Association of *HER-2* Copy Number and *HER-2/CEP-17* Ratio with Neoadjuvant Taxane-Containing Chemotherapy Sensitivity in Locally Advanced Breast Cancer

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Compare the clinical value of copy number–based fluorescence in situ hybridization (FISH) versus *HER-2/CEP-17* ratio-based FISH in identifying patients who may benefit from taxane-containing neoadjuvant chemotherapy.
2. Consider the implications of *HER-2* copy number and aneusomy 17 when making treatment decisions in patients with locally advanced breast cancer.

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ABSTRACT

Purpose. Aneusomy 17 causes inconsistency in fluorescence in situ hybridization (FISH)-based human epidermal growth factor receptor (*HER*)-2 status assessment using different algorithms (copy number or the *HER-2*/centromere enumerator probe 17 [*CEP-17*] ratio). We investigated the effects of FISH-based *HER-2* status assessment and aneusomy 17 on responsiveness to neoadjuvant chemotherapy (NAC).

Patients and Methods. This prospective study recruited 152 patients with locally advanced breast cancer who un-

derwent four-cycle weekly paclitaxel plus carboplatin without trastuzumab.

Results. The pathologic complete remission (pCR) rate in the breast and axilla was 24.3% (95% confidence interval [CI], 17.7%–32.0%). Although *HER-2* status, assessed by either *HER-2/CEP-17* ratio–based FISH or copy number–based FISH, was a predictor of NAC sensitivity, ratio–assessed *HER-2* status had a poorer performance in determining patients' responsiveness to NAC ($p = .029$). Patients who were not *HER-2* amplified when assessed us-

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ing the *HER-2/CEP-17* ratio but were *HER-2* amplified when assessed using copy number ($\sim 5\%$) were eventually proven to be responsive to NAC, with a pCR rate of 57% (95% CI, 18.4%–90.1%). In contrast, patients who were *HER-2* amplified when assessed by the ratio but not *HER-2* amplified when assessed using copy number ($\sim 3\%$) were completely unresponsive. Higher *HER-2* copy numbers rep-

resented increasing chances of a pCR (adjusted odds ratio, 3.09; 95% CI, 1.35–7.08), with an apparent gene–dose effect (p for trend $< .001$).

Conclusion. It is likely that *HER-2* copy number but not the *HER-2/CEP-17* ratio determines NAC sensitivity. Additional studies to validate our findings are warranted. *The Oncologist* 2012;17:792–800

INTRODUCTION

Human epidermal growth factor receptor (HER)-2 is involved in the pathophysiology of breast cancer, and *HER-2* gene amplification has been reported in $\sim 20\%$ – 30% of breast cancers. It is well documented that anti-HER-2–targeted therapy (e.g., trastuzumab, lapatinib) can induce responses in HER-2⁺ (immunohistochemistry [IHC] score of 3+ or excess gene copies by fluorescence in situ hybridization [FISH]) cases and prolong patient survival when combined with chemotherapy. Regarding chemotherapy, HER-2 overexpression has been shown to be associated with a higher response rate to anthracycline-containing chemotherapy regimens [1, 2], which might be because anthracyclines are topoisomerase inhibitors [3] and the topoisomerase-II gene (*TOP2A*) is coamplified with *HER-2* in $\sim 30\%$ – 40% of HER-2⁺ cases [4–6]. However, the benefit of taxane-containing chemotherapy in patients with HER-2⁺ tumors is still controversial. Some studies suggest better responses to docetaxel or paclitaxel in patients with HER-2⁺ tumors whereas others do not [7–10].

Neoadjuvant chemotherapy (NAC) has become the standard treatment for locally advanced breast cancer, though the optimal regimen is unknown. Pathologic complete remission (pCR) of the tumor is an important independent prognostic indicator of prolonged disease-free and overall survival times, and pCR has been widely used as the primary endpoint in NAC trials [11]. Because only 3%–30% of patients achieve a pCR after cytotoxic drugs and $\sim 20\%$ do not benefit from NAC [12], it is important to identify patients who would probably respond to NAC.

According to the recent American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines for HER-2 testing [13], *HER-2* amplification can be determined by either the *HER-2*/centromere enumerator probe 17 (*CEP-17*) ratio or *HER-2* copy number. Thus, there is a discrepancy between these two algorithms resulting from aneusomy 17. Because aneusomy 17 (monosomy 17 or polysomy 17) is detected in $\sim 30\%$ of breast cancer cases [14, 15], the inconsistency between copy number–based *HER-2* status and *HER-2/CEP-17* ratio–based *HER-2* status might account for 6%–9% of patients overall. Thus far, it is hard to tell which algorithm is superior in predicting NAC sensitivity. Moreover, although the *HER-2* amplification level seems not to influence the benefit from adjuvant trastuzumab [16, 17], whether it affects the benefit of NAC or not has not been revealed.

We performed this analysis in patients from a prospective study to clarify these issues. In our previous studies, we initiated a phase II trial in 2007 to evaluate the activity and safety of

a weekly paclitaxel plus carboplatin (PCb) regimen as NAC in women with locally advanced breast cancer or large operable disease (ClinicalTrials.gov identifier, NCT01203267) [18]. The clinical response rate was 86% with a complete remission rate of 32%, and 19% of patients achieved a pCR. Because the weekly PCb regimen is active and tolerable, it is now routinely administered to patients with locally advanced breast cancer in our institution. The patients recruited in this study were consecutive incident cases who had locally advanced breast cancer and had completed four cycles of PCb NAC.

PATIENTS AND METHODS

Patients

Patients with histological confirmation (by core needle biopsy) of locally advanced breast cancer (tumor stage 3–4 or nodal stage 2–3) and without prior chemotherapy, radiotherapy, or hormone treatment were considered eligible. Other eligibility criteria included [18]: age 18–70 years; the absence of metastases assessed using chest computed tomography scan, abdominal ultrasound, and bone scan; adequate hematologic, renal, and hepatic function; a normal echocardiogram without severe cardiac arrhythmia or heart failure; and a negative pregnancy test for premenopausal women. Exclusion criteria were [18]: previous malignancy history (except for inactive nonmelanoma skin cancer and in situ carcinoma of the cervix), sensorial neurotoxicity grade ≥ 1 according to National Cancer Institute Common Toxicity Criteria, version 3.0 scoring system, active infection, and other comorbid conditions that would affect drug tolerance or impair compliance.

The protocol was reviewed and approved by the independent ethical committee and institutional review board of Shanghai Cancer Center, Fudan University [18]. All patients provided written informed consent.

IHC and FISH

IHC assessment of estrogen receptor (ER), progesterone receptor (PR), and HER-2 was conducted with paraffin-embedded tumor samples biopsied before NAC. The cutoff for ER and PR positivity was 1% of tumor cells with positive nuclear staining [19]. All patients had their tumors tested for HER-2 status using IHC and FISH, regardless the IHC results. A positive HER-2 result on IHC of 3+ requires uniform, intense membrane staining of $>30\%$ of the invasive tumor cells according to the ASCO/CAP guidelines [13]. FISH analysis was performed on paraffinized 5- μ m tissue sections using the PathVysion HER-2 DNA probe kit (Abbott Laboratories, Abbott Park, IL). For each case, a parallel hematoxylin and eosin–

Table 1. Clinicopathologic characteristics and response results of patients

Characteristic	n of patients	%
Median (range) age, yrs	50 (26–70)	
≤50	77	50.7
>50	75	49.3
Menopausal status at diagnosis		
Premenopausal	77	50.7
Postmenopausal	75	49.3
Primary breast tumor stage		
T1–2	121	79.6
T3–4	31	20.4
Node status at baseline		
Negative	46	30.3
Positive	106	69.7
Grade		
I	16	10.5
II	102	67.1
III	34	22.4
Estrogen receptor status		
Negative	39	25.7
Positive	113	74.3
Progesterone receptor status		
Negative	35	23.0
Positive	117	77.0
HER-2 status		
Immunohistochemistry score		
0–2+	107	70.4
3+	45	29.6
FISH (<i>HER-2/CEP-17</i> ratio)		
<2.0	105	69.1
≥2.0	47	30.9
FISH (<i>HER-2</i> copy number)		
<6	103	67.8
≥6	49	32.2
Polysomy 17		
No ^a	113	74.3
Yes	39	25.7
Response		
Clinical response		
Not complete	101	66.4
Complete	51	33.6
Pathologic response in the breast (Miller-Payne Scale ^b)		
Progression and grade 1 (not pCR)	30	19.7
Grade 2 (not pCR)	13	8.6

(continued)

Table 1. (continued)

Characteristic	n of patients	%
Grade 3 (not pCR)	50	32.9
Grade 4 (not pCR)	17	11.2
Grade 5 (pCR)	42	27.6
No pCR	115	75.7
pCR	37	24.3

^aSeven cases (4.6%) were monosomy 17; the remainder were normal 17.^bMiller-Payne Scale: progression, off study prior to surgery for clinical progression or additional nonprotocol therapy; grade 1, no or minimal reduction in tumor; grade 2, up to 30% reduction; grade 3, 30%–90% reduction; grade 4, >90% reduction but with some residual invasive (or axillary) disease; grade 5, no residual invasive carcinoma (or axillary) metastasis.

Abbreviations: FISH, fluorescence in situ hybridization; HER-2, human epidermal growth factor receptor 2; pCR, pathologic complete response.

Figure 1. Flow chart of patients in the study.

Abbreviations: BC, breast cancer; FISH, fluorescence in situ hybridization; HER-2, human epidermal growth factor receptor 2; NAC, neoadjuvant chemotherapy; PCb, paclitaxel and carboplatin; PCbH, PCb plus trastuzumab.

stained slide was examined for regions of invasive carcinoma by pathologists. The FISH assessment was conducted by two practiced pathologists in the Department of Pathology of Shanghai Cancer Center (X.Y.Z. and X.L.Z.). The pathologists reported average copy numbers of *HER-2* and *CEP-17*, and the *HER-2/CEP-17* ratio of each case. Quality control of the *HER-2* FISH test was assessed routinely according to standard CAP and the American College of Medical Genetics guidelines [20]. For convenience, we classified a *HER-2/CEP-17* ratio ≥ 2.0 and an average *HER-2* copy number ≥ 6 as *HER-2* amplified in this study, because oncologists begin treating patients with anti-*HER-2*–targeted therapy at a ratio of 2.0 [13].

Of note, when polysomy 17 increases, the *HER-2/CEP-17* ratio decreases even if the *HER-2* copy number does not

Table 2. Relation among HER-2 expression, *HER-2* and *CEP-17* copy number, and the *HER-2/CEP-17* ratio

HER-2 status	IHC score 0–2+		IHC score 3+		<i>p</i> -value ^a
	<i>n</i> of patients	%	<i>n</i> of patients	%	
FISH (<i>HER-2/CEP-17</i> ratio)					
<2.0	97	90.7	8	17.8	<.001
≥2.0	10	9.3	37	82.2	
FISH (<i>HER-2</i> copy number)					
<6	100	93.5	3	6.7	<.001
≥6	7	6.5	42	93.3	
Polysomy 17					
No	89	83.2	24	53.3	<.001
Yes	18	16.8	21	46.7	

^a*p*-value by Pearson χ^2 test.

Abbreviations: CEP-17, centromere enumerator probe 17; FISH, fluorescence in situ hybridization; HER-2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

change. We categorized three levels of *CEP-17* signal according to criteria previously reported [16]: polysomy 17, three or more *CEP-17* signals in >30% of nuclei; monosomy 17, one or fewer *CEP-17* signals in >60% of nuclei; and normal 17, the other cases.

Treatment

Detailed information on NAC with the PCb regimen has been described elsewhere [18]. Briefly, all eligible patients received four cycles of paclitaxel (Taxol®; Bristol-Myers Squibb, New York) at a dose of 80 mg/m² in a 1-hour infusion immediately followed by carboplatin at an area under the curve (AUC) of 2 mg·min/mL in a 30-minute infusion given on days 1, 8, and 15 of a 28-day cycle. Creatinine clearance was calculated using the Jelliffe formula; AUC was calculated using the Calvert formula.

Patients with operable disease after NAC received breast surgery with level II–III axillary dissection within 4 weeks of the last scheduled chemotherapy cycle. Breast surgery type was at the surgeon's discretion; conservation was allowed. Postoperative treatments were performed according to our previous report.

Clinical and Pathological Evaluation

Response Evaluation Criteria in Solid Tumors guidelines were used to evaluate clinical response. Pathologic response was assessed postoperatively using the Miller-Payne scoring system [21, 22] (Table 1 footnote). Grade 5 of the Miller-Payne scale is considered as a pCR; grades 1–4 are not considered to be a pCR.

Statistical Analysis

Differences in clinicopathologic variables between subgroups were checked using χ^2 tests. Logistic regression analysis was used to calculate the odds ratio (OR) with its 95% confidence interval (CI) and for the multivariate anal-

ysis. Kruskal–Wallis analysis was used to compare continuous variables among three or more groups. To further evaluate the discrimination performance of HER-2 status according to different algorithms in predicting NAC sensitivity, we drew receiver operating characteristic (ROC) curves and calculated the AUC with its 95% CI. In this procedure, the continuous values of *HER-2* copy number and *HER-2/CEP-17* ratio were tested for their sensitivity and specificity for NAC response (pCR or not) at different cut-offs. The AUCs of two ROC curves were compared under nonparametric assumption. The ROC curve analysis and AUC comparison procedures were conducted using STATA software (StataCorp LP, College Station TX). A *p*-value ≤.05 was considered statistically significant. Statistical analyses were performed using STATA version 10.0 and SPSS version 12.0 (SPSS, Inc., Chicago, IL).

RESULTS

Patient Characteristics

We initially recruited 197 consecutive patients with locally advanced breast cancer in January 2009 to October 2010. They were administered the weekly PCb regimen or PCb plus trastuzumab (the PCbH regimen) (for HER-2⁺ patients when economically affordable) for four cycles. Among these patients, 34 who were treated with neoadjuvant PCbH and 11 who did not complete four cycles of NAC or were unavailable for FISH *HER-2* assessment were excluded from this study. Finally, 152 patients who completed four cycles of PCb NAC were included (Fig. 1). Clinicopathologic characteristics are shown in Table 1. The median age was 50 years (range, 26–70 years). About half of the patients (50.7%) were premenopausal. The median size of breast tumors was 5.0 cm, 74.3% were ER⁺, 77.0% were PR⁺, and 29.6% were HER-2⁺ based on an IHC score of 3+. In the FISH *HER-2* assessment, 30.9% and 32.2% of patients were classified as *HER-2* amplified using the *HER-*

Figure 2. pCR rates in the breast and axilla in subgroups according to *CEP-17* copy number and *HER-2* status by different algorithms. The diagonal, $y = 2x$, indicates the borderline with a *HER-2/CEP-17* ratio of 2.0. The area above the line $y = 2x$ indicates a *HER-2/CEP-17* ratio >2.0 (*HER-2* amplified) and the area under the line indicates a *HER-2/CEP-17* ratio <2.0 (*HER-2* not amplified). The pCR rate with 95% CI is shown. **(A):** Area in yellow denotes *HER-2* amplification with a ratio >2.0 ; the area in blue and green denotes no *HER-2* amplification with a ratio <2.0 . The vertical dashed line denotes the borderline of polysomy 17 with a *CEP-17* number of three. The area in green denotes cases without polysomy 17 and *HER-2* amplification; the area in blue denotes cases with polysomy 17 and *HER-2* amplification. **(B):** The vertical dashed line denotes the borderline of polysomy 17 with a *CEP-17* number of three; the horizontal dashed line denotes the borderline of a *HER-2* copy number of six. Areas in yellow, purple, green, and blue represent a *HER-2/CEP-17* ratio >2.0 and *HER-2* copy number >6 , *HER-2/CEP-17* ratio >2.0 and *HER-2* copy number <6 , *HER-2/CEP-17* ratio <2.0 and *HER-2* copy number <6 , and *HER-2/CEP-17* ratio <2.0 and *HER-2* copy number >6 , respectively. **(C):** ROC curves of *HER-2* status using ratio-based FISH and copy number-based FISH in discriminating patients responsive to neoadjuvant chemotherapy. The area under the ROC curve of the ratio-based FISH was 60% whereas that of the copy number-based FISH was 66% ($p = .029$).

Abbreviations: CEP-17, centromere enumerator probe 17; CI, confidence interval; FISH, fluorescence in situ hybridization; *HER-2*, human epidermal growth factor receptor 2; pCR, pathologic complete remission; ROC, receiver operating characteristic.

2/CEP-17 ratio and copy number, respectively. The incidence of polysomy 17 was 25.7% (95% CI, 18.9%–33.3%) in our study population.

Association of *HER-2* Protein Expression with *HER-2* Status

Comparisons between *HER-2* status assessed using IHC and that assessed using FISH (based on the *HER-2/CEP-17* ratio and copy number) are displayed in Table 2. Although ratio-based FISH and copy number-based FISH both had high concordance with IHC results—concordance rates of 88.2% (95% CI, 81.9%–92.8%) between IHC and ratio-based FISH and 93.4% (95% CI, 88.2%–96.8%) between IHC and copy number-based FISH—copy number-based FISH analysis might be more accurate than ratio-based FISH in determining the genetic basis of an IHC score of 3+ (in cases with an IHC score of 3+, 93.3% had a *HER-2* gene copy number ≥ 6 and 82.2% had a *HER-2/CEP-17* ratio ≥ 2.0 ; $p = .036$). In addition, polysomy 17 was more common in tumors with overexpression of *HER-2* (IHC score of 3+) than those with low-to-normal *HER-2* expression (IHC score of 0–2+) ($p < .001$). However, in the subgroup with no *HER-2* amplification (copy number <6), polysomy 17 was not related to a higher expression level of *HER-2* (IHC score 3+) ($p = .49$). Overall, polysomy 17 was associated with *HER-2* overexpression in the entire population but not in the group without *HER-2* amplification, which reveals a genetic linkage between the *CEP-17* locus and *HER-2* gene rather than a causal relationship between the *CEP-17* locus and *HER-2* expression.

Treatment Response and Predictors of Response

In terms of clinical response, there were 51 (33.6%; 95% CI, 26.1%–41.6%) cases of complete remission and 63 (41.4%; 95% CI, 33.5%–49.7%) cases of partial remission, for a total of 114 (75.0%; 95% CI, 67.3%–81.7%) objective clinical responses. Evaluation of pCR included patients whose disease had progressed. Forty-two (27.6%; 95% CI, 20.7%–35.5%) cases of pCR occurred in the breast, and 37 (24.3%; 95% CI, 17.7%–32.0%) pCRs occurred in both the breast and axilla (Table 1).

Regarding predictors of pCR, patients who were older, had higher grade disease, and were ER⁺, PR⁺, and *HER-2*⁺ (IHC score, 3+) achieved higher rates of pCR in the breast and in the breast and axilla, but tumor size and nodal status at baseline had no significant effect on tumor response. Remarkably, tumors with polysomy 17 had a relatively higher pCR rate than cases without polysomy 17, though this was not statistically significant in either the breast ($p = .35$) or the breast and axilla ($p = .13$). After Bonferroni adjustment for multiple comparisons, grade ($p = .035$), ER status ($p = .011$), PR status ($p = .002$), and *HER-2* status by IHC ($p = .028$) were still strongly associated with a pCR in the breast and axilla.

Table 3. Effects of *HER-2* status assessed by FISH and *CEP-17* copy number on neoadjuvant chemotherapy response

	No pCR in breast and axilla		pCR in breast and axilla		Unadjusted OR (95% CI)	<i>p</i> -value	Adjusted OR ^a (95% CI)	<i>p</i> -value ^a
	<i>n</i> of patients	%	<i>n</i> of patients	%				
<i>HER-2</i> copy number								
<6	87	75.7	16	43.2	1 (reference)		1 (reference)	
≥6	28	24.3	21	56.8	4.08 (1.87–8.87)	3.9 × 10 ^{−4}	3.09 (1.35–7.08)	.008
<i>HER-2/CEP-17</i> ratio								
<2.0	85	73.9	20	54.1	1 (reference)		1 (reference)	
≥2.0	30	26.1	17	45.9	2.41 (1.12–5.20)	.025	1.86 (0.82–4.21)	.140
<i>HER-2</i> copy number and <i>CEP-17</i> copy number								
<i>HER-2</i> copy number <6 and <i>CEP-17</i> <3	73	83.0	15	17.0	1 (reference)		1 (reference)	
<i>HER-2</i> copy number <6 and <i>CEP-17</i> ≥3	14	93.3	1	6.7	0.35 (0.04–2.85)	.32	0.35 (0.04–2.96)	.33
<i>HER-2</i> copy number ≥6 and <i>CEP-17</i> <3	15	62.5	9	37.5	2.92 (1.08–7.90)	.035	2.22 (0.78–6.35)	.10
<i>HER-2</i> copy number ≥6 and <i>CEP-17</i> ≥3	13	52.0	12	48.0	4.49 (1.72–11.75)	.002	3.42 (1.21–9.64)	.02
<i>HER-2</i> copy number and <i>HER-2/CEP-17</i> ratio								
<i>HER-2</i> copy number <6 and <i>HER-2/CEP-17</i> ratio <2.0	82	83.7	16	16.3	1 (reference)		1 (reference)	
<i>HER-2</i> copy number <6 and <i>HER-2/CEP-17</i> ratio ≥2.0	5	100.0	0	0.0	NA	NA	NA	NA
<i>HER-2</i> copy number ≥6 and <i>HER-2/CEP-17</i> ratio <2.0	3	42.9	4	57.1	6.83 (1.39–33.51)	.018	5.69 (1.02–31.74)	.047
<i>HER-2</i> copy number ≥6 and <i>HER-2/CEP-17</i> ratio ≥2.0	25	59.5	17	40.5	3.48 (1.54–7.88)	.003	2.64 (1.11–6.30)	.028

The OR indicates the likelihood of responsiveness to neoadjuvant chemotherapy relative to reference group.

^aAdjusted for estrogen receptor and progesterone receptor status.

Abbreviations: CEP-17, centromere enumerator probe 17; FISH, fluorescence in situ hybridization; HER-2, human epidermal growth factor receptor 2; NA, not applicable; OR, odds ratio; pCR, pathologic complete response.

Different Algorithms of *HER-2* Status and NAC Response

Subsequently, we investigated the response to PCb NAC according to *HER-2* copy number, aneusomy 17 status, and the *HER-2/CEP-17* ratio. Figure 2A shows that, among patients with *HER-2*–amplified tumors assessed using a *HER-2/CEP-17* ratio ≥2.0 (area in yellow), 36.2% achieved a pCR whereas only 19.0% of patients without *HER-2* amplification achieved a pCR ($p = .023$). When we defined *HER-2* amplification by copy number, the difference was more significant. Patients with a high *HER-2* copy number (≥6) had a 4.08-fold (95% CI, 1.87–8.87; $p < .001$) greater chance of achieving a pCR; after adjustment for ER and PR status, this was still 3.09-fold greater (95% CI, 1.35–7.08; $p = .008$) (Table 3). The effect of aneusomy 17 on responsiveness was further evaluated by dividing patients into a polysomy 17 group and a group without polysomy 17. In the overall population, polysomy 17 tended to be a predictor of higher NAC sensitivity (OR, 1.85; 95% CI, 0.83–4.14; $p = .13$); in cases that were not *HER-2* amplified as assessed by

the *HER-2/CEP-17* ratio, patients with polysomy 17 and those without polysomy 17 had comparable pCR rates ($p = .53$) (Fig. 2A).

Notably, the varying *HER-2* status according to different algorithms might result in different response results (Fig. 2B). In *HER-2*–amplified cases assessed using the *HER-2/CEP-17* ratio, five patients (Fig. 2B, area in purple) who were proven to not have *HER-2* amplification according to copy number did not achieve a pCR at all. In contrast, in patients assessed as not having *HER-2* amplification using the *HER-2/CEP-17* ratio, seven (Fig. 2B, area in blue) had a mean copy number ≥6, with a pCR rate of 57.1% (95% CI, 18.4%–90.1%). It is thus likely that, at least in patients undergoing PCb NAC, it is the *HER-2* copy number that determines chemosensitivity whereas the *HER-2/CEP-17* ratio is of lower importance in some cases. High *HER-2* copy number (≥6) was an indicator of a two- to sixfold greater chance of a pCR than in patients with a low-to-normal *HER-2* copy number regardless of the *HER-2/CEP-17* ratio and polysomy 17 status (Table 3), and even after adjustment for ER and PR status (Table 3), which are strongly and

Figure 3. Dose effect of *HER-2* copy number on response. (A): Locally weighted least squares plot of relation between *HER-2* copy number and pCR rate based on univariate nonparametric smoothing method. (B): Distribution of *HER-2* copy number in our consecutive patients with locally advanced breast cancer. (C): Forest plot of the relative benefit from neoadjuvant PCb chemotherapy according to *HER-2* copy number. Intergroup comparisons were performed using logistic regression analysis. (D): Distribution of *HER-2* copy number according to the degree of pathologic response using the Miller-Payne scale. Horizontal lines represent median values. *p*-value is .0004 for heterogeneity by Kruskal-Wallis test and *p* for trend is 9.4×10^{-5} .

Abbreviations: CI, confidence interval; *HER-2*, human epidermal growth factor receptor 2; HR, hazard ratio; PCb, paclitaxel and carboplatin; pCR, pathologic complete remission.

independently associated with NAC resistance [9, 18, 23]. In the ROC curve analysis, the AUC of the ROC curve of the ratio-based FISH assay was 60%, whereas that of the copy number-based FISH assay was 66% ($p = .029$) (Fig. 2C), suggesting better discrimination performance of copy number-based FISH in determining patients responsive to taxane-containing NAC.

Dose Effect of *HER-2* Copy Number on Response

Although current evidence suggests that patients with higher *HER-2* copy numbers or *HER-2/CEP-17* ratios receive similar benefits from adjuvant trastuzumab as patients with lower *HER-2* test results [16, 17, 24]; whether or not this is also true for NAC without trastuzumab is unclear. The locally weighted least squares smoothing plot displays a gene-dose effect of *HER-2* copy number on the pCR rate (Fig. 3A). Figure 3B displays the distribution of *HER-2* copy number in the unselected consecutive population. There was an apparent trend toward higher *HER-2* copy numbers with increasing probabilities of a pCR, with *p*-value < .001 for the trend (Fig. 3C). Analogously,

when we divided the patients into five groups according to their degree of pathologic response assessed using the Miller-Payne Scale, a high-grade (i.e., 4 and 5) response in the breast correspond to a higher *HER-2* copy number ($p = .0004$ by Kruskal-Wallis test) (Fig. 3D).

DISCUSSION

The current study indicates that *HER-2* status assessed using copy number-based FISH is superior to that assessed using *HER-2/CEP-17* ratio-based FISH in identifying patients who will probably benefit from taxane-containing NAC, and increasing *HER-2* copy numbers correspond to incrementally higher response rates. Currently, *HER-2/CEP-17* ratio-based FISH is more prevalent than copy number-based FISH in evaluating *HER-2* status, and physicians might pay more attention to the relative value of the *HER-2/CEP-17* ratio rather than the absolute values of *HER-2* and *CEP-17* copy numbers. Therefore, we propose that adequate information on *HER-2* copy number and aneusomy 17 helps us to predict cases likely to be responsive to NAC.

Because of a technical flaw of the FISH assay, pathologists could only count the copy number of *CEP-17* but not chromosome 17. In this study, we treated the *CEP-17* copy number as the chromosome 17 copy number although they are not completely coincident [25, 26]. We defined aneusomy 17, polysomy 17, and monosomy 17 according to *CEP-17* copy number. Typically, monosomy 17 is detected much less often than polysomy 17 (<10% and >25%, respectively) [14]. In our study, 5% ($n = 7$) of patients were monosomy 17 and 26% were polysomy 17, consistent with former findings [14, 16]. Polysomy 17 is usually more prevalent in *HER-2*-amplified tumors than in tumors without *HER-2* amplification [16, 27]. We observed polysomy 17 in 38% (by ratio) and 48% (by copy number) of *HER-2*-amplified tumors and in 22% (by ratio) and 13% (by copy number) of nonamplified tumors.

Polysomy 17 might be a cause of the observation that some patients test negatively for *HER-2* amplification by ratio-based FISH analysis and still respond to trastuzumab [10]. Similarly, we found that nearly 5% of patients who were *HER-2*⁺ according to the *HER-2/CEP-17* ratio (because of polysomy 17) but *HER-2*⁺ by copy number were eventually proven to be responsive (57%) to NAC. In contrast, about 3% of patients who were *HER-2*⁺ according to the *HER-2/CEP-17* ratio but *HER-2*⁻ according to copy number (because of monosomy 17) were completely nonresponsive to neoadjuvant PCb. Accordingly, for patients with moderate *HER-2/CEP-17* ratios (but still ≥ 2.0) and monosomy 17 (*CEP-17* copy number ≤ 1), precautions should be taken and absolute gene copy number might be more reliable than the *HER-2/CEP-17* ratio alone as a indicator of their true *HER-2* status. Following the flow chart of algorithms for FISH in the ASCO/CAP guideline [13], a clinician may pay more attention to the final result of *HER-2* testing presented as a *HER-2/CEP-17* ratio but ignore the absolute *HER-2* and *CEP-17* copy numbers. We stress that reporting the *HER-2* and *CEP-17* copy number is very important, and clinicians should consider the results of the *HER-2/CEP-17* ratio as well as the *HER-2* and *CEP-17* copy numbers before making a clinical decision.

Recently, data from the Herceptin® Adjuvant trial [17], National Surgical Adjuvant Breast and Bowel Project B-31 trial [24], and N9831 trial [16] consistently showed that the *HER-2* amplification level assessed using either the *HER-2/CEP-17* ratio or *HER-2* copy number does not influence the benefit from adjuvant trastuzumab. However, in the neoadjuvant setting, Arnould et al. [28] claimed that there is a correlation between the *HER-2* amplification level and response to trastuzumab. To our best knowledge, only very few studies have been conducted to test the effect of *HER-2* amplification level on responsiveness to NAC. Gonzalez-Angulo et al. [29] failed to observe an association between *HER-2* amplification and a pathologic response to NAC with taxanes in patients with early-stage breast carcinoma. In contrast, we discovered a gene-dose effect between *HER-2* copy number and pCR rate, with higher *HER-2* copy numbers corresponding to higher response rates in locally advanced breast cancer patients.

The strengths of the current work include the fact that the study was conducted within a prospective population, the pa-

tients underwent a unified NAC regimen, and FISH results were obtained from every patient. Furthermore, considering that *HER-2* and *TOP2A* are in close proximity on chromosome 17 and abnormality in the *TOP2A* gene (its protein is the target of anthracyclines [3]) is observed in ~35% of *HER-2*-amplified breast tumors, we used a nonanthracycline-containing regimen to reveal the intrinsic interaction between *HER-2* and chemotherapy, with confounding factors that affect chemosensitivity excluded. Several limitations exist. First, because of the limited sample size, some subset analyses are exploratory and underpowered to detect between-subgroup differences. As well, because of the low number of patients, our results should be further validated in an independent and larger patient cohort. Second, aneusomy 17 is reported as a predictor of cancer aggressiveness [15]; however, in the current study, we had no survival data available, and the effect of polysomy 17 on survival needs further study.

In conclusion, our data indicate that it is the *HER-2* copy number that actually determines the benefit from NAC. *HER-2* status tested using *HER-2/CEP-17* ratio-based FISH analysis might be unreliable in some cases (8% in this study) because *CEP-17* copy number is not always a stable internal reference. Adequate information on *HER-2* copy number and aneusomy 17 could help us to predict responsiveness to taxane-containing NAC as well as the degree of response in patients with locally advanced breast cancer. Additional studies to validate our findings are warranted.

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