Clinical Cancer Research

Pathological Complete Response to Neoadjuvant Trastuzumab Is Dependent on HER2/CEP17 Ratio in HER2-Amplified Early Breast Cancer

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Abstract

Purpose: To evaluate whether pathologic complete response (pCR) to neoadjuvant trastuzumab is dependent on the level of HER2 amplification.

Experimental Design: 114 HER2-overexpressing early breast cancer patients who had received neoadjuvant trastuzumab were included in this study. Absolute HER2 and chromosome 17 centromere (CEP17) were measured by *in situ* hybridization analysis, and associations were examined between HER2/CEP17 ratio and tumor pCR status (commonly defined by ypT0 ypN0, ypT0/is ypN0, and ypT0/is).

Results: In trastuzumab-treated patients, ypT0 ypN0 was achieved in 69.0% of patients with high-level amplification (HER2/CEP17 ratio > 6), but only in 30.4% of tumors with low-level amplification (ratio $\leq$ 6; $P = 0.001$). When pCR was defined by ypT0/is ypN0 or ypTis, 75.9% and 82.8% of tumors with high-level amplification had a complete response, whereas only 39.1%, and 38.3% with low-level amplification achieved pCR ($P = 0.002$ and $P < 0.001$, respectively). Logistic regression revealed that tumors with high-level amplification had a significantly higher probability achieving ypT0 ypN0 (OR, 5.08; 95% confidence interval, 1.86–13.90; $P = 0.002$) than tumors with low-level amplification, whereas no other clinicopathologic parameters were predictive of pCR. The association between high-level HER2 amplification and pCR was almost exclusively confined to hormone receptor (HR)–positive tumors (ypT0 ypN0: 62.5% vs. 24.0%, $P = 0.014$; ypT0/is ypN0: 75.0% vs. 28.0%, $P = 0.005$; and ypT0/is: 87.5% vs. 28.0%, $P < 0.001$), and was largely absent in HR-negative tumors.

Conclusions: An HER2/CEP17 ratio of >6 in the pretherapeutic tumor biopsy is associated with a significantly higher pCR rate, particularly in HER2/HR copositive tumors, and can be used as a biomarker to predict response before neoadjuvant trastuzumab is initiated. *Clin Cancer Res; 23(14); 3676–83. ©2017 AACR.*

Introduction

HER2 is amplified in 18% to 20% of human breast carcinomas and has been intensively evaluated as a therapeutic target in recent years (1–4). The subsequent development of the HER2-targeting antibody trastuzumab has profoundly improved the outcome in the very subgroup of HER2-positive breast cancers that had been previously considered to have a particularly poor prognosis (5, 6). Trastuzumab-based regimen has therefore become the standard treatment for HER2-positive tumors in the adjuvant and metastatic setting. More recently, several randomized trials have also investigated its efficacy in the neoadjuvant setting, using pathologic complete response (pCR) as primary endpoint, and consistently found that 4–8 cycles of preoperative trastuzumab improved pCR rate (7–11). Furthermore, in HER2-positive breast cancer, the achievement of pCR was also associated with a highly favorable long-term outcome, thus supporting the use of pCR as an endpoint for neoadjuvant trials: in a recent pooled analysis, Cortazar and colleagues (12) have demonstrated that on a patient-level, pCR (defined as ypT0 ypN0 or ypT0/is ypN0) correlates with

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Translational Relevance

Our study is the first to determine the optimum HER2/CEP17 ratio cut-off value for tumor response (pathologic complete response, pCR), commonly defined by ypT0 ypN0, ypT0/is ypN0, and ypT0/is, for HER2-positive breast cancer patients treated with neoadjuvant trastuzumab. Results from our study show convincingly that a HER2/CEP17 ratio of >6 in the pretherapeutic tumor biopsy is associated with a significantly higher pCR rate, particularly in HER2/hormone receptor copositive tumors, and can be used as a biomarker to predict response before neoadjuvant trastuzumab-based therapy is initiated. Our findings, therefore, have significant implications for the treatment and clinical management of this subgroup of breast cancer patients.

significantly improved survival. Consequently, strategies that allow for the prediction of a ypT0 ypN0 or ypT0/is ypN0 status in HER2-overexpressing tumors may allow for identification of patients who will derive the greatest benefit from preoperative HER2-targeted treatment over those who will not (13).

To date, the identification of cancers that are likely to respond to trastuzumab is based on IHC and *in situ* hybridization (ISH) analyses. IHC detects the level of HER2 protein expression on the cell surface and allows for a semi-quantitative score ranging from 0 to 3+, with 3+ indicating a positive HER2 status. ISH, in contrast, identifies the number of *HER2* gene copies on chromosome 17 as well as the number of centromere 17 (CEP17) copies per nucleus (14). According to current ASCO/CAP clinical practice guidelines (15), a tumor exhibits HER2 amplification if the HER2/CEP17 ratio exceeds 2, or absolute HER2 copy number exceeded 6. Although both tests determine whether a tumor is a potential target for trastuzumab, treatment response in the metastatic setting is only achieved in about 25% of cases, and more than 25% of women treated with neoadjuvant trastuzumab-based chemotherapy relapse within the first ten years of diagnosis (16). As a consequence, an optimal method to assess the likelihood of response to trastuzumab remains to be determined.

We have previously measured the *HER2*/CEP17 ratio in a cohort of 120 HER2-overexpressing patients with metastatic breast cancer who had not received (neo)adjuvant trastuzumab, but were treated with HER2-directed therapy in the metastatic setting. In this population, we found that a *HER2*/CEP17 ratio of >6 independently predicted a shorter time to first metastasis, but was also associated with a higher response rate and an improved progression-free survival during trastuzumab treatment (17). On the basis of these results, we performed *HER2*/CEP17 ratio measurements in pretherapeutic biopsies from HER2-positive tumors of women participating in the prospective neoadjuvant ABCSG-24 and 32 trials. We hypothesized that tumors were more likely to achieve pCR in response to trastuzumab-based treatment, based on three commonly used definitions of complete pathologic response, if pretherapeutic *HER2*/CEP17 ratio exceeds 6.

Patients and Methods

The current investigation is part of the ABCSG translational research program (18). Women included in this study had been randomized into the prospective neoadjuvant ABCSG-24 and 32 trials between 2004 and 2014. Trial design, inclusion criteria, and the main clinical results of these trials have been reported previously (19). Briefly, in ABCSG-24, 536 patients with invasive breast cancer were randomly assigned 1:1 to receive six cycles of preoperative epirubicine/docetaxel (both 75 mg/m^2) every 3 weeks with or without capecitabine (1,000 mg/m^2, bid, days 1–14). Patients with HER2-positive disease were further randomized to receive neoadjuvant trastuzumab (8 mg/kg loading dose, then 6 mg/kg every 3 weeks; $n = 44$) or not ($n = 49$). In ABCSG-32, 100 pre- and postmenopausal patients with invasive HER2-overexpressing breast cancer were randomly assigned 1:1:1:1 to either of the following therapy arms: 6 cycles of docetaxel 100 mg/m^2 every 3 weeks (Arm A); 6 cycles of docetaxel 100 mg/m^2 every 3 weeks and bevacizumab 15 mg/kg every 3 weeks (Arm B); 6 cycles of docetaxel 100 mg/m^2 every 3 weeks and non-pegylated liposomal Doxorubicin (NPLD) 50 mg/m^2 (Arm C); 6 cycles of docetaxel 100 mg/m^2 every 3 weeks, bevacizumab 15 mg/kg every 3 weeks, and NPLD 50 mg/m^2 (Arm D); all randomized patients also received 6 cycles of trastuzumab every 3 weeks (8 mg/kg loading dose, then 6 mg/kg), irrespective of the treatment arm they had been randomized to. pCR was a primary or secondary endpoint in both trials (20). Approval was obtained from Institutional Review Boards.

HER2/CEP17 ISH analysis

Assessment of the HER2 gene and chromosome 17 status was performed in paraffin-embedded tissue from core needle biopsies of the primary tumor using the Vysis PathVysion DNA-based fluorescence ISH or Ventana INFORM Dual ISH (bright field) technologies as per manufacturer's instructions. Assessment was performed by six board-certified pathologists (M. Rudas, K.A. Tendl, Z. Bago-Horvath, A. Reiner, F. Moinfar, and C. Gruber) specialized in breast pathology with at least 10 years' experience in ISH testing for HER2 in local pathology laboratories affiliated to study centers. HER2 and CEP17 signals of $\geq$20 nuclei of tumor cells within invasive tumor areas were measured to determine the *HER2*/CEP17 ratio (R). If the *HER2*/CEP17 ratio exceeded 2, or absolute HER2 copy number exceeded 6, tumors were considered to be ISH-amplified, thereby following current ASCO/CAP guidelines.

Endpoint assessment

We measured the effect of neoadjuvant trastuzumab by the three most commonly used pCR definitions: ypT0 ypN0 (absence of invasive cancer and *in situ* cancer (DCIS) in breast and axillary lymph nodes), ypT0/is ypN0 (absence of invasive cancer in breast and in axillary lymph nodes, irrespective of remaining DCIS in the primary tumor), and ypT0/is (absence of invasive cancer in the breast irrespective of the presence of DCIS or nodal involvement). All trials had standard operating procedures for pCR assessments and the assessments were performed locally by experienced pathologists. Specimens locally judged as pCR were reviewed centrally by a reference pathologist.

Statistical analysis

Values for quantitative variables in normal distribution were expressed as the mean $\pm$ 95% confidence interval (CI), whereas in skewed distribution, the values were expressed as median $\pm$ range. χ^2 and Fisher exact (for smaller sample size) tests were used to compare tumors achieving pCR with *HER2*/CEP17 ratios. On the

basis of the findings from previously published articles (21–23), including findings of our own that showed a high-level of HER2 amplification (defined by HER2/CEP17 ratio of >6) is associated not only with shorter time to metastasis in trastuzumab-untreated breast cancer but is also predictive of longer progression-free survival upon initiation of trastuzumab-based treatment (17), the cut-off value of >6 was chosen as our study hypothesis. To test robustness, we also explored ratios >4 and >5 in this study. The Mann–Whitney test was used to test for difference between pCR subgroups and ratios when considered as continuous variable. All variables were made categorical, and associations between clinicopathologic parameters, including age, height, BMI, tumor grade, tumor stage, nodal status, hormone receptor [HR, i.e. estrogen receptor (ER) or progesterone receptor (PR)] status and menopause status with the *HER2*/CEP17 ratio were analyzed using the Spearman correlation test. Univariate logistic regression was conducted for all clinicopathologic parameters with pCR as outcome. The parameters found to be significant in univariate analysis were assessed for the multivariate analysis using enter logistic regression model, to evaluate which parameters were independent. The parametric statistical Wald test was also used. OR estimates and the relative 95% CI were calculated. For all analyses, a P value of <0.05 (two-tailed) was considered statistically significant. All statistical analysis was performed with SPSS version 23.0 (SPSS, Inc.).

Results

Patient characteristics

The total number of eligible patients included in this study was 135. Of these, 65 had been randomized to ABCSG-24 and 70 had been randomized to ABCSG-32. Twenty-one individuals were then excluded from the study because they had received trastuzumab as adjuvant therapy, leaving the remaining 114 in the study. However, due to the unsuccessful staining in either *HER2* or *CEP17* copy number signals/cell ($n = 34$) and missing pCR status for ypT0is ($n = 6$), ypT0is+ypN0 ($n = 9$) and ypT0+ypN0 ($n = 9$), the total number of cases per subgroup available for analyses varied (Fig. 1). The median *HER2*/CEP17 ratio was 4.68 (range: 0.81–28.00). Women had a median age of 50.3 years (range: 25.4–76.9 years), and a median BMI of 24.2 (17.6–43.5). The majority of tumors was pT2 (56%), with 20% of the tumors being smaller than 2 cm (pT1) and 18% larger than 5cm (pT3). Chest wall or skin was affected in 4% of cases and were included in our analysis. The majority of tumors with known tumor grade were G3 (73%), whereas only 5% were classified as G1 and 3% as G2. Grading was not available in 19% of cases. Fifty-five percent of patients in our analysis did not have nodal involvement (pN0), whereas 42% were classified as pN1, and 3% pN2 or pN3. At least one of the two steroid hormone receptors (HR-positive/luminal HER-amplified) was detected in 62 samples (54.4%) whereas both ER and PR were absent in 52 tumors (45.6%, HER2-amplified, nonluminal; Table 1 and Fig. 1).

HER2/CEP17 ratio and pathologic complete response

Women who experienced a pCR as defined by ypT0 ypN0 had a median *HER2*/CEP17 ratio of 7.07 (range: 1.15–28.00) in their diagnostic tumor biopsy, whereas those who did not had a median ratio of 4.58 (range: 0.81–21.07; $P = 0.010$, Mann–Whitney test, Fig. 2A). Similarly, tumors which responded to trastuzumab-based neoadjuvant treatment with ypT0/is ypN0 had a median ratio of 7.00 (range: 1.15–28.00) in comparison with a median ratio of 3.82 (range: 0.81–21.07) if they did not ($P = 0.001$, Fig. 2b). The most pronounced difference in the median *HER2*/CEP17 ratio was detected when pCR was defined as ypT0/is: tumors achieving ypT0/is had a median ratio of 7.07 (range: 1.15–28.00) whereas the median ratio in tumors that did not respond with ypT0/is had a pretreatment *HER2*/CEP17 ratio of 3.80 (range: 0.81–17.33; $P < 0.001$; Fig. 2C). The robustness analysis did not reveal strong qualitative differences between ratio >5 versus >6 (Supplementary Tables S1–S3). We have therefore used and reported results for the cut-off value at 6 from here onwards for consistency with other previously published findings where a higher ratio in general predicts for better response.

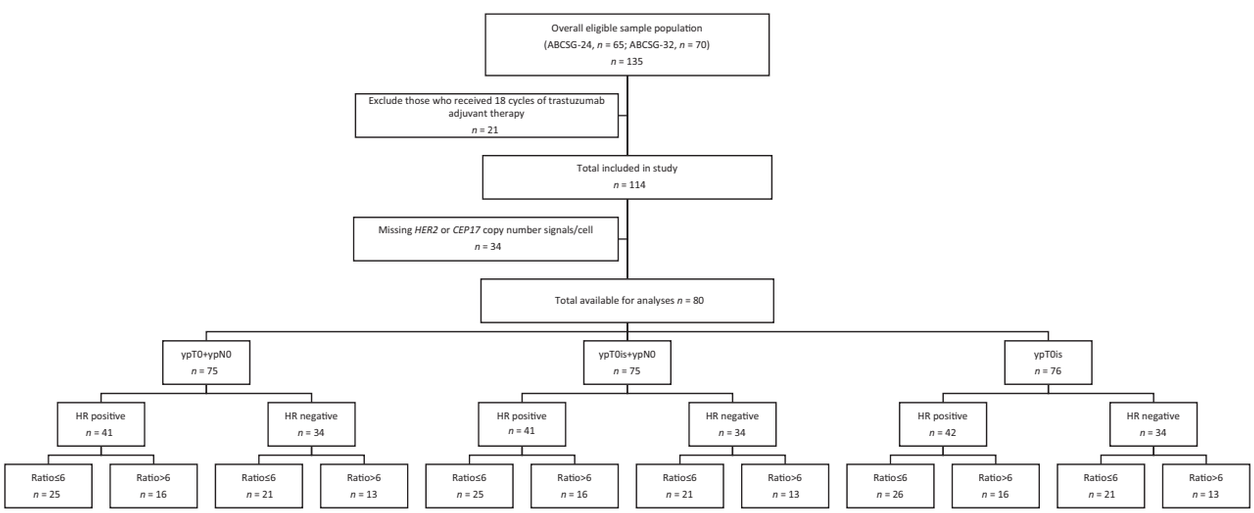

Figure 1.
REMARK diagram describing sample origin and characteristics.

Table 1. Patient demographics

Variable		*N* (%) Total = 114
Age, y	Median (years, range)	50.3 (25.4–76.9)
Height	Median (meter, range)	1.66 (1.50–1.79)
BMI	Median (range)	24.2 (17.6–43.5)
Tumor size	<2 cm	23 (20)
	2-5 cm	64 (56)
	>5 cm	20 (18)
	Any size, with extension to chest wall or skin	5 (4)
	Unknown	2 (2)
Grade (pretherapy)	G1	7 (5)
	G2	4 (3)
	G3	99 (73)
	Unknown	25 (19)
Stage (pretherapy)	T1	23 (20)
	T2	64 (56)
	T3	20 (18)
	T4	5 (5)
	Unknown	2 (2)
Nodal status (pretherapy)	N0	63 (55)
	N1	48 (42)
	N2	2 (2)
	N3	1 (1)
ER/PR status (pretherapy)	Negative	52 (46)
	Positive	62 (54)
HER2 Ratio (pretherapy)	Median (range)	4.68 (0.81–28.00)
Menopause status	Premenopause	58 (51)
	Perimenopause	2 (2)
	Postmenopause	54 (47)

Overall, trastuzumab-based neoadjuvant chemotherapy resulted in pCR rates of 55.3% in ypT0/is, 53.3% in ypT0/is ypN0, and 45.3% in ypT0 ypN0. Using the cut-off value at 6 to distinguish between low ($\leq$6) and high (>6) *HER2*/CEP17 ratios, we correlated the level of HER2 overexpression with pCR. When ypT0/is was selected as an endpoint, tumors with a ratio of >6 achieved a pCR in 24 of 29 cases (82.8%), which was significantly higher than the pCR rate in tumors with a ratio of <6 (18/47 cases; 38.3%; $P < 0.001$, χ^2 test, Fig. 3). In univariate logistic regression analyses, we found an improved pCR rate in tumors with a ratio of >6 (OR, 7.73; 95% CI, 2.50–23.91; $P < 0.001$). An improved ypT0/is rate was also seen when the *HER2*/CEP17 ratio was analyzed as a continuous variable (OR, 1.22; 95% CI, 1.06–1.40; $P = 0.004$). No other histopathologic or clinical parameters such as age, menopausal status, BMI, grade, stage, nodal status, or HR status was significantly associated with ypT0/is (data not shown).

When the more restrictive pCR definition ypT0/is ypN0 was used, tumors with a ratio>6 achieved pCR in 22 of 29 cases (75.9%), which was again significantly higher than the pCR rate of 18 in 46 (39.1%) observed in tumors with a ratio of $\leq$6 ($P = 0.002$, Fig. 3). Tumors with a ratio of >6 had a 4.89 (95% CI, 1.73–13.78; $P = 0.003$) fold higher chance to achieve ypT0/is ypN0 than tumors with a ratio of $\leq$6. When the *HER2*/CEP17 ratio was considered as continuous variable, women were also more likely to achieve pCR if tumors expressed higher *HER2*/CEP17 ratio (OR, 1.14; 95% CI, 1.02–1.26; $P = 0.016$). The probability to achieve pCR is also contingent on tumor grade; tumor grade 3 is more likely to achieve pCR compared with those with grade 1 or 2 (OR, 8.76; 95% CI, 1.06–72.69; $P = 0.044$). No other clinicopathologic parameters was associated with ypT0/is ypN0 in a univariate logistic regression model (data not shown). When *HER2*/CEP17 ratio (continuous) and tumor grade (grade 3 vs. 1/2) were included in the multivariate model, only HER2 ratio was predictive of pCR, with an OR of 1.18 (95% CI, 1.03–1.35; $P = 0.017$).

When the most stringent pCR definition, that is, ypT0 ypN0 was used, tumors with a high *HER2*/CEP17 ratio achieved a pCR in 20 of 29 cases (69.0%), which was significantly higher than the pCR rate in tumors with a low ratio in which 14 of 46 tumors (30.4%) achieved a ypT0 ypN0 ($P = 0.001$, Fig. 3). In univariate analyses, we found an improved pCR rate in tumors with a ratio of >6 (OR, 5.08; 95% CI, 1.86–13.90; $P = 0.002$). When *HER2*/CEP17 was treated as a continuous variable, we found an OR of 1.12 (95% CI, 1.02–1.23; $P = 0.017$) for achieving pCR. None of the other parameters that were subjected to this model (stage, nodal status, menopausal status, HR status, age or BMI) was significantly associated with ypT0 ypN0 (data not shown).

Hormone receptor status and pCR

Although pCR was more common in HR-negative nonluminal tumors, the HR status (ER- and/or PR-positive cases vs. ER- and PR-negative cases) was not significantly correlated with pCR, irrespective of pCR definition (Fig. 4 and data not shown).

Interestingly, within the subgroup of HR-positive luminal tumors, those with a *HER2*/CEP17 ratio of >6 achieved ypT0/is in 14 of 16 (87.5%) cases while pCR was only achieved in 7 of 26 (26.9%) tumors with a low ratio ($P < 0.001$; Fig. 4). HR-positive tumors with a high *HER2*/CEP17 ratio had an OR of 18.00 (95% CI, 3.22–100.49; $P = 0.001$) for ypT0/is, when compared with tumors with a low ratio. No significant difference was observed in pCR rates of HR-negative tumors when a high *HER2*/CEP17 ratio was compared with a low ratio (76.9% vs. 52.4%; $P = 0.276$) (Fig. 5).

With the more restrictive pCR definition of ypT0/is ypN0, we also observed that within the subgroup of HR-positive luminal tumors, 12 of 16 (75.0%) cancers with a high *HER2*/CEP17 ratio achieved ypT0/is ypN0, whereas only 7 of 25 (28.0%) tumors with a low ratio ($P = 0.005$) had a pCR. HR-positive luminal tumors with a ratio of >6 had an OR of 7.71 (95% CI, 1.85–32.21; $P = 0.005$) for ypT0/is ypN0, when compared with tumors with a low ratio. Again, no significant difference in pCR rates was observed in HR-negative tumors when tumors with a high *HER2*/CEP17 ratio was compared with tumors with a *HER2*/CEP17 ratio of $\leq$6 (76.9% vs. 52.4%; $P = 0.276$) Fig. 5.

When pCR was defined by the absence of histologically detectable tumor cells in both primary tumor and ipsilateral lymph nodes (i.e., ypT0 ypN0), we found that in tumors that expressed either ER and/or PR, a high *HER2*/CEP17 ratio resulted in a pCR in 10 of 16 cases (62.5%), whereas tumors with a low ratio had a pCR in 6 of 25 (24.0%) cases ($P = 0.014$). HR-positive tumors with a ratio of >6 had an OR of 5.28 (95% CI, 1.35–20.69; $P = 0.017$) for ypT0 ypN0, when compared with tumors with a ratio of $\leq$6. In contrast with the two less stringent definitions of pCR, HR-negative tumors also exhibited a significant difference in pCR rates when tumors with a high *HER2*/CEP17 ratio were compared with tumors with a *HER2*/CEP17 ratio of $\leq$6 (76.9% vs 38.1%; $P = 0.039$). In this population, univariate logistic regression revealed an OR of 5.42 (95% CI, 1.14–25.83; $P = 0.034$) of achieving a pCR for tumors with a high *HER2*/CEP17 ratio when compared with tumors with a low ratio.

Discussion

We have evaluated the effect of the *HER2*/CEP17 ratio on the pCR rate in early HER2-positive malignant breast tumors, which

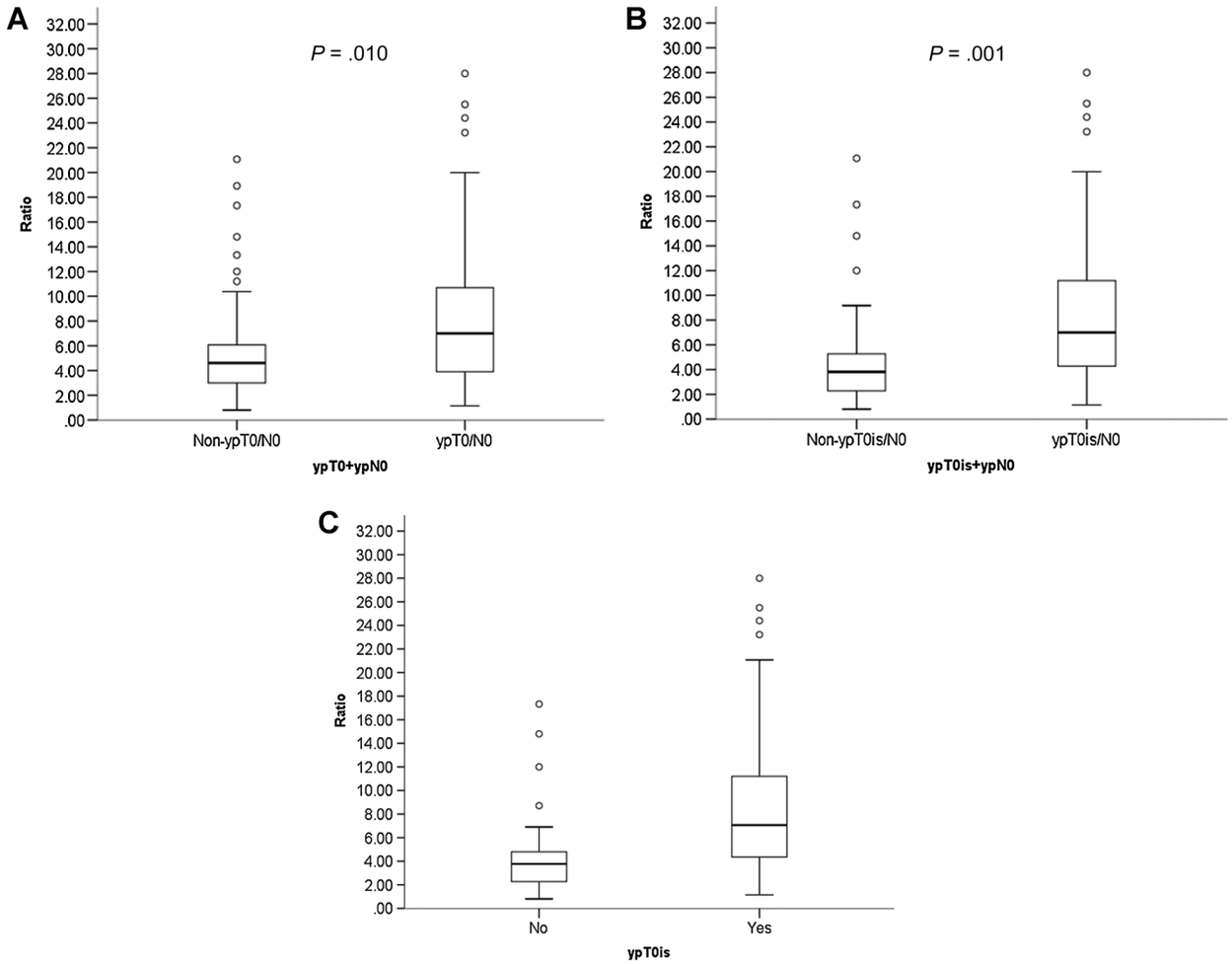

Figure 2.
Median (range) HER2/CEP17 ratios in diagnostic tumor biopsies from women who did not (left box blot) or did (right box blot) experience pCR as defined by ypT0/is, ypT0/is N0, or ypT0 ypN0; (o outliers).

were treated with trastuzumab-based chemotherapy in two prospectively randomized neoadjuvant trials. The cut-off ratio at 6 was chosen to distinguish a high *HER2*/CEP17 ratio from a low ratio, and is based on our previous publication where we have demonstrated that a ratio of >6 in HER2-overexpressing primary breast tumor is an independent predictor for a shorter time to first metastasis in the absence of adjuvant trastuzumab, but for an improved progression free survival when trastuzumab-based chemotherapy is initiated upon detection of metastatic disease (17). We have used three different definitions of pCR: The ypT0/is criterion is widely used and has been the endpoint in several older trials (24). More recently, a pooled analysis of more than 11,000 patients from 12 international trials has suggested that eradication of tumors from both breast and lymph nodes (ypT0 ypN0 or ypT0/is ypN0) has a stronger association with improved event-free survival (EFS) and overall survival (OS) than eradication of tumor from the breast alone (ypT0/is). Notably, the strongest association between pCR and long-term outcome was found in patients with aggressive breast cancer subtypes such as HER2-positive tumors with or without concomitant HR expression (12).

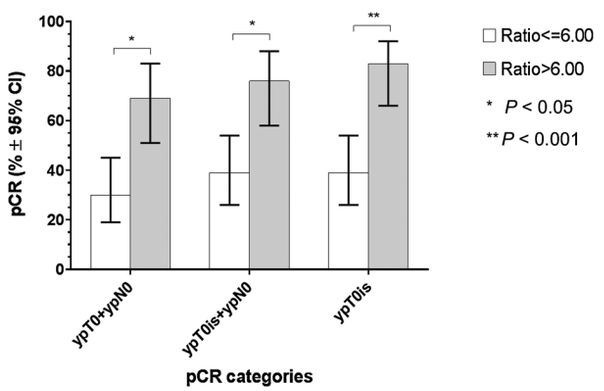

Figure 3.
HER2/CEP17 ratio and pCR as defined by ypT0/is, ypT0/is N0, or ypT0 ypN0 (HER2/CEP17 ratio ≤6: white bar, HER2/CEP17 ratio >6: gray bar).

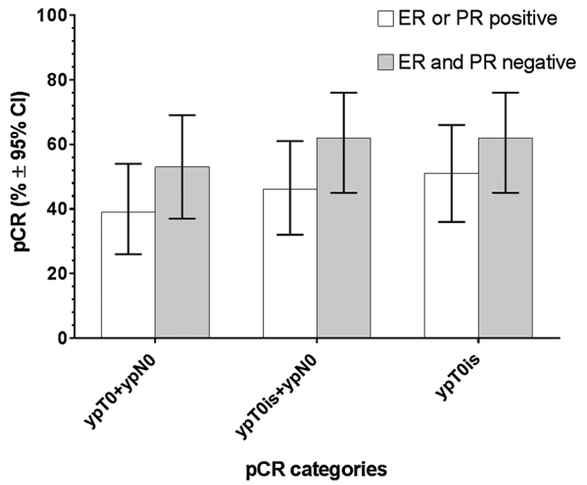

Figure 4.
HR status and pCR as defined by ypT0/is, ypT0/is N0, or ypT0 ypN0 (ER and/or PgR positive: white bar, ER and PgR negative: gray bar).

Recently, two prospective randomized phase II studies have investigated the efficacy and safety of a dual blockade with trastuzumab and pertuzumab in the neoadjuvant setting: The primary endpoint of the Neosphere trial was the pCR defined by ypT0/is, and 4 cycles of neoadjuvant docetaxel (75 mg/m^2, every 3 weeks) in combination with trastuzumab every 3 weeks given to women with a FISH ratio of 2.0 or greater resulted in a pCR rate of 21.5%. When pertuzumab was added to the regimen, the pCR rate was increased to 39.3% (25). In the three-armed Tryphaena trial, all patients with IHC3+ or FISH-positive HER2 status received trastuzumab and pertuzumab either concomitantly every 3 weeks together with 6 cycles of neoadjuvant carboplatin/docetaxel, or together with 6 cycles of sequential 5-fluorouracil/epirubicine/cyclophosphamide (FEC) and docetaxel, or during 3 cycles of docetaxel after 3 cycles of FEC. Achievement of ypT0/is and ypT0 ypN0 were reported as secondary endpoints. ypT0/is was achieved in 66.2%, 61.6%, and 57.3%, respectively, in each treatment subgroups, whereas ypT0 pN0 was achieved in 51.9%, 50.7% and 45.3% in each subgroup (26). Our overall pCR rates of 55.3% (ypT0/is), 53.3% (ypT0/is ypN0), and 45.3% (ypT0 ypN0) with trastuzumab alone-based regimen are comparable with the published double-combinations. This might be attributable to the fact that we have used 6 cycles of q3d-scheduled chemotherapy backbones, which, in some patients, also included capecitabine (ABCSG-24), or bevacizumab with or without NLPD (ABCSG-32). Nevertheless, within our population, tumors with a *HER2*/CEP17 ratio of >6 achieved a pCR approximatively twice as often as tumors with a ratio of $\leq$6. Within the high *HER2*/CEP17 group, the pCR rates of 82.8% (ypT0/is), 75.9% (ypT0/is ypN0), and 69.0% (ypT0 ypN0) even exceeded the respective pCR rates reported for the double combination in Neosphere and Tryphaena.

Because either IHC3$^+$ status or HER2 amplification of $\geq$2.0 were inclusion criteria for ABCSG-24 and 32, 8 of 75 (10.7%) tumors received trastuzumab, which would not fulfill current ASCO/CAP criteria (15). It is thus not surprising that only one patient (12.5%) whose tumor exhibited a ratio of <2.2 achieved ypT0/is ypN0 / ypT0 ypN0. The pCR rates, however, increase considerably with increasing *HER2*/CEP17 ratios.

Limitations of our study include: (i) lack of centralized testing. It has been suggested that a lack of centralized testing might lead to misclassification of participants within a study (27, 28); however, a nationwide assessment on practice of HER2 testing in 34 histology laboratories in the departments of pathology in Austria using FISH testing as the gold standard demonstrated high level of accuracy, with an overall sensitivity of 90.5% and specificity of 99.2% (29). All centers successfully completed the assessment and all the pathologists involved in this study participated in the ring trial. In addition, all centers involved in this study participated in external quality assurance programs (such as UK NEQAS, QuIP, NordiQC) for routine diagnostic purpose, and is a common practice in Austria; (ii) two different ISH technologies used might affect the results of our study. However, the two technologies used in our study are both endowed by an international quality assurance program (30) and previous studies that evaluated the concordance of various ISH testing methods have shown that the overall agreement between ISH methods is around 97% (31–33). Cases with disagreement were in many cases caused by heterogeneity of tumors; and (iii) small sample size to be repeated in a larger cohort.

Conclusion

Taken together, we have analyzed the *HER2*/CEP17 ratio in early-stage breast cancers from pre- and postmenopausal women who had received trastuzumab-based neoadjuvant treatment. We have demonstrated that a *HER2*/CEP17 ratio of >6 is associated with a significantly higher probability of achieving pCR, regardless of absence or presence of DCIS. Recently, the neoadjuvant approval of pertuzumab has improved pCR rates when the antibody is added to trastuzumab-based regimen. However, the dual blockage is expensive and burdened by left ventricular systolic dysfunction (LVSD) in up to 5.6% and additional EGFR-mediated side effects such as diarrhea, or rash (26, 34–36). Reporting of the *HER2*/CEP17 ratio in the histopathologic analysis might therefore be useful in identifying those patients who are sufficiently treated with trastuzumab alone, and who could thus be spared from dual blockage regimen and its associated side effects. In this context, it would be of great interest to

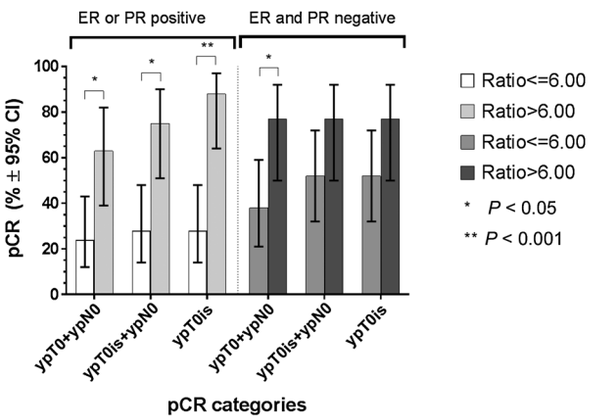

Figure 5.
pCR and HER2/CEP17 ratio in HR-positive versus HR-negative breast cancer (HER2/CEP17 ratio $\leq$6: white and light blue bars, HER2/CEP17 ratio >6: gray and blue bars).

investigate in larger studies whether the superior pCR rates that can be achieved by adding pertuzumab to trastuzumab-based neoadjuvant chemotherapy are independent of the intratumoral *HER2*/CEP17 ratio, or if the use of pertuzumab is highly beneficial in tumors with a lower ratio.

Disclosure of Potential Conflicts of Interest

G.G. Steger reports receiving commercial research grants, speakers bureau honoraria from, and is a consultant/advisory board member for Roche. E. Petru is a consultant/advisory board member for and reports receiving speakers bureau honoraria from Roche. D. Fuchs is a consultant/advisory board member for Gilead, Novartis, and Roche. M. Balic reports receiving other commercial research support from Celgene, speakers bureau honoraria from Amgen, Celgene, Novartis, and Roche, and is a consultant/advisory board member for Amgen, Astra Zeneca, and Pierre Fabre. M. Gnant is an employee of Sandoz, reports receiving commercial research grants from AstraZeneca, Novartis, Pfizer, and Roche, speakers bureau honoraria from Amgen, AstraZeneca, Celgene, GlaxoSmithKline, Novartis, OBI-Pharma, and Roche, and is a consultant/advisory board member for Accelsoirs. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The sponsor had no role in any aspect pertinent to the study, including study design/conduct, patient recruitment, data analysis/interpretation, and writing/publication of the manuscript.

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