Original Article

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Clinicopathological Features of Breast Cancer with Polysomy 17 and Its Response to Neoadjuvant Chemotherapy

Hongxia Sun[1], Hui Chen[1], James Crespo[2], Guilin Tang[1], Melissa Robinson[1], Bora Lim[2], Ayşegül A. Şahin[1]

[1]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas
[2]Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas

ABSTRACT

Objective: The interpretation of human epidermal growth factor receptor 2 *(HER2)* fluorescence *in situ* hybridization (FISH) results may be challenging in tumors with polysomy 17, which is defined as increased signals of chromosome enumeration probe 17 (CEP17). The effect of polysomy 17 on *HER2* protein expression and tumor treatment response has not been established. In this retrospective study, we investigated the clinicopathological features of breast cancer with polysomy 17 and determined the tumors' response to neoadjuvant chemotherapy (NACT).

Materials and Methods: The study included 366 patients with primary breast cancer whose tumors had a CEP17 count of ≥ three/nucleus based on *HER2* FISH studies. These cases were categorized according to *HER2*/CEP17 ratio and *HER2* signals/nucleus using the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. We compared the clinicopathological characteristics and tumor response to NACT among different groups.

Results: There was a statistically significant difference in patients' age at diagnosis, tumor pathological grade, estrogen and progesterone receptor status, and NACT response among different *HER2* FISH groups. Polysomy 17 tumors in group 1 had a higher rate of response (pathological complete response and residual cancer burden class I) to NACT containing anti-*HER2* reagent than did those in other groups (p = 0.004), whereas polysomy 17 tumors in group 3 did not show a significant response to anti-*HER2* treatment.

Conclusion: Polysomy 17 tumors in different *HER2* FISH groups have different pathological features and respond to NACT differently. These results may help us identify patients who will benefit from anti-*HER2* therapy.

Keywords: Polysomy 17, breast cancer, *HER2* FISH study, neoadjuvant chemotherapy

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Key Points

- Polysomy 17 tumors show different clinicopathological characteristics among different *HER2* FISH groups.
- Polysomy 17 tumors show a different response to NACT among different *HER2* FISH groups.
- Polysomy 17 tumors in group 3 did not show a significant response to anti-*HER2* treatment.

Introduction

Human epidermal growth factor receptor 2 (HER2) is a member of the human epidermal growth factor receptor family, encoded by the gene *HER2* on 17q12-21.32 (1). Around 20% of invasive breast cancer cases overexpress or exhibit amplification of *HER2* (HER2+ breast cancer). HER2+ breast cancer is known to be an aggressive disease, with a poor clinical outcome (2). Trastuzumab, a monoclonal antibody that targets HER2, has demonstrated efficacy against HER2+ primary and metastatic breast cancer, both as a single agent and combined with chemotherapy (3, 4). Treatments that include anti-HER2 reagent have become the standard of care for patients with early or advanced HER2+ breast cancer (5, 6).

Accurate detection of HER2 overexpression or gene amplification is crucial in determining patients' eligibility for anti-HER2 treatment and predicting disease prognosis. According to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, HER2 testing is performed using immunohistochemical (IHC) assessment of HER2 protein overexpression and *in situ* hybridization

Corresponding Author:
Ayşegül A. Şahin; asahin@mdanderson.org

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(ISH) analysis of *HER2* gene amplification (7). ISH is conducted using either a single probe to enumerate HER2 copies per nucleus only or a dual-probe technique in which the *HER2*/CEP17 ratio is determined via hybridization to the chromosome 17 centromere region using chromosome enumeration probe 17 (CEP17). Although ASCO/CAP provides clear guidance on HER2 assessment, the test results can be difficult to interpret for various reasons, including copy number alterations in different foci on chromosome 17.

Chromosome 17 polysomy is associated with equivocal HER2 results. True polysomy is defined as the presence of extra copies of one or a whole chromosome. However, according to recent studies, true chromosome 17 polysomy is very rare in breast cancer. Focal amplifications encompassing the centromere are a common cause of the increase in CEP17 signals in ISH testing (8). Currently, the commonly adopted threshold for polysomy 17 is a mean of ≥3 CEP17 signals per nucleus (9). Increased CEP17 copies can alter the *HER2*/CEP17 ratio and subsequently influence the interpretation of the final HER2 ISH result. Consequently, 2013 ASCO/CAP recommends the use of a reflex test with alternative chromosome 17 probes for resolving equivocal HER2 ISH results (7).

HER2 FISH testing using alternative chromosome 17 probes can be performed by testing for additional genes on chromosome 17 that are not expected to coamplify with HER2. The commonly used commercially available probes include *SMS* (Smith-Magenis syndrome, also called *RAI1*), *RARA* (retinoic acid receptor alpha), and *TP53* (8). On using these different chromosome 17 genes, *HER2* gene status has been reported to be upgraded from equivocal to amplified or positive in a significant percentage of cases (8, 10, 11). However, in these studies, there were no clinical outcome data available in these patients with "revised" HER2+ breast cancer. The benefit of HER2-targeted therapy in these patient populations was also unknown. A recent study by Sneige et al. (12) demonstrated that the "revised" HER2 status due to the use of alternative chromosome 17 probes was unreflective of patient outcome. They concluded that these alternative chromosome 17 genes might overestimate the number of HER2-positive cases and lead to an erroneous upgrade of HER2 status to "positive."

A better understanding of the biological features of polysomy 17 breast cancer and how polysomy 17 affects *HER2* gene copy number and protein expression could help select patients who will respond to anti-HER2 treatment. In this study, we determined the clinicopathological characteristics of patients with breast cancer with polysomy 17. We also investigated the tumors' response to neoadjuvant chemotherapy (NACT), with and without anti-HER2 reagent.

Materials and Methods

Patient cohort

This retrospective study was conducted in a cohort of 366 patients with primary invasive breast cancer. Tumor HER2 FISH testing was performed at our institution between April 1st, 2013, and March 31st, 2018, and a CEP count ≥3/nucleus was required. Patients with a prior history of breast cancer, those diagnosed with de novo stage IV disease, and those who had multiple HER2 FISH tests using the same specimen were excluded from the study.

We reviewed patients' medical charts to determine clinical variables, including age at diagnosis, tumor pathological characteristics (tumor size, lymph node status, pathological staging, and histological grade), tumor biomarker features [estrogen receptor (ER), progesterone receptor (PR), Ki-67 value, and HER2 immunohistochemical (IHC) analysis], and treatment (with or without NACT and with or without anti-HER2 reagent in the NACT regimen). In patients who received NACT, tumor response to NACT was evaluated according to the pathological residual cancer burden (RCB) (13). A pathological complete response (pCR) and RCB class I were interpreted as a good response, while RCB classes II and III were interpreted as a poor response. Approval was obtained from the Institutional Review Board at our institution (no: PA18-0021) before the initiation of this study.

Immunohistochemical analysis

The IHC analysis performed at our department was processed using formalin-fixed, paraffin-embedded tumor sections (4 μm) with ER Clone 6F11 (Leica Biosystems, Inc., Buffalo Grove, IL) and PR Clone PgR 1294 (Agilent DAKO, Santa Clara, CA). The HER2 IHC analysis was performed using antibody clone AB8 (NeoMarkers) from April 1st, 2013 until August 31st, 2016, and clone 4B5 (Ventana Medical Systems, Inc., Tucson, AZ) from September 1st, 2016, to March 31st, 2018, due to institutional antibody change. The IHC studies (ER, PR, and HER2) conducted at outside institutions were reviewed at our department. ER, PR, and HER2 IHC statuses were interpreted according to the ASCO/CAP guidelines (7, 14).

FISH analysis

HER2 FISH analysis was performed using the Vysis PathVysion probe kit, which includes a SpectrumGreen-conjugated probe for the alpha satellite DNA located at the centromeric region of chromosome 17 (17p11.1-q11.1) and SpectrumOrange-conjugated probe for the *HER2* gene locus (Abbott Molecular/Abbott Laboratories, Abbott Park, IL). The same specimen blocks used for the HER2 IHC study were selected for the FISH study. *HER2* and CEP17 signals in 60 representative invasive cell nuclei were examined. *HER2* FISH result was interpreted according to the ASCO/CAP guidelines (7). For cases that were interpreted as equivocal for *HER2* amplification, another 60 representative invasive cell nuclei were examined.

Polysomy 17 tumors are classified into four *HER2* FISH groups according to the *HER2*/CEP17 ratio and *HER2* signals/nucleus, on the basis of ASCO/CAP guidelines: group 1 had a *HER2*/CEP17 ratio ≥2.0 and *HER2* signals/nucleus ≥4.0; group 3 had a *HER2*/CEP17 ratio <2.0 and *HER2* signals/nucleus ≥6.0; group 4 had a *HER2*/CEP17 ratio <2.0 and *HER2* signals/nucleus ≥4.0 and <6.0; group 5 had a *HER2*/CEP17 ratio <2.0 and *HER2* signals/nucleus <4.0 (15).

Statistical analysis

Categorical variables were summarized using frequencies and percentages. For most categorical clinical and pathological features, group differences were assessed using chi-square and Fisher's exact test. The correlation between the age at diagnosis and tumor *HER2* FISH group was examined by ANOVA analysis. A p-value <0.05 (two-sided) was considered statistically significant.

Results

Group distribution of polysomy 17 tumors

Among the 366 primary invasive breast cancer tumors included in this study, 128 (35.0%) were classified as *HER2* FISH group 1, 21

(5.7%) as group 3, 69 (18.9%) as group 4, and 148 (40.4%) as group 5. Representative *HER2* FISH images of polysomy 17 tumors in each group are shown (Figure 1).

Clinicopathological characteristics of polysomy 17 tumors

The clinicopathological characteristics of invasive breast cancer with polysomy 17 are shown in Table 1. In our study cohort, the mean age of patients at diagnosis was 56 years (range: 24–92 years). Most tumors were of a ductal type [340 of 366 (92.9%)] and histological grade 2 [151 of 365 (41.4%)] or [201 of 365 (55.1%)] with ER expression in over two-thirds of the patients [260 of 366 (71.0%)] and PR expression in half of the patients [184 of 365 (50.4%)].

We investigated the clinicopathological characteristics of polysomy 17 tumors in 4 *HER2* FISH groups. The histological types did not differ significantly; however, there were statistically significant differences in the age of the patients at diagnosis, tumor nuclear and histological grade, and ER/PR status. Patients with group 1 polysomy 17 tumors were diagnosed at a slightly younger age (mean age: 54 years) than were those with groups 4 (mean age: 57 years) and 5 (mean age: 58 years) tumors ($p<0.05$). Histological grade 3 tumors were more frequently observed in group 1 [81 of 127 (63.8%)] than in groups 3 [6 of 21 (28.6%)] and 5 [73 of 148 (49.3%)] ($p<0.05$). ER negative tumors were more common in group 1 [52 of 128 (40.6%)] than in groups 4 [14 of 69 (20.3%)] and 5 [35 of 148 (23.6%)] ($p<0.05$). PR negative tumors were more common in group 1 [86 of 128 (67.2%)] than in the other three groups [5 of 21 (23.8%) in group 3; 26 of 69 (37.7%) in group 4; 64 of 147 (43.5%) in group 5] ($p<0.001$).

Next, we evaluated tumor size and axillary lymph node status in 185 polysomy 17 tumors without NACT treatment (Table 2). These tumors were predominantly low stage [109 (58.9%) were pT1 and 63 (34.1%) were pT2], and only 13 (7%) were pT3 or pT4. No differences were observed in tumor pathological staging among the four groups. One-third of the tumors [52 of 185 (30.2%)] had metastasized to the axillary lymph nodes at the time of surgery. Tumors in group 1 had a significantly lower metastatic rate than did tumors in group 5 (16.7% vs 37.0%, $p<0.05$).

Comparison of IHC and FISH for HER2 status in polysomy 17 tumors

The HER2 IHC and FISH test results of the 366 tumors are outlined in Table 3: 92 tumors (26.3%) were positive (score 3+) on HER2 IHC testing, 145 (41.4%) were equivocal (score 2+), and 113 (32.3%) were negative, with a score of 1+ [92 of 113 (81.4%)] or 0 [21 of 113 (18.6)].

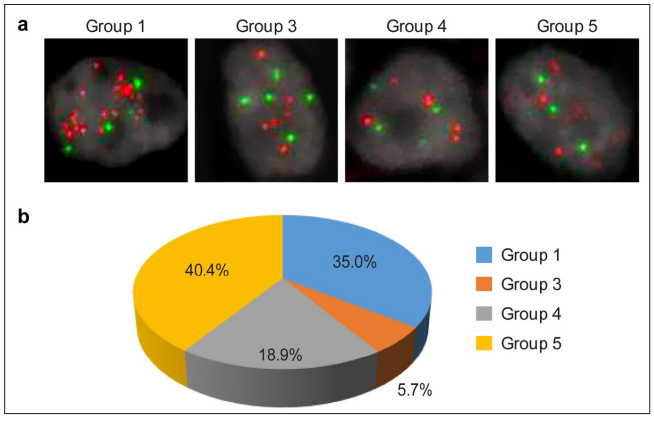

Figure 1. Representative *HER2* FISH images of polysomy 17 tumors in different *HER2* FISH groups (a); proportions of each group (b)

HER2: Human epidermal growth factor receptor 2; FISH: Fluorescence in situ hybridization

The distribution of HER2 IHC results was significantly different among tumors in the four *HER2* FISH groups ($p<0.001$). In group 1, most tumors [86 of 123 (69.9%)] were positive (score 3+) on HER2 IHC testing, about a quarter [34 of 123 (27.6%)] were equivocal (score 2+), and only a small number [3 of 123 (2.5%)] were negative (score 1+). No tumors had a HER2 IHC score of 0 in this group.

In contrast, most tumors [75 of 138 (54.4%)] in group 5 were negative for HER2 IHC staining, with a score of 1+ [60 of 138 (43.5%)] or 0 [15 of 138 (10.9%)]. Fewer tumors in this group [62 of 138 (44.9%)] were equivocal (score 2+), and only one tumor (0.7%) was positive (score 3+). In group 3, five tumors (23.8%) were positive (score 3+) on HER2 IHC testing; this was significantly lower than the number of tumors in group 1 ($p<0.001$) but higher than that in groups 4 ($p<0.05$) and 5 ($p<0.001$).

NACT response in polysomy 17 tumors

In our study cohort, 181 patients underwent NACT after initial diagnosis. Of these, 97 patients received an anti-HER2 therapy, and 84 did not. RCB was calculated for 175 tumors to evaluate the response to NACT (13). RCB could not be calculated for six tumors because of insufficient parameters of residual cancer in the breast or lymph nodes after NACT (all cases were from outside facilities). As shown in Table 4, 82 of 175 patients (46.9%) experienced a good response, while 93 (53.1%) had a poor response to NACT.

Tumors in different *HER2* FISH groups had different responses to NACT ($p<0.001$). Overall, a significant number group 1 tumors had a good response compared with tumors in other groups: 56 tumors (70.9%) in group 1 had a pCR or RCB I compared with three tumors (30.0%) in group 3 ($p<0.05$), eight (30.8%) in group 4 ($p<0.001$), and 15 (25%) in group 5 ($p<0.001$). Most patients with group 1 or 3 tumors received an anti-HER2 reagent containing NACT. Of these, 55 tumors (72.4%) in group 1 had a good response compared with three of nine in group 3 (33.3%) ($p<0.004$). In contrast, most patients with group 4 or 5 tumors did not receive an anti-HER2 reagent; 33.3% and 26.8% of these tumors had a good response, respectively.

We also investigated the pathological features associated with tumor response to NACT, with or without anti-HER2 therapy. *HER2* FISH group 1, ER negativity, PR negativity, and HER2 overexpression were associated with a good response to treatment with anti-HER2 reagent (Table 5). A high nuclear and histological grade, ER negativity, PR negativity, and high proliferative index Ki-67 were associated with a good response to treatment without anti-HER2 reagent (Table 6).

Discussion and Conclusion

Polysomy has been proposed to explain the increased rates of *HER2* amplification or discordance between IHC and FISH results. The results of recent studies have suggested that, detected by FISH, the major contributor to polysomy 17 is a significantly increased copy number of CEP17 secondary to the amplification of larger segments of chromosome 17, involving both *HER2* and the centromere (8). An elevated CEP17 count is frequently observed in invasive breast cancer. Using the cut-off of ≥3 CEP17 copies per cell, reported prevalence

rates of polysomy 17 tumors have ranged from 3% to 46% across various studies (16-23).

Polysomy 17 contributes to increased *HER2* FISH equivocal results. To resolve this problem, the 2013 ASCO/CAP guidelines advocate additional testing in these cases. Many studies have tested for additional genes on chromosome 17 and have upgraded a significant portion of equivocal cases to *HER2* amplified or positive (10-12). However, recent studies have demonstrated the presence of frequent complex structural alterations of chromosome 17 in patients with breast cancer, with losses and gains of genetic material at different loci of the chromosome (10, 24). As a consequence, the use of additional FISH probes is not sufficient for correcting the *HER2* gene status. Currently, there is no standard method to detect the *HER2* gene status in polysomy 17 breast cancer.

The reported clinicopathological features of polysomy 17 tumors are controversial. Several studies have linked polysomy with unfavorable pathological features, such as high proliferative activity, high Nottingham Prognostic Index Score, and nodal involvement (19, 23, 25), while other studies have found no significant differences between polysomic and nonpolysomic primary breast cancer in terms of clinicopathological variables and patient survival (18, 21). In our study cohort, most of the polysomy 17 tumors are histological grade

Table 1. Clinical and pathological characteristics of patients with primary breast cancer with polysomy 17 tumors

Characteristic	Total (n = 366)	Group 1 (n = 128)	Group 3 (n = 21)	Group 4 (n = 69)	Group 5 (n = 148)	p-value
Age, years						
Mean	56	54	55	57	58	
Range	24–92	24–90	34–75	32–92	24–85	**0.044⁺**
				*	*	
Histological type, n (%)						
IDC, NOS	340 (92.9)	122 (95.3)	20 (95.2)	65 (94.2)	133 (89.9)	
ILC	9 (2.5)	2 (1.6)	1 (4.8)	1 (1.4)	5 (3.4)	0.701⁺⁺
Other	17 (4.6)	4 (3.1)	0 (0)	3 (4.4)	10 (6.7)	
Nuclear grade, n (%)						
I	2 (0.5)	1 (0.7)	0 (0)	0 (0)	1 (0.7)	
II	151 (41.3)	39 (30.5)	15 (71.4)	27 (39.1)	70 (47.3)	**0.004⁺⁺**
III	213 (58.2)	88 (68.8)	6 (28.6)	42 (60.9)	77 (52)	
			**		*	
Histological grade, n (%)						
1	13 (3.5)	2 (1.6)	1 (4.7)	3 (4.4)	7 (4.8)	
2	151 (41.4)	44 (34.6)	14 (66.7)	25 (36.2)	68 (45.9)	
3	201 (55.1)	81 (63.8)	6 (28.6)	41 (59.4)	73 (49.3)	**0.04⁺⁺**
NA	1	1	0	0	0	
			*		*	
ER, n (%)						
Positive	260 (71.0)	76 (59.4)	16 (76.2)	55 (79.7)	113 (76.4)	
Negative	106 (29.0)	52 (40.6)	5 (23.8)	14 (20.3)	35 (23.6)	**0.005⁺⁺**
				*	*	
PR, n (%)						
Positive	184 (50.4)	42 (32.8)	16 (76.2)	43 (62.3)	83 (56.5)	
Negative	181 (49.6)	86 (67.2)	5 (23.8)	26 (37.7)	64 (43.5)	
NA	1	0	0	0	1	**<0.001⁺⁺**
			**	**	**	

Important p-values shown in bold.

*p<0.05 compared with group 1; **p<0.001 compared with group 1; ⁺: one-way ANOVA analysis; ⁺⁺: Chi-square analysis; IDC: Invasive ductal carcinoma; NOS: Invasive ductal carcinoma of no specific type; ILC: Invasive lobular carcinoma; ER: Estrogen receptor; PR: Progesterone receptor; NA: Not available; n: Number

2 or 3. ER positivity was seen in 71.4% of tumors, which is a slightly lower rate than the known 75%–80% rate in invasive breast cancer (Table 1). This indicates that polysomy 17 tumors display unfavorable pathological features.

In addition, polysomic *HER2*-amplified tumors have been reported to have more unfavorable pathological features than polysomic *HER2*-nonamplified tumors (20). Since *HER2* FISH group 1 tumors are HER2 amplified and *HER2* FISH group 5 tumors are HER2 nonamplified, we compared the clinicopathological features of the tumors in these two groups. Group 1 tumors were more likely to be diagnosed in younger patients ($p<0.01$), have a higher histological grade ($p<0.05$), and be ER negative ($p<0.01$) and PR negative ($p<0.001$) than were group 5 tumors (Table 1). These findings agree with previous findings that HER2-positive breast cancer is more likely to be diagnosed in younger patients and be more aggressive (2). However, when the tumor stage and axillary lymph node status in these two groups were compared, group 1 tumors were found to have a lower lymph node metastatic rate ($p<0.05$). No significant difference was observed in tumor stage between these two groups (Table 2). The reason why polysomic *HER2*-amplified tumors had a lower risk of lymph node metastasis needs to be further studied.

The effect of polysomy 17 on HER2 alteration also needs to be further investigated. Some studies have shown that polysomy 17 alone might not significantly contribute to the variation in *HER2* copy number and HER2 protein overexpression (16), while other studies have correlated polysomy 17 with an increased HER2 IHC score in tumors without *HER2* amplification (22, 26-28). For example, Hyun et al. (27) reported a significantly higher incidence of elevated CEP17 count in tumors with HER2 IHC score 2+/3+ compared with tumors with score 0/1+. In addition, Varshney et al. (22) and Petroni et al. (23) found that high CEP17 counts were associated with HER2 IHC score 3+ staining. Our results showed that 67.7% of polysomy 17 tumors were HER2 IHC score 2+/3+, and 32.3% of tumors were score 0/1+. In group 5, 44.9% of tumors were HER2 IHC score 2+, and one tumor (0.7%) was score 3+ (Table 3). This percentage was dramatically higher than that reported by the BCIRG clinical trial, in which 0.55% of group 5 tumors (including polysomy 17 and non-polysomy 17 tumors) had a HER2 IHC score 2+/3+ (14). Although this difference could be due to variations in the population under study

Table 2. Pathological stage of polysomy 17 tumors that had not received NACT

	Total	Group 1	Group 3	Group 4	Group 5	
Stage	**(n = 185)**	**(n = 46)**	**(n = 11)**	**(n = 42)**	**(n = 86)**	**p-value**
Tumor stage, n (%)						
pT1	109 (58.9)	30 (65.2)	6 (54.5)	24 (57.1)	49 (57)	
pT2	63 (34.1)	15 (32.6)	4 (36.4)	16 (38.1)	28 (32.6)	0.665
pT3 + T4	13 (7.0)	1 (2.2)	1 (9.1)	2 (4.8)	9 (10.4)	
Lymph node stage, n (%)						
pN0	120 (69.8)	35 (83.3)	7 (77.8)	27 (67.5)	51 (63)	
pN1 + N2 + N3	52 (30.2)	7 (16.7)	2 (22.2)	13 (32.5)	30 (37.0)	0.121
NA	13	4	2	2	5	
					*	

*p<0.05 compared with group 1.
NACT: Neoadjuvant chemotherapy; NA: Not available; n: Number

Table 3. HER2 protein expression in polysomy 17 tumors

	Total	Group 1	Group 3	Group 4	Group 5	
	(n = 366)	**(n = 128)**	**(n = 21)**	**(n = 69)**	**(n = 148)**	**p-value**
HER2 IHC score, n (%)						
0	21 (6.0)	0 (0)	2 (9.6)	4 (5.8)	15 (10.9)	
1+	92 (26.3)	3 (2.5)	7 (33.3)	22 (32.4)	60 (43.5)	
2+	145 (41.4)	34 (27.6)	7 (33.3)	42 (61.8)	62 (44.9)	**<0.001**
3+	92 (26.3)	86 (69.9)	5 (23.8)	0 (0)	1 (0.7)	
NA	16	5	0	1	10	
			**	**	**	

**p<0.001 compared with group 1. Important p-values are shown in bold.
HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemical; NA: Not available; n: Number

Table 4. Treatment response of polysomy 17 tumors in HER2 FISH group to NACT

	Total	Group 1	Group 3	Group 4	Group 5	
Treatment	**(n = 181)**	**(n = 82)**	**(n = 10)**	**(n = 27)**	**(n = 62)**	**p-value**
NACT overall, n (%)						
pCR + RCB I	82 (46.9)	56 (70.9)	3 (30)	8 (30.8)	15 (25)	
RCB II + III	93 (53.1)	23 (29.1)	7 (70)	18 (69.2)	45 (75)	**<0.001**
NA	6	3	0	1	2	
			*	**	**	
NACT with anti-HER2, n (%)						
pCR + RCB I	59 (62.8)	55 (72.4)	3 (33.3)	1 (20)	0 (0)	
RCB II + III	35 (37.2)	21 (27.6)	6 (66.7)	4 (80)	4 (100)	**0.004**
NA	3	2	0	1	0	
			*	*	*	
NACT without anti-HER2, n (%)						
pCR + RCB I	23 (28.4)	1 (33.3)	0 (0)	7 (33.3)	15 (26.8)	
RCB II + III	58 (71.6)	2 (66.7)	1 (100)	14 (66.7)	41 (73.2)	0.856
NA	3	1	0	0	2	

Important p-values are shown in bold.

*p<0.05 compared with group 1; **p<0.001 compared with group 1; NACT: Neoadjuvant chemotherapy; FISH: Fluorescence *in situ* hybridization; NA: Not available; RCB: Residual cancer burden; n: Number; HER2: Human epidermal growth factor receptor 2

and test methods, the high percentage of tumors with HER2 IHC score 2+/3+ in our patients with *HER2*-nonamplified tumors indicates that polysomy 17 is associated with an increased HER2 IHC score.

The potential association between polysomy 17 and HER2 expression raises the question of whether polysomy 17 influences anti-HER2 treatment response. Some data indicate that polysomy 17 tumors are sensitive to anti-HER2 treatment. In a study by Hofmann et al. (17), two patients with HER2 overexpression (IHC 3+) due to polysomy rather than HER2 amplification experienced a response to trastuzumab. In contrast, phase III EGF30001 trial revealed that lapatinib had no significant benefit in patients with *HER2*-nonamplifed, polysomic metastatic breast cancer (29). We evaluated the response of polysomy 17 tumors to NACT in the presence or absence of anti-HER2 reagent. Our results indicated that patients with *HER2* FISH group 1 tumors who received NACT containing an anti-HER2 reagent had a higher good response rate than did patients with other groups of tumors who received NACT, with or without anti-HER2 reagent. In group 5 tumors, one patient had a tumor that showed HER2 overexpression (IHC score 3+). However, this patient did not undergo NACT. In reviewing the pathological features associated with tumor response to NACT, our results revealed that, for tumors treated with NACT containing an anti-HER2 reagent, the *HER2* FISH group, ER and PR status, and HER2 expression level were associated with treatment response. For tumors treated without anti-HER2 reagent, tumor nuclear and histological grade, proliferative index Ki-67, and ER and PR status were correlated with response.

Another finding was that, in our study cohort, *HER2* FISH group 3 tumors, which were designated as *HER2*-amplified tumors according to 2013 ASCO/CAP guidelines, did not demonstrate a significant response to NACT containing anti-HER2 reagent. This finding supports the recently published update to the ASCO/CAP guidelines that HER2 status in group 3 tumors should be interpreted combined with the FISH result and HER2 protein expression level (30). To our knowledge, this is the first report of the treatment response of polysomy 17 tumors in the NACT setting. Further study in a larger population is needed to confirm these findings.

In summary, we studied the clinicopathological features and tumor response to NACT treatment of polysomy 17 breast cancer on the basis of tumor *HER2* FISH groups. We conclusively demonstrated that group 1 polysomy 17 tumors have more unfavorable pathological features but have the best response to NACT with anti-HER2 treatment. Polysomy 17 tumors in other groups did not significantly benefit from anti-HER2 treatment in the NACT setting. These results could help identify patients who may benefit from a more intensive targeted therapy regimen.

Table 5. Pathological features associated with response to NACT with anti-HER2 reagent in polysomy 17 tumors

	pCR + RCB I	RCB II + RCB III	
Feature	**(n = 59)**	**(n = 35)**	**p-value**
HER2 FISH group, n (%)			
1	55 (93.2)	21 (60.0)	**-**
3	3 (5.1)	6 (17.1)	**0.02**
4	1 (1.7)	4 (11.4)	**0.02**
5	0 (0)	4 (11.4)	**0.008**
HER2 IHC score, n (%)			
0	0 (0)	2 (5.7)	
1+	1 (1.8)	6 (17.1)	
2+	15 (26.8)	10 (28.6)	**<0.05**
3+	40 (71.4)	17 (48.6)	
NA	3	0	
ER, n (%)			
Positive	28 (47.5)	26 (74.3)	**<0.05**
Negative	31 (52.5)	9 (25.7)	
PR, n (%)			
Positive	13 (22.0)	19 (54.3)	**<0.05**
Negative	46 (78.0)	16 (45.7)	
Ki-67, n (%)			
<15	5 (9.8)	1 (4.5)	
15–<35	15 (29.4)	7 (31.8)	0.821
≥35	31 (60.8)	14 (63.6)	
NA	8	13	
Nuclear grade, n (%)			
II	18 (30.5)	15 (42.9)	0.225
III	41 (69.5)	20 (57.1)	
Histological grade, n (%)			
1	1 (1.7)	0 (0)	
2	19 (32.2)	19 (54.3)	0.063
3	39 (66.1)	16 (45.7)	

Important p-values are shown in bold.

NACT: Neoadjuvant chemotherapy; HER2: Human epidermal growth factor receptor 2; FISH: Fluorescence *in situ* hybridization; IHC: Immunohistochemical; RCB: Residual cancer burden; NA: Not available. ER: estrogen receptor; PR: progesterone receptor; n: Number

Table 6. Pathological features associated with response to NACT without anti-HER2 reagent in polysomy 17 tumors

	pCR + RCB I	RCB II + RCB III	
Feature	**(n = 23)**	**(n = 58)**	**p-value**
Nuclear grade, n (%)			
II	2 (8.7)	28 (48.3)	**<0.001**
III	21 (91.3)	30 (51.7)	
Histological grade, n (%)			
1	0 (0)	1 (1.7)	
2	2 (8.7)	29 (50.0)	**<0.001**
3	21 (91.3)	28 (48.3)	
ER, n (%)			
Positive	8 (34.8)	39 (67.2)	**<0.05**
Negative	15 (65.2)	19 (32.8)	
PR, n (%)			
Positive	3 (13.0)	27 (46.6)	**<0.05**
Negative	20 (87)	31 (53.4)	
Her2 IHC score, n (%)			
0	4 (19)	5 (9.4)	
1+	9 (42.9)	25 (47.2)	0.521
2+	8 (38.1)	23 (43.4)	
NA	2	5	
HER2 FISH group, n (%)			
1	1 (4.3)	2 (3.4)	
3	0 (0)	1 (1.7)	0.837
4	7 (30.4)	14 (24.1)	
5	15 (65.2)	41 (70.7)	
Ki-67, n (%)			
<15	0 (0)	8 (15.4)	
15–<35	2 (11.8)	24 (46.2)	**<0.05**
≥35	15 (88.2)	20 (38.5)	
NA	6	6	

Important p-values are shown in bold.

NACT: Neoadjuvant chemotherapy; HER2: Human epidermal growth factor receptor 2; FISH: Fluorescence *in situ* hybridization; IHC: Immunohistochemical; RCB: Residual cancer burden; NA: Not available. ER: estrogen receptor; PR: progesterone receptor; n: Number

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Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Conception: H.S., J.C., H.C., A.A.Ş., G.T., M.R., B.L.; Design: H.S., J.C., H.C., A.A.Ş.; Supervision: A.A.Ş., B.L.; Data Collection or Processing: H.S., H.C., A.A.Ş., G.T.; Analysis or Interpretation: H.S., H.C., A.A.Ş., G.T.; Literature Search: H.S.; Writing: H.S., B.L.; Critical Review: H.C., A.A.Ş., G.T., B.L.

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