Influence of Neoadjuvant Chemotherapy on HER2/neu Status in Invasive Breast Cancer

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Abstract

Neoadjuvant anthracycline- and taxane-based chemotherapy for breast carcinoma might result in the decrease of HER2/neu protein expression which can indicate the sensitivity to chemotherapy and might be a predictive marker for pathologic complete response. However, they have the stable gene HER2/neu gene amplification status. Thus, retesting HER2/neu immunohistochemistry (IHC) status in residual tumors after neoadjuvant chemotherapy (NAC) should be considered in order to optimize adjuvant systemic therapy postsurgery.

Introduction: Reliably estimating HER2/neu expression in breast cancer is important for predicting patient prognosis and optimizing adjuvant therapeutic strategies. In this retrospective cohort study, effects of NAC on HER2/neu status in invasive breast cancer were evaluated, and the related factors were analyzed. Patients and Methods: One hundred thirty-one patients with primary breast cancer were treated with anthracycline- and/or taxane-based NAC. HER2/neu status was evaluated by IHC on core needle biopsies of primary tumors before NAC and surgical resection specimens of post-NAC residual breast cancers or tumor-positive axillary lymph nodes. Thirty-two pairs of specimens with discordant HER2/neu IHC scores were analyzed by fluorescence in situ hybridization (FISH). Results: A significant difference in HER2/neu status by IHC between core needle biopsies and surgical resection specimens in patients receiving NAC was observed. After NAC, 23.4% (29 of 124) of tumors showed downregulated HER2/neu expression by IHC. Alterations of HER2/neu IHC scores did not significantly correlate with tumor subtype, pathologic response to NAC, adjuvant regimen, or time interval from the last chemotherapy to surgery. HER2/neu protein overexpression level was associated with favorable pathologic response to anthracycline and taxane-based chemotherapy. However, tumors with altered HER2/neu IHC scores after NAC revealed stable HER2/neu gene amplification/nonamplification by FISH analysis. Conclusion: Neoadjuvant chemotherapy for breast carcinoma resulted in the HER2/neu status alteration by IHC, but they have stable gene amplification status by FISH. HER2/neu protein overexpression indicated greater sensitivity to neoadjuvant anthracycline- and taxane-based chemotherapy. Thus, retesting HER2/neu IHC status in residual tumors after NAC should be considered in order to optimize adjuvant systemic therapy.
and systemic dissemination of tumor cells into the circulation. Third, treatment efficacy can be monitored and allow chemosensitivity testing in vivo. Moreover, approximately 15% of patients have complete remis-
sion of the primary tumor and a better clinical outcome compared with
those with partial remission or no response.2

Although some patients undergoing neoadjuvant therapy might suffer from the side effects without benefitting from the treatment and even lose the valuable window of time for effective treatment, NAC for invasive breast cancer is now a relatively standard treat-
ment. This systemic adjuvant therapy is mainly based on expression of the
estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu) in the core needle biopsy before treatment. Little is known about the influence of NAC on those receptors and the possible consequences for subsequent adjuvant systemic therapy. Although statistically significant changes in ER/PR expression and Ki-67 labeling index after administration of NAC have been identified in many retrospective studies, its influence on HER2/neu status has not been adequately investigated. This issue has become important in recent years because of the development and approval of trastuzumab for
the treatment of HER2/neu overexpressing tumors, and decreased HER2/
neu expression in invasive breast cancer after NAC has been noted.4,5 How-
ever, contradictory findings also have been reported,10–13 and there is cur-
rently no consensus on whether post-NAC tumors should be reexamined for HER2/neu status. The present study attempts to document the effects of NAC on HER2/neu status in breast cancer using immunohistochemistry (IHC) and fluorescence in situ hybrid-
ization (FISH).

**Patients and Methods**

**Patients**

Breast cancer samples were selected retrospectively from archived files of the Department of Pathology, Xijing Hospital. All patients (n = 168) presented with primary invasive breast cancer with or without axillary lymph node involvement, and the diagnosis was established by core needle biopsy between January 2008 and December 2010. The test group comprised 131 patients who received combina-
ton NAC with docetaxel (T, 75 mg/m2), pirarubicin (40 mg/ m2) or epirubicin (E, 60 mg/m2) (both are anthracyclines with the same clinical efficacy,14 and ‘E’ represents pirarubicin or epirubicin in this article), cyclophosphamide (C, 600 mg/m2), fluorouracil (F, 500 mg/m2), and/or recombinant human endostatin injection (RHEI, 15 mg, brand name: Endostar) administered at 21-day inter-
vals. The neoadjuvant combinations included TEC (n = 52), TE (n = 46), TE plus RHEI (n = 18), CEF (n = 3), and TC (n = 2), and 5 patients changed therapy regimens because of nonsensitivity of tumor to previous agents (Table 1A). One to 9 cycles of chemother-
apy were administered preoperatively until the best possible response was achieved, and most patients (n = 95) received 3 cycles of treat-
ment. For 5 patients, details of NAC treatments could not be obtained. From 6 to 63 days (median, 20 days) after the final cycle of
chemotherapy, the patients underwent definitive surgery consisting of either a mastectomy or breast-conserving surgery and axillary lymph node dissection according to the standard protocols of the National Comprehensive Cancer Network clinical practice guideline
s (V.2.2008). In the control group, there were 37 patients who did not receive any presurgical neoadjuvant treatment (Table 1B).

The median time from diagnosis to surgery was 3 days (range, 1–16
days). All patients gave informed consent, and the trial was approved by the Medical Ethical Committee of the Xijing Hospital.

**Immunohistochemistry**

Using the Ventana BenchMark XT system (Roche Ltd), IHC staining for HER2/neu was performed on formalin-fixed paraffin-
embedded tissue sections of the core needle biopsies taken before treatment and the resection specimens after NAC in the same batch for each patient. Immunohistochemistry was carried out according to the protocol described by the manufacturer. The preanalytic process was completed according to the guidelines of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) that time to tissue fixation was kept to <1 hour and the length of fixation with 10% neutral-buffered formalin is >6 hours but <48 hours. Antigen retrieval was performed in 10 mM sodium citrate buffer (pH 6.0) for 30 minutes. The tissue sections were incubated with rabbit anti-human HER2/neu (GA048504; Dako Corp, Carpinteria, CA) for 32 minutes. In 10 tumors displaying a complete pathologic response or nearly complete primary tumor remission, the residual axillary node metastases were used to evaluate the HER2/neu status. For 37 patients in the control group, core needle biopsy samples obtained before surgery and the subsequent surgical resection specimens were available for IHC. Known positive control slides were run with each staining batch, and normal breast epithelial cells represented an internal negative control. Replacement of the primary antibody with phosphate-buffered saline (PBS) and 1% nonimmune serum solution in PBS also served as negative controls. Following our routine practice, at least 2 attending pathologists scored semiquantitatively the IHC staining as 0, 1+, 2+, or 3+ based on both the intensity and proportion of membrane staining according to the recent criteria recommended by ASCO/CAP, in which IHC staining scores of 0 and 1+ were considered as negative, 2+ as doubtful, and 3+ as positive. In case of disagreement, the scores were reassessed by discussion.

**Fluorescence In Situ Hybridization**

The PathVysion HER2 DNA Probe Kit (Vysis Inc, Downers Grove, IL) was used to detect the HER2/neu gene via FISH in 32 pairs of formalin-fixed paraffin-embedded breast cancer tissue specimens in which the HER2/neu IHC scores changed, except for those in which the score changed only from 1+ to 0 or from 0 to 1+. Assay procedures were performed according to the PathVysion protocol described in the package insert as approved by the US Food and Drug Administration. The paraffin-embedded sections were deparaffinized in Hemo-De and dehydrated in ethanol, followed by pretreatment of the slides, protease treatment, and fixation of the specimens. To denature the specimen DNA, the prepared slides were immersed in the denaturing solution at 72°C. The sections were hybridized with fluorescent-labeled DNA probes for the HER2/neu gene and α-satellite DNA sequence at the centromeric region of chromosome 17. Posthybridization washes were carried out with wash buffer (2 × SSC/0.3% NP-40). The nuclei were routinely counterstained with an intercalating fluorescent counterstain 4,6-diamidino-2-phenylindole (DAPI). Vysis ProbeChek control slides were processed concurrently with patients slides in each batch. Hybridization of the HER2/neu and chromosome 17 enumeration probes to their target regions were marked by orange and green fluorescence, respectively. All other DNA fluoresced blue with the DAPI stain. For each tumor, 60 nonoverlapping interphase nuclei of tumor cells with intact morphology were identified and scored for both HER2/neu gene and chromosome 17 centromere numbers using a Zeiss Axioplan 2 microscope with a magnification ×100 planar objective and a triple band-pass filter that permitted simultaneous blue, green, and red colors. HER2/neu gene amplification was defined as a HER2/neu-to-chromosome 17 signal ratio ≥2.2 as required by the manufacturer, and negative specimens showed a ratio of <1.8. Analysis was repeated on a fresh specimen slide if the results were at or near the cutoff point (1.8–2.2).

**Statistical Analysis**

Statistical analysis was carried out using SPSS 17.0 for Windows. P values were 2-sided, and the α-value was set at .05. For comparison and analysis of HER2/neu IHC status between paired samples (core needle biopsy vs. surgical resection specimen with or without receiving NAC), we used the McNemar–Bowker test as an extension of McNemar’s setup. Differences of HER2/neu IHC status between the NAC and non-NAC groups were assessed with the χ² test. For cross-tabulation, HER2/neu status was categorized dichotomously as altered (increased or decreased) or stable. Comparison of the decrease in HER2/neu IHC status between 2 groups was carried out with the χ² test. Associations between alteration in HER2/neu IHC status and various factors, such as the tumor subtype, pathologic response to NAC, adjuvant regimens, and time interval from the last chemotherapy to surgery were tested with Spearman’s test for nonparametric correlation. The association between pathologic response and HER2/neu IHC status pre- or post-NAC was also analyzed using Spearman’s test.

**Results**

**Characteristics of Patients and Tumors**

The mean age of the 131 patients at diagnosis was 49.6 years (median age, 49 years, ranging from 30 to 74 years) in the test group receiving NAC. Histologic classification could be made for all tumors: 111 (84.7%) cases were classified as invasive ductal carcinoma (IDC) (Figure 1A), 9 (6.9%) as invasive lobular carcinoma (ILC), 3 (2.3%) as mucinous carcinoma, 2 (1.5%) as basal-like breast carcinoma (BLBC), and 6 (4.6%) as other subtypes, including tubular carcinoma, micropapillary carcinoma, neuroendocrine carcinoma, and mixed invasive patterns. The data are summarized in Table 1A. In the control group, the mean age of 37 patients without NAC was 51.8 years (median age, 53 years, ranging from 32 to 81 years). The histopathologic subtypes included IDC (30 cases; 81.1%), ILC (2 cases; 5.4%), mucinous carcinoma (2 cases; 5.4%), BLBC (1 case; 2.7%), medullary carcinoma (1 case; 2.7%), and micropapillary carcinoma (1 case; 2.7%) (Table 1B).

**Response to NAC**

Pathologic responses of the primary tumor to NAC in 129 patients were semiquantified by analysis of hematoxylin and eosin-stained sections and the residual tumor volume of the surgical specimens according to Miller and Payne system (MP system) and the criteria of the Japanese Breast Cancer Society (JBCS) (Figure 1B), respectively. Pathological complete response (pCR) of the primary tumor after neoadjuvant treatment was observed in 15 patients (11.6%), among whom 7 had tumor-negative axillary lymph nodes with undetectable HER2/neu expression in the surgical resection specimens. Marked changes approaching pCR were found in 11 cases (8.5%), and 3 patients showed no response to NAC. The results are summarized in Table 2.
HER2/neu Protein Expression and Gene Status

The HER2/neu IHC scores of core needle biopsies and resection specimens of 124 patients receiving NAC were compared with those of 37 patients who had not received NAC. Seven patients had to be excluded from the analysis mainly because of the complete disappearance of tumor cells in both the primary site and axillary node after NAC. By IHC analysis of the core needle biopsy, 21.5% of breast carcinomas (28 of 130) were determined to be HER2/neu 3+1, compared with only 15.3% of patients (19 of 124) in postchemotherapy surgical resection specimens. The HER2/neu IHC score remained the same in 80 (64.5%) pre- and post-NAC paired samples, and 31 pairs (83.8%) remained stable in the control group. A decrease of HER2/neu IHC score was noted in 29 of 124 (23.4%) study cases (Figure 1C and D) and 2 of 37 (5.4%) control cases, and HER2/neu IHC scores increased in the excised specimens of 15 patients (12.1%) in the study group and 4 cases (10.8%) in the control group. As summarized in Table 3A, the difference in HER2/neu IHC status before and after chemotherapy exposure of the test group was statistically significant \((P = .021, \text{McNemar–Bowker test})\). Meanwhile, in the control group HER2/neu IHC status before and after surgery was not statistically significant \((P = .572, \text{McNemar–Bowker test})\) (Table 3B). However, the difference between the proportion of cases with altered HER2/neu status (increased or decreased) in the test group and that in the control group was statistically significant \((P = .026, \chi^2 \text{test})\). Simultaneously, the difference in the rate of decreased HER2/neu IHC scores between the 2 groups was statistically significant \((P = .015, \chi^2 \text{test})\), while the comparison between the increased HER2/neu IHC status in the test group (15 of 124; 12.1%) and those of the control group (4 of 37; 10.8%) was not \((P = .83, \chi^2 \text{test})\).

FISH was performed for paired samples with alterations of HER2/neu IHC scores, and none of the 32 pairs of specimens changed from positive to negative or from negative to positive (Figure 1E and F). The expression of HER2/neu protein in 14 patients with HER2/neu gene amplification decreased by IHC after NAC, and the HER2/neu
HER2/neu IHC Status and Tumor Subtypes

Alterations in HER2/neu IHC status were analyzed for correlation with subtypes of 124 tumor cases. Of the 104 IDC cases, the HER2/neu IHC score decreased in 25 resection specimens and increased in 13 cases after NAC, and that of 3 pairs of mucinous carcinoma specimens remained the same. Of the 9 ILC samples, HER2/neu IHC status decreased in 3 patients and increased in 2 cases. However, changes in HER2/neu IHC status did not correlate with subtypes of breast cancer (P = .237, Spearman’s test). Furthermore, the HER2/neu IHC status pre- and post-NAC were also not significantly correlated with the histologic subtypes of invasive breast cancer (P = .960 and .372, respectively, Spearman’s test).

HER2/neu IHC Status and Assessment of Pathologic Response to NAC

Pathologic responses to NAC were assessed in 129 patients, because 2 cases were excluded because of lack of available therapy data. The association between alteration of HER2/neu IHC status and pathologic response to NAC was analyzed in 122 patients, but no correlation was found (P = .748 and .831 according to MP system and JBCS criteria, respectively; Spearman’s test).

Additionally, to determine the predictive value of HER2/neu expression in the prechemotherapy biopsy, we compared the HER2/neu IHC score of core biopsies and resection specimens with the degree of pathologic response (based on the JBCS criteria) in 116 patients receiving neoadjuvant TE-based chemotherapy (TE or TEC or TE plus Endstar) (Tables 4 and 5). The results showed that HER2/neu protein overexpression levels both in core biopsies and in resection specimens were significantly associated with favorable pathologic responses to anthracycline and taxane-based chemotherapy (P = .046, r = 0.185 and P = .020, r = 0.222, respectively, Spearman’s test). Of the 35 patients with no response or mild response to NAC according to the JBCS criteria, the HER2/neu IHC score pre-NAC was ≤ 2+ in 29 patients (82.9%), and it was ≥ 2+ in 60.9% of patients (14 of 23) obtaining pCR or markedly high responses.

HER2/neu IHC Status and Neoadjuvant Regimens

In our trial, several regimens were used for NAC in breast cancer, of which the most common was the combination TEC (52 of 131 patients), followed by the combination TE (46 of 131 cases). There were 18 patients treated with TE plus RHEI. However, no correlation was detected between the alteration of HER2/neu IHC status and neoadjuvant cytotoxic agents (P = .981, Spearman’s test).

Furthermore, the influence of the number of chemotherapy cycles and time interval from the last chemotherapy to surgery on HER2/neu status were assessed. Because most patients received 3–4 cycles of chemotherapy, all cases were categorized into the following 3 subgroups: ≤ 2 cycles (n = 17), 3–4 cycles (n = 97), and ≥ 5 cycles (n = 6). Correlation tests demonstrated that the alteration of HER2/neu IHC status was not associated with the cycles of chemotherapy (P = .297, Spearman’s test). Likewise, the correlation between discordance of HER2/neu IHC score and time interval from the last

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**Table 3** Comparison of HER2/neu IHC Status

<table>
<thead>
<tr>
<th>Core Needle Biopsies</th>
<th>Surgical Resection Specimens</th>
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<tbody>
<tr>
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<tr>
<td>A. Pre- and Post-NAC</td>
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</tr>
<tr>
<td></td>
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<td>4</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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</tbody>
</table>

**Table 4** Relationship of Pathologic Response to HER2/neu IHC Status in Core Biopsy Pre-NAC in Patients Receiving TE-Based Chemotherapy

<table>
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<tr>
<th>HER2/neu IHC Status</th>
<th>Pathologic Response Assessment</th>
<th>Total</th>
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<tbody>
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<td>1a</td>
</tr>
<tr>
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<td>6</td>
</tr>
<tr>
<td>Total</td>
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<td>33</td>
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</tbody>
</table>

**Table 5** Relationship of Pathologic Response to HER2/neu IHC Status in Resection Specimens Post-NAC in Patients Receiving TE-Based Chemotherapy

<table>
<thead>
<tr>
<th>HER2/neu IHC Status</th>
<th>Pathologic Response Assessment</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
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<td>1a</td>
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<tr>
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<td>2</td>
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<tr>
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<tr>
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<tr>
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<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>33</td>
</tr>
</tbody>
</table>

Abbreviations: IHC = immunohistochemistry; JBCS = Japanese Breast Cancer Society; NAC = neoadjuvant chemotherapy; TE = docetaxel, and pirarubicin or epirubicin.

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chemotherapy to surgery was also not statistically significant (P = .531, Spearman’s test).

Discussion

Accurately assessing the change in HER2/neu status of invasive breast cancer specimens has become an important factor in formulating patient prognosis and providing individualized therapy. HER2/neu has been reported to be overexpressed in 20%–30% of invasive breast cancer cases. In our study, 21.8% of the test cases and 13.5% of the control group cases showed HER2/neu protein overexpression by IHC staining.

We analyzed the changes in HER2/neu IHC status in patients with invasive breast cancer receiving anthracycline- and/or taxane-based chemotherapy. Although the axillary node metastatic tumors had to be used to assess HER2/neu expression instead of surgical resection specimens in 10 cases because of near or complete remission, as it has been demonstrated that the HER2/neu status of primary tumors correlates with those of the lymph node metastatic tumor.18–20 A provoking finding in our study was that HER2/neu IHC scores increased in the resection specimens in 10.81% of patients (4 of 37) in the control group not treated with NAC and in 12.10% of those in the test group (15 of 124). In most cases that showed increased HER2/neu expression after NAC, the IHC scores changed from 0 to 1+ (9 of 19 patients, 47.4%) and from 1+ to 2+ (4 of 19 patients, 21.1%), and the rates of increased HER2/new IHC status between these 2 groups were not statistically significant (P = .83). In the test group, there were 4 patients with HER2/new IHC score alterations from 0 or 2+ to 3+, yet FISH analysis showed HER2/new gene amplification both in the core biopsy and the resection specimen. This result is in line with the published insight that there is a tendency to underestimate weakly positive cells in a core biopsy, and the false negative rate could be reduced by taking more core samples.21 Therefore, a likely explanation for the increase of HER2/new IHC status is that it is caused by sampling error within heterogeneous tumors or technical variables.3

In our trial, compared with only 5.41% of patients (2 of 37) in the control group (P = .572), HER2/new IHC scores decreased in 23.39% of patients (29 of 124) receiving NAC (P = .021). It was difficult to determine whether these differences were caused by the sampling error of the tumor or the therapeutic agents. Factors such as intratumor heterogeneity, differences in specimen processing and technical variables might have contributed to the HER2/new status variation, but published reports suggest that such differences induced by heterogeneity or technical artifacts are rare and have minor clinical significance.22,23 In our trial, the preanalytical process was carried out based on the guidelines of ASCO/CAP, and paired specimens from each patient were processed in the same staining batch for IHC analysis. Furthermore, our conclusion was based on the finding that the decrease of HER2/new IHC status in the study group was statistically significantly decreased compared with the well-matched control group without neoadjuvant therapy. Moreover, the HER2/new gene could be amplified by FISH in 14 patients with decreased HER2/new IHC score after chemotherapy in the test group. The results were consistent with some cases in the literature in which a tumor tissue showed loss of HER2/new protein expression yet the gene could be amplified by FISH after administration of combina-

tion adjuvant chemotherapy with F-epirubicin and/or C/T.24 Although some of the discordance observed in our series might have been caused by sampling error and technical variables, they did not account for the observed decrease in HER2/new IHC status seen in some cases. Therefore, the study demonstrated that therapeutic agents, at least in part, can downregulate tumor HER2/new expression. In a similar study by Burstein et al, the preoperative HER2/new status determined by IHC was reconfirmed using an enzyme-linked immunosorbent assay postoperatively.25 In their patient selection, all initially negative cases remained negative; however, 17% of the originally strong positive cases were found to be negative. Oldham et al demonstrated that paclitaxel downregulated HER2/new expression in MDA-MB453 cells by Western blot analysis and that HER2/new protein was decreased to 70% and 27% at 24 and 48 hours in paclitaxel-treated samples, respectively.26 However, the mechanism of downregulation of HER2/new expression after TE-based agent exposure is unclear and extremely variable. Because we found that the decrease of HER2/new IHC score was not related to the time interval difference from the last chemotherapy to surgery in our trial, it might not be because of a transient alteration in the metabolism of the cells related to treatment. However, it could not be determined whether the HER2/new receptor was actively downregulated in these cases or NAC was selecting for HER2/new negative tumor cells. Thus, a larger study is needed to identify whether the alteration of HER2/new IHC status is caused by TE-based chemotherapy via protein degradation. In contrast, Taucher et al analyzed tumors of patients on anthracycline/taxane-based neoadjuvant therapy regimens and found no significant change of HER2/new expression,13 and Sivara-jan et al reported a case showing conversion from HER2/new negative to positive.27 Thus, the findings in reported NAC studies are conflicting with respect to HER2/new status and response to anthracyclines. However, based on our results we recommend that the HER2/new IHC score should be reevaluated in posttreatment tissue, as clinical decisions regarding adjuvant cytotoxic treatment after NAC are frequently based on immunohistochemical analysis performed on surgical specimens.5 The decrease of HER2/new protein expression might be 1 of the mechanisms of secondary resistance to trastuzumab treatment because the drug targets the HER2/new protein.28,29

Although NAC was determined to induce changes in HER2/new IHC status in our study, differences between chemotherapy regimens were not statistically significant. In fact, most patients received TE-based chemotherapy in our trial (88.5%, 116 of 131), and comparison of other agents, such as trastuzumab and hormonal therapy, might reveal potential differential effects. Furthermore, the pathologic response was not related to the NAC regimens, and this result might be influenced by the doses of chemotherapy agents used. The number of chemotherapy cycles and time interval from the last chemotherapy to surgery also were not associated with HER2/new IHC status. These findings were consistent with previous studies reporting that there is no statistical difference between the altered and conserved HER2/new status groups in the number of chemotherapy cycles30 and that the time of assessment of the HER2/new status is not a critical factor with neoadjuvant therapy including epirubicin and docetaxel.13 Therefore, the dose intensity, cumulative dose, and in-
individual cytotoxic agents in NAC might not contribute to alterations of HER2/neu status.

It has been reported that clinical and pathologic responses are poorly associated, and clinical assessments often overestimate the number of complete responders and underestimate the number of nonresponders. Histopathologic changes of breast tumors as a result of chemotherapy might be a valid parameter of the response to chemotherapy exposure. In particular, the rate of pCR was shown to correlate well with disease-free survival and overall survival and thus can be used as a surrogate marker for the clinical outcome of disease. Two methods for pathologic response assessment were used in our trial, which showed good concordance, although the proportion of cases with no pathologic response to NAC appeared to be lower in the present study than previously reported. For 116 patients receiving anthracycline- and taxane-based chemotherapy, the pathologic response was related to HER2/neu IHC status both pre- and post-NAC, and the response rate increased with high HER2/neu IHC scores. Therefore, HER2/neu IHC status in core needle biopsies might be used to predict sensitivity to TE-based chemotherapy. It has been demonstrated that HER2/neu expression is strongly predictive of pathologic response or even pCR. Previous clinical data have also shown that patients with HER2/neu overexpressing tumors are more likely to achieve complete clinical response to anthracyclines and taxanes. However, the molecular mechanisms responsible for the better response of HER2/neu overexpressing invasive breast cancer to NAC is not completely clear. Thus, it was suggested that HER2/neu-positive tumors are more sensitive to anthracycline chemotherapy because of the frequent co-amplification of HER2/neu and topoisomerase II, which is the target of anthracyclines. However, data on this issue in various studies are conflicting. Although Yao et al demonstrated that HER2/neu-negative breast cancers benefit from anthracycline-based NAC, they also observed that HER2/neu-positive tumors are more likely to respond to the paclitaxel-containing NAC. In contrast, Taucher et al assessed 97 primary breast cancer cases administered NAC including epirubicin and T and found no correlation of the response to chemotherapy with HER2/neu status. Therefore, a larger number of cases should be investigated prospectively to clarify this issue.

Good concordance of HER2/neu amplification by FISH has been reported, although the HER2/neu expression measured with IHC was found to be more discordant. Our results also indicated that HER2/neu status was stable by FISH, consistent with a study by Hannemann et al reporting that HER2/neu was not included in a set of 88 genes distinguishing between tumor material obtained before treatment and remaining tumors after chemotherapy by microarray hybridization. However, a large retrospective study (n = 368) described a change in HER2/neu status in 9.5% of patients, which included positive and negative switches of HER2/neu expression in 6% and 3.5% of the patients, respectively. More significant decreases have been demonstrated in other studies with the loss of HER2/neu amplification in 32%–43% of patients with residual tumors treated with/trastuzumab, which was associated with poor recurrence-free survival. More subtle differences in HER2/neu gene are likely to be present but can only be reliably identified by studying a larger group of patients. Based on our data, exposure to anthracycline- and/or taxane-based chemotherapy did not result in significant changes of HER2/neu gene amplification by FISH which appeared to be more stable than its protein expression. Therefore, HER2/neu gene amplification might be a moot point as if the protein is being downregulated for NAC. We recommend that patients with alteration of HER2/neu IHC scores with at least a score of 2+ or 3+ should have FISH analysis performed on posttreatment tissue specimens to accurately determine the true HER2/neu gene Status.

**Conclusion**

In summary, we presented a clinicopathological study evaluating HER2/neu status before and after NAC with anthracycline and taxane. Neoadjuvant chemotherapy seemed to affect the HER2/neu IHC status in primary invasive breast cancer tissue, and a change in HER2/neu IHC status might have important clinical consequences for adjuvant systemic treatment. Based on our results, NAC for breast cancer might decrease the HER2/neu protein expression level, and we recommend that the HER2/neu status should be retested in residual tumors after NAC in order to optimize adjuvant systemic therapy postsurgery. Furthermore, exposure to anthracycline- and/or taxane-based chemotherapy did not result in significant changes of HER2/neu gene amplification by FISH which appeared to be more stable than its protein expression. Therefore, HER2/neu gene amplification might be a moot point as if the protein is being downregulated for NAC. Patients with alteration of HER2/neu IHC scores should have FISH analysis to accurately determine the true HER2/neu gene status.

**Clinical Practice Points**

- Neoadjuvant chemotherapy for breast carcinoma resulted in the decrease of the HER2/neu protein expression.
- Alterations of HER2/neu IHC scores did not significantly correlate with tumor subtype, pathologic response to NAC, adjuvant regimen, or time interval from the last chemotherapy to surgery.
- HER2/neu protein overexpression both in core biopsies and in resection specimens were associated with favorable pathologic response to anthracycline- and taxane-based chemotherapy, and it also indicated greater sensitivity to neoadjuvant anthracycline- and taxane-based chemotherapy and might be a predictive marker for pathologic complete response.
- Thus, retesting HER2/neu IHC status in residual tumors after NAC should be considered in order to optimize adjuvant systemic therapy postsurgery.
- This study can contribute to the clinical management of patients.

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Peifeng Li and Tantan Liu contributed equally to this work.

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**Disclosures**

All authors have no conflicts of interest.
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