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Pathological complete response to neoadjuvant trastuzumab and pertuzumab therapy is related to human epidermal growth factor receptor 2 (*HER2*) amplification level in *HER2*-amplified breast cancer

Jin Hyuk Choi, MD[a], Chang Wan Jeon, MD[a], Young Ok Kim, MD, PhD[b], Sungui Jung, MD[a,*]

Abstract

The human epidermal growth factor receptor 2 (*HER2*) is amplified in approximately 20% of breast cancers, and *HER2* receptor targeting therapy is associated with a significant improvement in disease-free and overall survival. In several clinical trials, the pathologic complete response (pCR) rate was significantly increased with combined pertuzumab and trastuzumab treatment in *HER2*-amplified breast cancer. Although the efficacy and safety of anti-*HER2* dual blockade therapy has been reported, the markers that predict the response are still unclear. This study aimed to investigate the relationship between the level of *HER2* amplification and the pCR in trastuzumab and pertuzumab neoadjuvant therapy.

Twenty-two *HER2*-amplified early breast cancer patients who had received neoadjuvant docetaxel, carboplatin, trastuzumab, and pertuzumab (TCHP) therapy were included in this study. *HER2/CEP17* ratio and average *HER2* copy number were measured by fluorescence in situ hybridization analysis. The relationship between level of *HER2* amplification and tumor pCR status was investigated.

The median age was 47.5 years (range, 36–62). 31.8% of the patients were hormone receptor (HR) positive and 68.2%% of the patients were HR negative. The pCR (ypN0/is ypN0) rate in the breast and axilla was 68.2%. The patients who experienced a pCR had a median *HER2/CEP17* ratio of 7.08 (range, 3.16–10.40) and average *HER2* copy number of 17.00 (range, 5.85–37.50). The patients who did not experience a pCR had a median ratio of 4.70 (range, 1.06–9.00) and median *HER2* copy number of 12.00 (range, 5.85–20.95) ($P = .030$, $P = .174$), respectively.

pCR was highly correlated with *HER2/CEP17* ratio in neoadjuvant anti-*HER2* dual blockade. This suggests that the *HER2/CEP17* ratio can be used as a predictive marker for pCR in neoadjuvant trastuzumab and pertuzumab therapy.

Abbreviations: ALND = axillary lymph node dissection, AUC = area under the curve, CEP17 = centromere enumerator probe 17, CNB = core needle biopsy, CT = computed tomography, DAPI = 4′,6-diamidino-2-phenylin-dole, DCIS = ductal carcinoma in situ, ECOG = Eastern Cooperative Oncology Group, FISH = fluorescence in situ hybridization, FNA = fine needle aspiration, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry, LVEF = left ventricular ejection fraction, MRI = magnetic resonance imaging, MUGA = multiple gated acquisition, pCR = pathologic complete response, SLNB = sentinel lymph node biopsy, USG = ultrasonography.

Keywords: breast cancer, fluorescence in situ hybridization, human epidermal growth factor receptor 2, neo adjuvant therapy, pathological response, pertuzumab, trastuzumab

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[a] Department of Surgery, [b] Department of Pathology, Kosin University Gospel Hospital, Busan, Republic of Korea.

[*] Correspondence: Sungui Jung, Department of Surgery, Kosin University Gospel Hospital, 262 Gamcheon-ro, Seo-gu, Busan, 49267, Republic of Korea (e-mail: ist2000good@daum.net).

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1. Introduction

The human epidermal growth factor receptor 2 (*HER2*) gene is amplified in approximately 20% of all breast cancers.[1,2] *HER2* gene over-expression is associated with poor clinical outcome.[3,4] Treatment with trastuzumab, a recombinant monoclonal antibody against *HER2*, in *HER2*-positive breast cancer patients significantly improved the effectiveness of treatment.[5–7] Accordingly, a trastuzumab-based chemotherapy regimen has become the standard treatment for *HER2*-amplified breast cancer in the adjuvant, neoadjuvant, and metastatic setting.[8–10]

More recently, the treatment of *HER2*-amplified breast cancer patients has been expanded with the introduction of dual *HER2* block therapy using pertuzumab. In the APHINITY study, the addition of pertuzumab to trastuzumab in adjuvant chemotherapy significantly improved invasive-disease-free survival in operable breast cancer patients.[11] The NeoSphere and TRYPHAENA studies confirmed the improvement of pathologic complete response (pCR) when pertuzumab was added in neoadjuvant treatment.[12,13] First-line treatment of recurrent *HER2* breast cancer patients also improved progression-free survival in the pertuzumab, trastuzumab, and docetaxel groups compared with the placebo, trastuzumab, and docetaxel groups.[14] Unfortunately, while treatment with pertuzumab has a higher pCR rate than previously, pertuzumab treatment is not effective in all *HER2*-amplified breast cancer patients. In luminal breast cancer patients, even if the hormone receptor is positive, the scores and responses are significant.[15] Similarly, even when the *HER2* gene is amplified, there is a difference in the level of *HER2* amplification. There have been few studies showing that the level of *HER2* amplification is correlated with response in treatment with trastuzumab in patients with *HER2*-positive breast cancer.[16–18] There is still no study showing the relationship between *HER2* amplification levels and response in dual *HER2* block therapy with pertuzumab and trastuzumab. We therefore investigated the relationship between *HER2* amplification and pCR in *HER2* dual blockade using fluorescence in situ hybridization (FISH) analysis.

2. Material and methods

2.1. Patients

We retrospectively analyzed *HER2*-positive breast cancer patients using trastuzumab and pertuzumab neoadjuvant chemotherapy among 22 patients diagnosed at Kosin University Gospel Hospital from January 2018 to December 2019. *HER2* status was ascertained by immunohistochemistry (IHC) test. Patients with *HER2* 2+ were diagnosed as positive; a *HER2*/*CEP17* ratio of 2.0 or higher or an average *HER2* copy number of 6.0 or higher was considered amplified using FISH analysis.[19] All patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. Patients previously treated with chemotherapy, radiation therapy, or targeted therapy were excluded. Patients whose left ventricular ejection fraction (LVEF) was 50% or less prior to chemotherapy as determined by echocardiography or multiple gated acquisition (MUGA) scan were excluded. Cases of metastatic disease, bilateral breast cancer, other malignancies, inadequate bone marrow function, and impaired liver function were also excluded. This study protocol was reviewed and approved by the institutional review board of Kosin University Gospel Hospital.

2.2. Procedure

All patients were diagnosed with invasive carcinoma through core needle biopsy (CNB) or excisional biopsy of breast lesions. Fine needle aspiration (FNA) cytology was performed when metastasis to an axillary lymph node was suspected. Breast ultrasonography (USG), breast magnetic resonance imaging (MRI), chest and abdominal computed tomography (CT), and bone scan were conducted for clinical staging.

All patients received 75 mg/m^2 docetaxel, carboplatin area under the curve (AUC) of 6, an 8 mg/kg loading dose of trastuzumab, followed by a 6 mg/kg maintenance dose, and an 840 mg pertuzumab loading dose, followed by 420 mg maintenance dose every 3 weeks for 6 cycles. No patients required dose reduction due to tolerance difficulties. Tumor clinical response was assessed by physical breast examination at every cycle. After 3 cycles, breast USG and breast MRI were taken to confirm the tumor response. There were no cases of conversion to surgery without completing the scheduled chemotherapy. All patients were evaluated for cardiac function through performance of an echocardiographic or MUGA scan before starting chemotherapy.

The type of surgery was determined after consultation with the patient. However, total mastectomy was performed for multicentric malignant lesions, nipple invasion, and expectation of cosmetic problems as well as in patients who refused conserving surgery. All reconstructions were performed immediately, and the opposite side was also performed depending on the patient's condition.

2.3. HER2 scoring

IHC staining was performed on 4-μm sections of formalin-fixed paraffin-embedded tissue by BOND-MAX autostainer (Leica Biosystems, Wertzlar, Germany). Antigen retrieval procedure was accomplished using Bond Epitope Retrieval Solution 2 (Leica Biosystems) for 20 minutes at 100°C. The sections were incubated with primary polyclonal antibody for *HER2* (1: 700, DAKO, Glostrup, Denmark). A negative control stain without primary antibody was performed. *HER2* immunoreactivity was assessed using the following scoring approach: 0, no immunoreactivity or weak incomplete membrane staining within <10% of tumor cells; 1+, weak incomplete membrane staining within >10% of tumor cells; 2+, weak to moderate incomplete membrane staining within >10% of tumor cells or strong complete membrane staining within <10% of tumor cells; 3+, strong complete membrane staining within >10% of tumor cells.

2.4. Fluorescence in situ hybridization analysis

HER2 gene copy number was evaluated using the PathVysion *HER2* DNA Probe kit (Abbott Molecular, Des Plaines, IL/ Inter Medico, Markham, Canada). The deparaffinized 4-μm section was immersed in 0.2N HCl for 20 minutes. The Slide was placed in pretreatment solution at 98°C for 30 minutes and then proteaseized at 37°C for 5 minutes. Slides were hybridized with a PathVysion *HER2* DNA probe mixture containing *HER2* DNA probe (labeled with spectrum red) and *CEP17* DNA probe (labeled with spectrum green) (Fig. 1). The *CEP17* DNA probe allows correction of *HER2* gene copy number relative to the copy number of chromosome 17. Slide glass cover slip was applied and sealed with rubber. The slides were then denatured at 74°C for 2 minutes and hybridized overnight at 37°C in a humidified hybridization chamber (ThermoBrite, Abbott Vysis, Downers

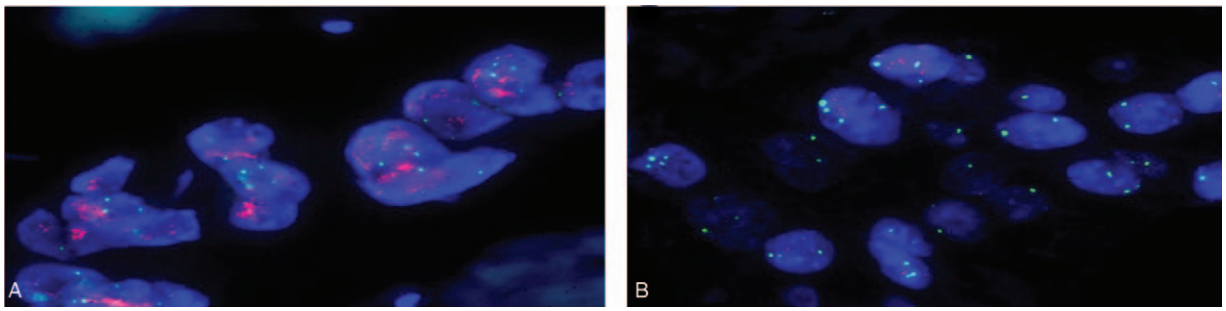

Figure 1. Fluorescence in situ hybridization (FISH) is shown for *HER2* gene amplification on Thin-Prep specimens and on corresponding histologic sections (*HER2*: red signal, *CEP17*: green signal). (A) A case with positive *HER2* amplification status (DAPI counterstain; ×1000). (B) A case with negative *HER2* amplification status (DAPI counterstain; ×1000). DAPI = 4′,6-diamidino-2-phenylin-dole.

Grove, IL). The nest day, the slides were washed in a post-hybridization buffer at 73.5 °C for 2 minutes and dried in the dark. The nuclei were then subsequently counterstained with 10 μL of 4′,6-diamidino-2-phenylin-dole (DAPI). Slides were stored in the dark at 4 °C until signal enumeration.

2.5. Statistics

General characteristics of study subjects are presented as number (%). The *HER2/CEP17* ratio and average number of *HER2* copies were tested for normality through the Kolmogorov–Smirnov test and Shapiro-Wilk test, and we verified that these 2 variables were normally distributed. According to pCR status, we compared *HER2* amplification using Fisher exact test, and data are presented as mean value with 95% confidence interval using box plot. For all analyses, a P value of $<.05$ was considered statistically significant. All statistical analysis was done with SPSS version 25.0 (IBM Co., Armonk, NY).

3. Results

3.1. Patient characteristics

The baseline characteristics of all 22 patients are summarized in Table 1. Median age was 47.5 years (range, 36–62). The number of patients in tumor stage T1 was 2 (2.9%), in T2 was 13 (59.1%), and in T3–4 was 7 (31.8%). The number of patients in nodal stage N1 was 15 (68.2%), in N2 was 4 (18.2%), and in N3 was 3 (13.6%). Seven patients (31.8%) were hormone receptor positive; 15 were hormone receptor negative (68.2%). In tissue samples obtained from core needle biopsy, the histologic grade was G2 in 19 patients (86.4%) and G3 in 3 patients (13.6%). The Ki-67 value was over 20% in most patients (n = 16, 72.7%). There were 12 premenopausal patients (54.5%) and 10 postmenopausal patients (45.5%). Ten patients (45.5%) underwent total mastectomy, and 9 of these underwent reconstruction immediately after nipple areolar complex-preserving mastectomy. Twelve patients (54.5%) underwent partial mastectomy. Sixteen patients (72.7%) had sentinel lymph node biopsy (SLNB) and 4 (18.2%) had axillary lymph node dissection (ALND). When postoperative pathologic examination was confirmed, 15 patients (68.2%) were ypT0 ypN0 (absence of invasive cancer and in situ cancer in breast and axillary lymph nodes), 19 (86.4%) were ypT0/is ypN0 (absence of invasive cancer in breast and in axillary lymph nodes, irrespective of remaining ductal carcinoma in situ (DCIS) in the primary tumor) and 20 (90.9%) were ypT0/is (absence of invasive cancer in the breast irrespective of the presence of DCIS or nodal involvement).

3.2. HER2/CEP17 ratio and pathologic response

Patients who experienced a pCR as defined by ypT0 ypN0 had a median *HER2/CEP17* ratio of 7.08 (range, 3.16–10.40). Those who did not have a pCR had a median *HER2/CEP17* ratio of 4.70 (range, 1.06–9.00; $P = .030$, Fig. 2A). Patients who experienced pCR with ypT0/is ypN0 had a median ratio of 6.87 (range, 2.54–10.40). Those who did not have a pCR had a median *HER2/CEP17* ratio of 3.15 (range, 1.06–5.90; $P = .029$, Fig. 2B). The tumors which responded with ypT0/is had a median *HER2/CEP17* ratio of 6.51 (range, 2.54–10.40). The median ratio in those that did not have a pCR were 2.11 (range, 1.06–3.15; $P = .017$, Fig. 2C) (Table 2).

Table 1

Baseline characteristics of patients.

	Total, N = 22
Age (yrs), mean (range)	47.5 (36–62)
Clinical stage	
Tumor stage, no. (%)	
T1	2 (9.1)
T2	13 (59.1)
T3–T4	7 (31.8)
Nodal stage, no. (%)	
N1	15 (68.2)
N2	4 (18.2)
N3	3 (13.6)
Hormone receptor status, no. (%)	
Positive	7 (31.8)
Negative	15 (68.2)
Histologic grade, no. (%)	
G1	0 (0)
G2	19 (86.4)
G3	3 (13.6)
Ki-67, no. (%)	
<10%	3 (13.6)
10–20%	3 (13.6)
≥20%	16 (72.7)
Menopause status, no. (%)	
Pre-menopause	12 (54.5)
Post-menopause	10 (45.5)

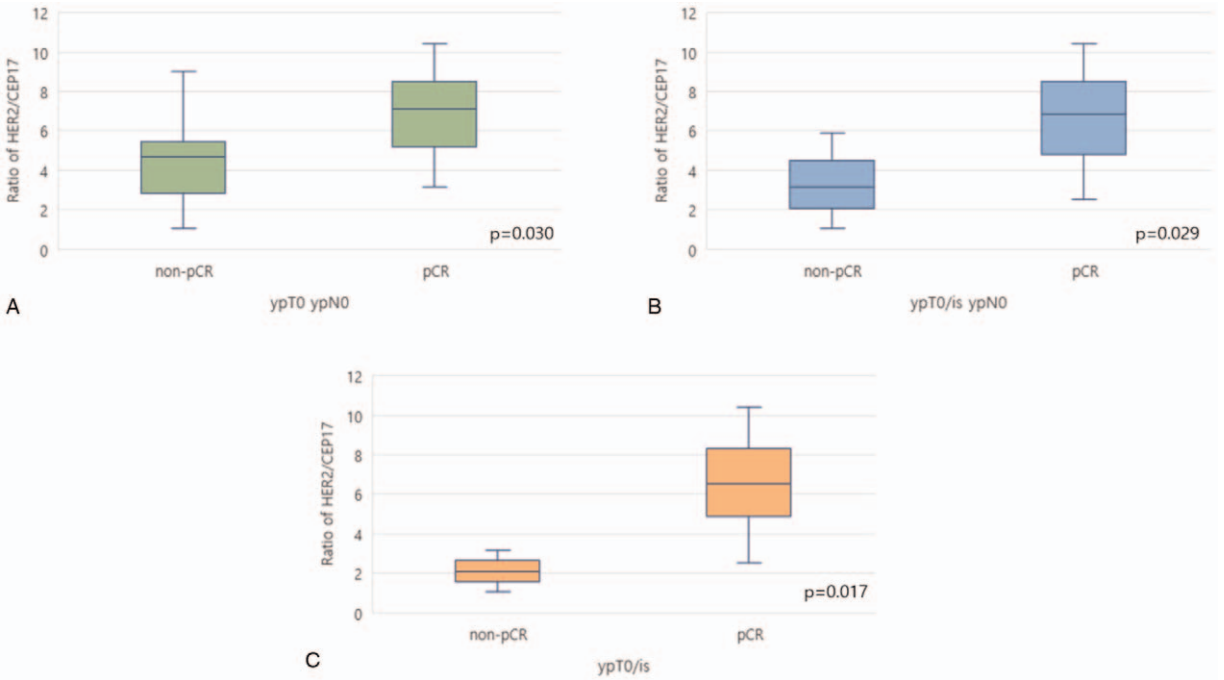

Figure 2. *HER2/CEP17* ratios in tumors from patient who did not (left box blot) or did (right box blot) experience pCR as defined by ypT0 ypN0 (A), ypT0/is ypN0 (B), ypT0/is (C). pCR = pathologic complete response.

3.3. Average HER2 copy number and pathologic response

Patients who did have a pCR with ypT0 ypN0 had average *HER2* copy number of 17.00 (range, 5.85–37.50). Those who did not have a pCR had average *HER2* copy number of 12.00 (range, 5.85–20.95; $P = .174$, Fig. 3A). Patients who experienced pCR with ypT0/is ypN0 had average *HER2* copy number of 17.00 (range, 5.85–37.50). Those who did not have a pCR had average *HER2* copy number 11.30 (range, 2.50–12.00; $P = .070$, Fig. 3B). The tumors which responded with ypT0/is had average *HER2* copy number of 16.40 (range, 5.85–37.50). The average copy number in those that did not have a pCR were 7.25 (range, 2.50–12.00; $P = .093$, Fig. 3C) (Table 2).

4. Discussion

When large-scale trials of trastuzumab were first reported, many studies reported that the level of *HER2* amplification was not related to the benefit from the adjuvant. In the HERA trial, *HER2/CEP17* ratio, *HER2* copy number, and *CEP17* copy number were not associated with disease-free survival.[20] The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial also did not find a relationship between *HER2* copy number and therapeutic benefits.[21] However, some studies in neoadjuvant therapy have focused on the level of *HER2* amplification and its relationship to pCR. Arnould et al[16] first reported a relationship between level of *HER2* amplification and rate of pCR in trastuzumab-based neoadjuvant treatment. Signal per nuclei was classified into no amplification, low amplification, and high amplification groups; pCR rate was high in the high amplification group. In a study by Singer et al,[17] *HER2/CEP17* ratio was measured in patients undergoing neoadjuvant therapy including trastuzumab, and a higher pCR rate was reported when the cut-off value was higher than 6. In our study, only a correlation between *HER2/CEP17* ratio and pCR rate could be confirmed because the number of patients was not large enough to divide by grade of amplification or to set a cut-off value. In addition, Singer et al[17] reported that pCR is more common in hormone receptor negative tumors. However, our study had too few patients to understand the relationship between hormone receptor staus and pCR.

Table 2

Median (range) level of *HER2* amplifications and pCR as defined by ypT0 N0, ypT0/is N0, ypT0/is.

	ypT0 ypN0			ypT0/is ypN0			ypT0/is		
	pCR	Non-pCR	*P*	pCR	Non-pCR	*P*	pCR	Non-pCR	*P*
HER2/CEP17 ratio, mean (range)	7.08 (3.16–10.40)	4.70 (1.06–9.00)	.030	6.87 (2.54–10.40)	3.15 (1.06–5.90)	.029	6.51 (2.54–10.40)	2.11 (1.06–3.15)	.017
Average *HER2* copy number, mean (range)	17.00 (5.85–37.50)	12.00 (5.85–20.95)	.174	17.00 (5.85–37.50)	11.30 (2.50–12.00)	.070	16.40 (5.85–37.50)	7.25 (2.50–12.00)	.093

CEP17 = centromere enumerator probe 17, *HER2* = human epidermal growth factor receptor 2, non-pCR = absence of pathological complete response, pCR = pathological complete response.

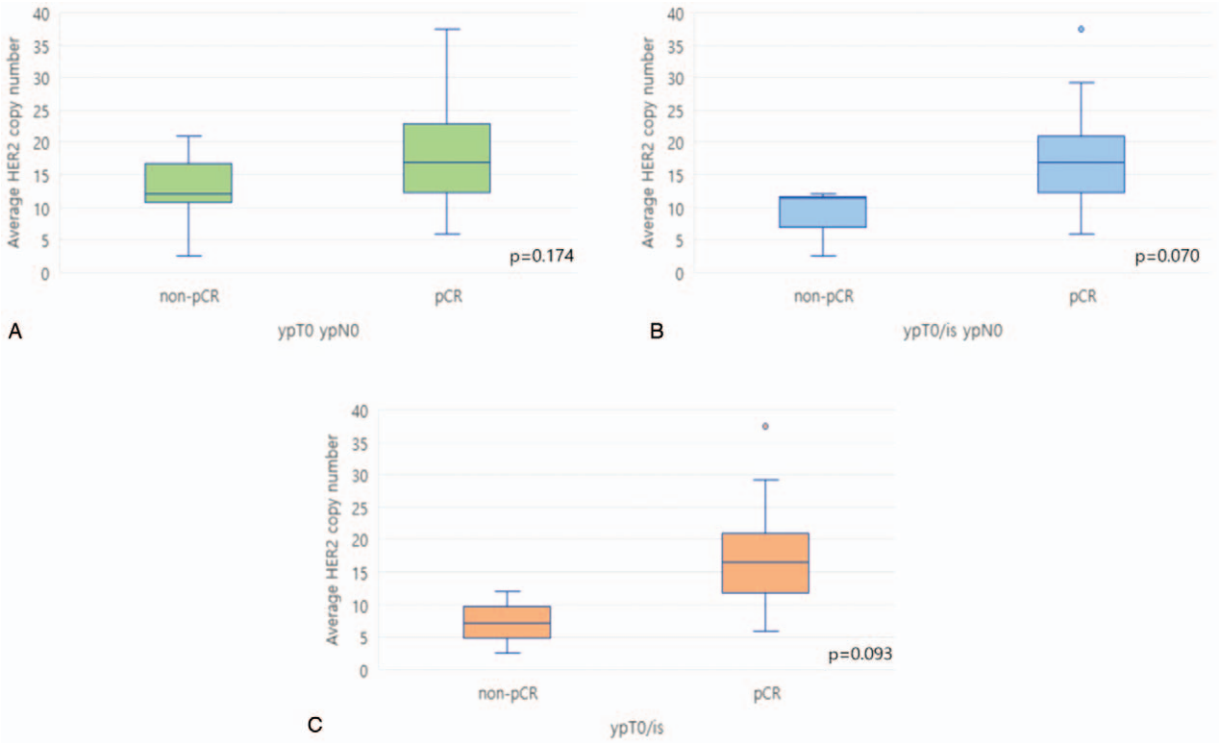

Figure 3. Average *HER2* copy number in tumors from patient who did not (left box blot) or did (right box blot) experience pCR as defined by ypT0 ypN0 (A), ypT0/is ypN0 (B), ypT0/is (C). pCR = pathologic complete response.

There are various studies that have proven the concept that dual *HER2* blockade will be more effective than single anti-*HER2* block therapy. The NeoALTTO trial showed that combined therapy using an anti-*HER2* agent, the tyrosine kinase inhibitor lapatinib, and trastuzumab had a higher pCR rate than therapy with trastuzumab or lapatinib alone.[22] In the NeoSphere study, the pCR rate was about twice as high with dual *HER2* blockade with anti-*HER2* antibodies trastuzumab and pertuzumab than with a single block.[12] In the TRYPHAENA study, the combination of pertuzumab with trastuzumab and standard chemotherapy resulted in a high response rate.[13] In this study, when pertuzumab was added to docetaxel, cisplatin and trastuzumab treatment, pCR (ypT0/is ypN0) occurred in 19 of 22 patients (86%). This is much higher than the responses seen in the NeoALTTO trial (51.3%) and NeoSphere trial (45.8%). This discrepancy may be due to the small number of participants in our study or to differences in treatment regimen.

In the NOAH study, pCR rate increased when trastuzumab was added to conventional chemotherapy in *HER2*-positive, locally advanced or inflammatory breast cancer, which improved event-free survival.[23,24] In addition, in the TECHNO study, pCR rate was higher in the group in which trastuzumab was added, which improved disease-free survival and overall survival.[25] The NeoSphere trial also showed a benefit in long term efficacy in the group in which pertuzumab was added to trastuzumab and docetaxel in a 5-year analysis study.[26] By securing a larger number of samples and having a sufficient follow-up period, this study not only confirmed survival in patients with pCR after dual *HER2* blockade but also confirmed the frequency and characteristics of recurrence according to the *HER2/CEP17* ratio.

Being a retrospective single center study with a relatively small sample size, our study has some limitations. Therefore, our results must be interpreted with caution. Studies conducted on larger sample sizes are required. Nevertheless, this study provided evidence of the clinical impact of the *HER2/CEP17* ratio in patients with *HER2*-positive breast cancer that were treated with double *HER2* blockade. Our study provides direction for future preliminary clinical trials.

Recently, an antibody–drug conjugate, trastuzumab emtansine, with reduced side effects compared with conventional anticancer agents, has been introduced. Treatment with this antibody–drug conjugate has been evaluated in various studies. In patients with *HER2*-positive metastatic breast cancer, overall survival was improved in patients previously treated with trastuzumab and taxane.[27] A study comparing neoadjuvant TCPH and trastuzumab emtansine-added pertuzumab showed that the pCR rate was lower than the dual *HER2* blockade group. However, serious adverse events were significantly reduced.[28] Current practice standards consider not only the efficacy of treatment, but also the effect of treatment on the cancer patient's quality of life. Therefore, developing a therapy with high efficacy and low toxicity is a research priority. In the future, we plan to study the relationship between *HER2* targeted chemotherapy response and *HER2/CEP17* ratio.

In conclusion, the *HER2/CEP17* ratio was associated with pCR in dual *HER2* blockade neoadjuvant treatment in *HER2*-positive breast cancer patients. Determining the degree of *HER2* amplification by FISH analysis will help predict the effectiveness of treatment and will help in the selection of therapeutic agents.

Author contributions

All authors conceived and designed the study. Jin Hyuk Choi conducted the experiments, analyzed the data and wrote the paper. All authors contributed to manuscript revisions. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

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