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Original Article

Treatment of HER2+ breast cancer: a retrospective of disease prognosis with loss of HER2 amplification on residual disease after neoadjuvant treatment in a community hospital setting

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Abstract: Neoadjuvant chemotherapy (NAC) with Anti-Human Epidermal Growth Factor Receptor 2 (Anti-HER2) agents increase rates of pathologic complete response (pCR) in stage II-III, HER2+ breast cancer (BC). Several retrospective studies show HER2 amplification discordance from biopsy to post-NAC residual disease (RD). This phenomenon has unclear prognostic significance. This data was obtained from patients with HER2+ BC treated with NAC between 2018-2021 at our institution. Patients with biopsy and surgical specimens at our institution were analyzed. PCR was defined as ypT0/is N0, and HER2 status on RD was evaluated. 2018 HER2 ASCO/CAP definitions were used. In total, 71 patients were identified. 34/71 patients had pCR and were not included in further analysis. 37/71 patients had RD and HER2 was analyzed. 17/37 had HER2 loss and 20/37 remained HER2 positive. Mean follow-up time for HER2 loss was 43 months and 27 months for patients remaining HER2 positive, but neither group met 5-year Overall Survival as follow-up is ongoing. Recurrence Free Survival (RFS) was 35 months for HER2+ and 43 months for HER2 loss (P = 0.007). However, short follow-up time since diagnosis likely contributed to the under-representation of the true RFS of both groups. Therefore, at our institution, retained HER2 positivity on RD after NAC was associated with a statistically worse RFS. Although limited by sample size and follow-up time, further prospective investigation into the significance of HER2 discordance on RD assessed by 2018 definitions could clarify true RFS and if next-generation tumor profiling on RD will yield changes in tailored management.

Keywords: Targeted therapy, chemotherapy, hormone therapy, next-generation sequencing, triple positive, HER2+

Introduction

With the discovery of the Human Epidermal Growth Factor Receptor 2 (HER2/neu) gene in the 1980s and its elucidated role in breast cancer pathogenesis, the incidence of its amplification has been observed in about 15-30% of breast cancers in the current clinical landscape [1, 2]. Characterized as a predictor of poor prognosis, refined efforts in pharmacologic development of HER2-targeted agents such as trastuzumab and pertuzumab have led to significantly improved overall survival and disease-free survival with their implementation into the standard of care [3].

In combination with HER2 targeting agents, NAC has further improved the probability of achieving pCR with the added benefit of increasing the likelihood of breast conservation surgery with no additional toxicity when compared to chemotherapy alone [4, 5]. However, in RD, expressional changes in hormone and HER2 receptors after NAC have been reported on surgical specimens prompting discussion of clinical significance. Previously, studies have proposed that changes in HER2 amplification may be due to tumor heterogeneity, neoadjuvant therapy-induced clonal replacement, sampling error, or interoperator variability in HER2 assessment [6]. This discordance in receptor sta-

tus has led to unfavorable RFS and overall survival (OS) reports. Several studies, such as Mittendorf et al. and Dieci et al., have reported phenotypic discrepancies with poor RFS with loss of HER2 amplification [6]. Other studies, however, report no difference in prognosis with HER2 status change after NAC [7, 8].

Of note, all of the mentioned studies interpreted HER2 amplification on both biopsy and surgical specimens using 2007-2013 ASCO/CAP guidelines for HER2 assessment which were subsequently updated in 2018 [9]. This is of significant importance as demonstrated by a retrospective study performed by Gordian-Arroyo et al., who retrospectively identified 1,350 core biopsies between 2014-2017, reclassified them under 2018 ASCO guidelines, and found a change in HER2 status in 6% of the biopsies primarily from HER2 equivocal status (2013 guidelines) to HER2-negative (2018 guidelines).

Our study aimed to determine the prognostic impact of HER2 amplification loss on RD using HER2 ASCO/CAP 2018 definition guidelines on biopsy and post-NAC RD in patients with HER2+ breast cancer treated with NAC and anti-HER2 treatment.

Patients and methods

Patients with HER2+ breast cancer diagnosed between 2018 and 2021 at our institution and treated with NAC and anti-HER2 antibodies were eligible.

Other significant inclusion criteria included: Biopsy and surgical specimens obtained strictly within our institution; all specimens were assessed by two independent pathologists using 2018 HER2 ASCO/CAP guidelines.

Clinical characteristics compared between HER2+ post-NAC and HER2- post NAC surgical specimens were age, ethnicity, TNM stage, date of diagnosis, Tumor grade, ER/PR status on biopsy, IHC on biopsy, FISH HER2/CEP17 ratio on biopsy, FISH HER2 gene Copy Number (CN) on biopsy, ER/PR status on the surgical specimen, IHC on the surgical specimen, FISH HER2/CEP17 ratio on the surgical specimen, FISH HER2 gene Copy Number on the surgical specimen, median follow up time from the start of neoadjuvant therapy until recurrence/metastasis or last follow up, type of NAC and pCR.

HER-2 positive disease was defined as Immunohistochemical (IHC) 3+ or 2+ and amplified by Fluorescent In-Situ Hybridization (FISH). Further detailed in **Tables 1** and **2** [10].

pCR was defined as ypT0/is N0. Hormone receptor-positive was defined as staining in more than 1% of tumor cells.

Statistical Analysis was performed using XLSTAT and Minitab statistics. When appropriate, clinical characteristics between groups were compared using the χ^2 or Fisher exact tests. Survival was estimated using the Kaplan-Meier method, and the log-rank test was used to compare survival between the groups of patients. Recurrence Free-Survival was also calculated using the Kaplan-Meier method. Statistical significance was defined if $P < 0.05$.

Results

A retrospective chart review of pre-treatment biopsy and post-NAC surgical pathologic specimens was reviewed on all HER2+ breast cancer patients treated with TCHP between 2018-2021 in a community hospital system in New Jersey. In total, 71 patients were identified. However, 34/71 (48%) had pCR (ypT0/is N0) after total NAC and were not included due to not having the residual disease for analysis.

Thirty-seven of the remaining patients were subsequently analyzed. The median age for the 37 analyzed patients was 61 (33-79). Clinical Staging was performed via the American Joint Committee of Cancer (AJCC) TNM 2018 system. 9 patients had stage I, 24 stage II, and five stage III. Thirty-five patients were HR+ (hormone receptor-positive; Estrogen receptor and progesterone receptor) on pretreatment diagnostic biopsy. HER2 IHC 3+ status on pretreatment biopsy was reported in 24 patients, and IHC 2+ in 13 patients. All 13 patients with IHC 2+ were FISH amplified. A complete summarization of the patients and their biopsy characteristics are included in **Table 3**.

All patients received a neoadjuvant TCHP regimen. All patients after TCHP underwent lumpectomy or mastectomy. Residual disease was analyzed with 20/37 (54%) patients remaining

Table 1. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update

HER2 IHC status	2007 ASCO/CAP guidelines	2013 ASCO/CAP guidelines	2018 ASCO/CAP guidelines
Positive (3+)	Uniform intense membrane staining of > 30% of invasive tumor cells	Circumferential membrane staining that is complete, intense, and in > 10% of tumor cells	Circumferential membrane staining that is complete, intense, and in > 10% of tumor cells
Equivocal (2+)	Complete membrane staining that is neither non-uniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells	Circumferential membrane staining that is incomplete and/or weak to moderate and within > 10% of the invasive tumor cells. Complete circumferential membrane staining that is intense and within 10% of the invasive tumor cells	Weak to moderate complete membrane staining observed in > 10% of tumor cells
Negative (1+)	Weak incomplete membrane staining in any proportion of tumor cells	Incomplete membrane staining that is faint or barely perceptibly and within > 10% of the invasive tumor cells	Incomplete membrane staining that is faint or barely perceptible and within > 10% of the invasive tumor cells
Negative (0)	No staining	No staining observed Incomplete membrane staining that is faint or barely perceptible and within 10% of the invasive tumor cells	No staining observed Incomplete membrane staining that is faint or barely perceptible and within 10% of the invasive tumor cells

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, Jenkins RB, Press MF, Spears PA, Vance GH, Viale G, McShane LM and Dowsett M. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. Arch Pathol Lab Med 2018; 142: 1364-1382.

Table 2. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update

HER2 ISH status	2007 ASCO/CAP guidelines	2013 ASCO/CAP guidelines	2018 ASCO/CAP guidelines
ISH positive	HER2/CEP17 ratio > 2.2	HER2/CEP17 ratio 2.0	HER2/CEP17 ratio 2.0 and average HER2 copy number 4.0 (group 1)
		HER2/CEP17 ratio < 2.0 and average copy number 6.0	HER2/CEP17 ratio 2.0 and average HER2 copy number < 4.0 (group 2) with concurrent IHC 3+ HER2/CEP17 ratio < 2.0 and average HER2 copy number 6.0 (group 3) with concurrent IHC 2+[a] HER2/CEP17 ratio < 2.0 and average HER2 copy number 6.0 (group 3) with concurrent IHC 3+ HER2/CEP17 ratio < 2.0 with average copy number 4.0 and < 6.0 (group 4) with concurrent IHC 3+
ISH equivocal	HER2/CEP17 ratio 1.8-2.2	HER2/CEP17 ratio < 2.0 with average HER2 copy number 4.0 and < 6.0	No equivocal category
ISH negative	HER2/CEP17 ratio < 1.8	HER2/CEP17 ratio < 2.0 with average HER2 copy number < 4.0	HER2/CEP17 ratio < 2.0 with average HER2 copy number < 4.0 (group 5) HER2/CEP17 ratio 2.0 and average HER2 copy number < 4.0 (group 2) with concurrent IHC 2+[b] HER2/CEP17 ratio < 2.0 with average HER2 copy number 4.0 and < 6.0 (group 4) with concurrent IHC 2+[b] Groups 2, 3, and 4 with concurrent IHC 0 or 1 +

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; ISH, in situ hybridization; CEP17, chromosome enumeration probe 17; IHC, immunohistochemistry. [a]An additional pathologist is blinded to previous results and recounts ISH. If the repeated ISH result is categorized to the same group, it is finally regarded as HER2 positive; [b]An additional pathologist is blinded to previous results and recounts ISH. If the repeated ISH results are designated to the same ISH group, it is finally regarded as HER2 negative. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, Jenkins RB, Press MF, Spears PA, Vance GH, Viale G, McShane LM and Dowsett M. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. Arch Pathol Lab Med 2018; 142: 1364-1382.

HER2+ on surgical resection and 17/37 (46%) patients losing HER2 amplification on surgical resection. Surgical specimens that lost HER2 amplification and those that remained HER2+ were compared between their tumor profiles, highlighted below in **Table 4**.

Patients with pretreatment biopsy IHC 2+ and IHC 3+ and HER2 loss on post-NAC RD were compared with patients with pretreatment biopsy IHC 2+ and IHC 3+ patients who remained HER2 positive on post-NAC RD ($P = 0.047$, X^2 test). Surgical specimens with HER2 loss consistent with IHC 1+ revealed a statistically significant change in their HER2 status from positive to negative ($P = 0.001$, X^2 test). Surgical IHC 3+ for patients with HER2 loss and those who remained HER2+ was also com-

Table 3. Baseline characteristics of patients and their pathologic samples on biopsy

Age	Median (range)	61 years (33-79)
Race	African American	1 (2%)
	Asian	2 (5%)
	Hispanic	8 (22%)
	White	26 (70%)
Clinical stage	Stage I	9 (24%)
	IA	7
	IB	2
	Stage II	24 (65%)
	IIA	16
	IIB	8
	Stage III	5 (14%)
	IIIA	4
	IIIB	1
Grade	2	20 (54%)
	3	17 (46%)
Hormonal receptor status	HR positive (ER and/or PR)	35 (95%)
	ER+/PR+	33
	ER+/PR-	1
	ER-/PR+	1
	HR negative (ER and PR)	3 (8%)
	ER	34 (92%)
	1-9%	2
	10-40%	2
	> 40%	30
	PR	34 (92%)
	1-9%	6
	10-40%	11
	> 40%	17
HER2 status on Biopsy	IHC 3+	24 (65%)
	IHC 2+/FISH+	13 (35%)

pared ($P \leq 0.001$, X^2 test). Finally, surgical FISH HER2/CEP17 ratio medians for HER2 loss patients and for patients who remained HER2 positive showed a statistically significant change ($P \leq 0.001$, X^2 test) as well as Surgical FISH HER2 gene CNs for HER2 loss patients and for patients who remained HER2 positive ($P \leq 0.001$, X^2 test).

There were two deaths, one breast cancer related and one non-breast cancer-related, all within the HER2-positive RD group. Three relapse events occurred in the patients with HER2-positive RD. The median OS has yet to be reached in either group. Survival analysis was calculated with a mean follow-up time of 43 months for post-TCHP HER2 loss and 27 months for patients remaining HER2 positive after TCHP. RFS was compared with a mean of 43 months for HER2 loss and 35 months for patients remaining HER2 positive. When comparing the two groups, P = 0.007, signifying patients remaining HER2 positive were statistically significant regarding recurrence-free survival, highlighted in **Figure 1**.

Discussion

The effects of neoadjuvant chemotherapy in early-stage breast cancer have been studied extensively, with randomized clinical trials demonstrating similar DFS compared to post-operative chemotherapy [12]. However, the I-SPY 1 TRIAL would further cement the relationship between NAC and pCR, which was demonstrated as a high predictor of RFS in every established receptor subset [13]. Although, patients that do not obtain pCR and have hormonal or HER2 receptor discordance on post-NAC residual disease need to be more clearly understood regarding their survival metrics.

Several reports of discordance and conflicting data have arisen on the prognostic implications of these changes. HR status conversion after NAC in one study of 267 stage II-III breast cancer patients found a worse progression-free survival (PFS) and OS for patients that converted from HR+ to HR- (hazard ratio 6.88, P: 0.001) [13]. HER2 receptor discordance is hypothesized to be due to many factors, including anthracycline/anti-her2 antibody treatment-induced clonal selection, pre-analytical and analytical pitfalls, sampling errors, inter-operator variability, and tumor heterogeneity [14].

Previous studies using versions antecedent to the 2018 ASCO/CAP guidelines for HER2 identification found that discordant HER2 status in the metastatic breast cancer setting was associated with shorter overall survival (hazard ratio

Table 4. Characteristics of patient's postsurgical samples

	HER2 loss (n = 17)	HER2+ (n = 20)	*p*-value
Biopsy ER			
Negative	2 (11.76%)	2 (10%)	> 0.999
Positive	15 (88.24%)	18 (90%)	
Biopsy PR			
Negative	2 (11.76%)	2 (10%)	> 0.999
Positive	15 (88.24%)	18 (90%)	
Biopsy ER/PR			
Negative/negative	2 (11.76%)	1 (5%)	0.584
Negative/positive	0 (0%)	1 (5%)	> 0.999
Positive/negative	0 (0%)	1 (5%)	> 0.999
Positive/positive	15 (88.24%)	17 (85%)	> 0.999
Biopsy IHC			
2+	9 (52.94%)	4 (20%)	0.047
3+	8 (47.06%)	16 (80%)	
Biopsy FISH HER2/CEP17 ratio, median	4.3 (2.35, 5.65)	5 (4.225, 6.025)	0.2
Biopsy FISH HER2 gene CN, mean	10.85 (4.74)	14.46 (5.22)	0.059
Surgical ER			
Negative	1 (5.88%)	3 (15%)	0.609
Positive	16 (94.12%)	17 (85%)	
Surgical PR			
Negative	3 (17.65%)	8 (40%)	0.169
Positive	14 (82.35%)	12 (60%)	
Surgical ER/PR			
Negative/negative	1 (5.88%)	3 (15%)	0.609
Positive/negative	2 (11.76%)	5 (25%)	0.416
Positive/positive	14 (82.35%)	12 (60%)	0.169
Surgical IHC			
0	1 (5.88%)	0 (0%)	0.459
1+	7 (43.75%)	0 (0%)	0.001
2+	9 (56.25%)	7 (35%)	0.194
3+	0 (0%)	13 (65%)	< 0.001
Surgical FISH HER2/CEP17 ratio, median	1.2 (1.1, 1.55)	4.85 (3.425, 6.275)	< 0.001
Surgical FISH HER2 gene CN, median	3.1 (2.55, 3.85)	13.45 (8.5, 17.85)	< 0.001

Hormone receptor status is defined as positive if > 1% tumor cell staining. HER2 loss if the absence of HER2 overexpression in the residual tumor at surgical resection after Neoadjuvant treatment. FISH, Fluorescent In-Situ Hybridization; IHC, Immunohistochemistry; CEP17 ratio, chromosome enumeration probe 17; CN, Copy number.

0.43, P: 0.003) [16]. Retrospective studies in the non-metastatic environment with HER2 discordance on residual disease have demonstrated worse RFS and OS. However, due to the small sample sizes and retrospective study designs, the total prognostic value of the changes has yet to be fully understood [15, 17, 18].

While we understand that our study's results are limited by the small sample size and retrospective design, we utilized the 2018 ASCO/CAP HER2 identification guidelines for all biopsy and surgical specimens, differing from all previously reported studies. This is of particular significance since a previous retrospective study that identified 1,350 breast cancer biopsy specimens from 2014-2017 and re-classified them under 2018 guidelines found a 6% discordance rate in HER2 amplification and a HER2 positive rate which decreased by 0.4% [19].

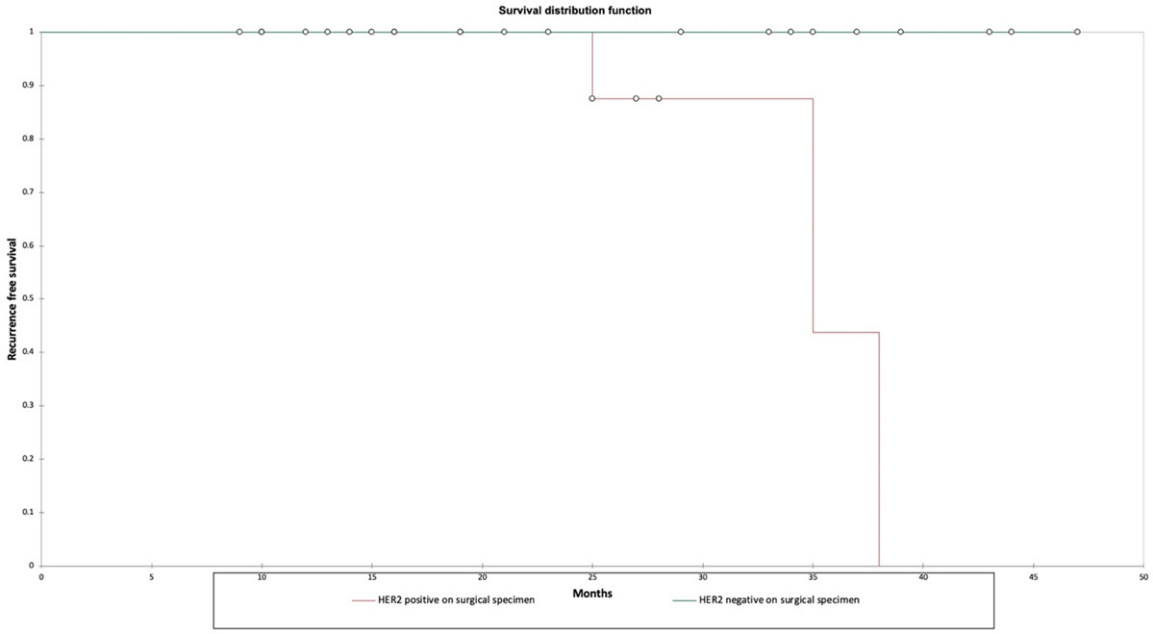

Figure 1. Kaplan-Meier recurrence-free survival curve (RFS) according to residual tumor HER2 status after Neoadjuvant treatment for non-pathologic complete response patients. RFS according to loss of HER2 overexpression/amplification after neoadjuvant treatment in patients with residual disease, comparison by a log-rank test with 95% CI for the hazard rate (HR) and *p*-value. Limited data with *p*-value 0.007 and incalculable HR/CI. Patient baseline characteristics are highlighted in **Tables 3** and **4**. Censored data points marked by "o".

Using the 2018 guidelines, we observed statistically significant differences in HER2 status changes from HER2+ to HER2- after NAC concerning biopsy specimen IHC, surgical specimen IHC, surgical FISH HER2/CEP17 ratios, and surgical FISH HER2 gene copy numbers.

However, the clinical significance of each parameter has yet to be fully elucidated without adequate follow-up of this sample. Assessment of HER2 using the current guideline definitions, tumor heterogeneity, and NAC may have attributed to some of these changes. However, causal relationships as they pertain to the changes and their relevance to survival outcomes still need to be clarified. Although, prospective analysis of these patients moving forward may reveal additional clinical relevance after the total survival outcomes are made available for analysis.

Additionally, due to these patients' limited follow-up time since diagnosis, a 5-year OS could not be met, and longer follow-up will be needed. Notably, though, this study also found a statistically worse RFS for patients who remained HER2 positive on residual disease (P: 0.007); however, this is prefaced by the short follow-up time since diagnosis, which may likely contribute to the underrepresentation of the true RFS of both residual disease groups.

Conclusion

In summary, our findings on the prognostic impact of NAC-induced HER2 loss on residual disease could not be definitively associated with worse RFS or OS. This is due to the retrospective study design, small sample size, and short length of time for follow-up. An explanation for HER2 status change from biopsy to post-NAC surgical resection was also not definitively explored and may require next-generation sequencing to elucidate more profound genetic changes associated with HER2 loss and prognosis, for which this limited retrospective study design could not provide. However, with a more extensive data set assessed by 2018 HER2 pathologic definitions and increased follow-up time, these results could contribute to the design of more extensive prospective trials and their evaluation of the prognostic implications of HER2 loss on residual disease as well as tailored approaches to adjuvant therapy on residual tumor profiles for patients in the future.

Disclosure of conflict of interest

None.

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