Rezai M, Loibl S, et al. Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the Gepar-Quattro study. J Clin Oncol 2010;28:2024–31.

[37] Pernas S, Tolaney SM. Targeting HER2 heterogeneity in early-stage breast cancer. Curr Opin Oncol 2020;32:545–54.