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PRECLINICAL STUDY

Pathologic complete response rate according to HER2 detection methods in HER2-positive breast cancer treated with neoadjuvant systemic therapy

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Abstract

Purpose Human epidermal growth factor receptor 2 (HER2)-positive breast cancers are known to have significant clinical and pathological response to neoadjuvant systemic therapy (NST). The aim of this study was to identify factors associated with pathological complete response (pCR), defined as no residual invasive carcinoma in the breast and axillary lymph nodes (ypT0/is ypN0), among patients with HER2-positive breast cancer and to compare pCR rates between breast cancers with HER2 protein overexpression by immunohistochemistry (IHC) versus *HER2* gene amplification by fluorescence in situ hybridization (FISH) in the absence of protein overexpression by IHC.

Methods We conducted a retrospective review of HER2-positive breast cancer patients treated with NST and surgery at Memorial Sloan Kettering Cancer Center between January 2013 and May 2018. Estrogen receptor (ER), progesterone receptor (PR), and HER2 status were assessed according to the 2018 ASCO/CAP guidelines.

Results During the study period, 560 patients were identified. Of 531 patients with IHC results available, 455 patients had HER2 IHC 3+, and 76 had IHC < 3+ but HER2 amplification detected by FISH. The overall pCR rate was 59% (330/560). The pCR rate among patients with HER2 protein overexpression (IHC 3+) was 67%, compared to 17% among patients with HER2 amplification by FISH (IHC < 3+). On univariate and multivariate analyses, HER2 protein overexpression by IHC (IHC 3+) was a significant predictor of pCR, along with grade 3 histology, PR-negative status, and dual anti-HER2 therapy.

Conclusion Although both HER2 IHC and FISH are standard HER2 testing methods in breast cancer, achievement of pCR is associated with HER2 IHC expression level, among other factors.

Keywords Pathologic complete response · HER2 assessment · Neoadjuvant systemic therapy · HER2-positive breast cancer · Human epidermal growth factor receptor 2

Introduction

Neoadjuvant systemic therapy (NST) offers the benefits of downstaging the tumor, allowing for less extensive surgery and the possibility of avoiding axillary dissection. Pathologic complete response (pCR) is predictive for long-term outcomes in human epidermal growth factor receptor 2 (HER2)-positive and triple-negative breast cancer [1]. Rates of pCR vary significantly by breast cancer receptor subtypes, with the highest pCR rate in HER2-positive, hormone receptor-negative breast cancer patients receiving dual HER2-targeted therapy [1].

HER2 belongs to the epidermal growth factor receptor family. It is a transmembrane glycoprotein with tyrosine kinase activity. Amplification of *ERBB2* gene is the primary

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mechanism of HER2 (the protein translated from *ERBB2*) overexpression. HER2 protein overexpression assessed by immunohistochemistry (IHC) and gene amplification assessed by in situ hybridization (ISH) are the primary predictors and validated biomarkers for HER2-targeted therapies in breast cancer. Adding trastuzumab, a humanized monoclonal antibody against the extracellular domain of HER2, to chemotherapy significantly increased pCR rate after NST in HER2-positive breast cancer [2]. The combination of pertuzumab, a humanized monoclonal antibody against the dimerization domain of HER2, with trastuzumab plus chemotherapy further improved pCR rate in HER2-positive breast cancer [3, 4].

The aim of this study was to evaluate factors associated with pCR among patients with HER2-positive breast cancer, and to compare the pCR rates between breast cancers with HER2 protein overexpression (IHC 3+) and with HER2 gene amplification by ISH in the absence of protein overexpression.

Materials and methods

Patient population and ER, PR, HER2 testing

This retrospective study was approved by the institutional review board. Patients with HER2-positive invasive breast cancer treated with neoadjuvant systemic therapy and surgical resection at Memorial Sloan Kettering Cancer Center between January 2013 and May 2018 were included in this study. All patients received neoadjuvant systemic therapy with trastuzumab (H) or dual anti-HER2 agents with trastuzumab and pertuzumab (HP) in combination with standard chemotherapy. Immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR), and HER2 and dual-probe fluorescence in situ hybridization (FISH) for HER2 were assessed according to the 2018 ASCO/CAP guidelines using FDA approved assays [5, 6]. ER and PR positivity was defined as at least 1% of nuclear staining in tumor cells [5]. HER2 IHC was scored as positive (3+), equivocal (2+ or 1+ to 2+), or negative (0 or 1+) according to the 2018 ASCO/CAP guidelines [6]. The revised definition of IHC 2+ (equivocal) in the 2018 ASCO/CAP guideline did not affect the HER2 IHC scores of our cohort, comparing to the assessment according to the 2013 ASCO/CAP guideline. At our center, we routinely assess HER2 expression by IHC in all invasive breast cancers. HER2 IHC 3+ cases require no further testing. All HER2 IHC equivocal (2+ or 1+ to 2+) cases were subject to reflex HER2 dual-probe FISH assay (HER2 IQFISH pharmDx; DAKO; PathVysion HER2 DNA Probe Kit, Vysis). On rare occasions, HER2 IHC 1+ cases were tested by HER2 FISH in our laboratory when there were discrepant results from those at the outside institution (IHC equivocal, FISH amplified per outside report). HER2 FISH-positive result was defined as *HER2/CEP17* ratio ≥ 2.0 or average *HER2* copy number ≥ 6.0 signals per cell [6].

We retrospectively reviewed clinicopathologic characteristics, HER2 assessment methods, and results in pretreatment core biopsy, chemotherapy, HER2-targeted therapy modalities, and pathologic response. Pathologic complete response (pCR) was defined as no residual invasive carcinoma in the breast and axillary lymph nodes (ypT0/is ypN0) upon surgical resection.

Statistical analysis

The association between clinicopathologic features and pCR was assessed using a two-tailed student *t* test for continuous variables, Fisher's exact test for categorical variables, and multivariable logistic regression. A p value < 0.05 was considered as statistically significant.

Results

Patient baseline characteristics

A total of 560 consecutive patients with HER2-positive invasive breast cancer treated with NST followed by surgical resection at Memorial Sloan Kettering Cancer Center in the study period were identified. Baseline characteristics, chemotherapy, and HER2-targeted treatments are summarized in Table 1. The mean and median age at diagnosis was 51 years (range 24–88). Over half of the patients had grade 3 breast cancer. ER was positive in 60% (336/560) of the cohort and PR was positive in 51% (283/560). HER2 IHC results in the pretreatment biopsies were positive (3+) in 455 (81%), equivocal (2+ or 1+ to 2+) in 73 (13%), and negative (1+) in 3 (0.5%). HER2 IHC was not performed (HER2 amplification was detected by FISH at outside institutions) in 29 (5%) patients and these patients were excluded in the multivariate analysis.

Among the 76 patients with HER2 amplification detected by FISH in the absence of HER2 protein overexpression, the median *HER2/CEP17* ratio by FISH was 2.6 (range 1.4–9) and the median *HER2* signals/cell was 6.1 (range 4.2–23.6). Most (74/76, 97%) HER2 amplified cases had *HER2/CEP17* ratio ≥ 2.0 and *HER2* signals/cell ≥ 4.0 (ASCO/CAP ISH Group 1). Two (2.6%) cases had *HER2/CEP17* ratio < 2.0 but *HER2* signals/cell ≥ 6.0 (ASCO/CAP ISH Group 3). Cases with heterogeneous amplification, defined as focal amplification in a small subset of tumor cells in an overall non-amplified tumor, were not included in this study.

Compared with patients with HER2 protein overexpression (IHC 3+) breast cancers, patients with HER2 amplification by FISH in the absence of HER2 protein overexpression

Table 1 Baseline characteristics overall and by pathologic complete response (pCR)

	Overall ($n=560$)		By pCR				
			No ($n=230$)		Yes ($n=330$)		p value
Age							
Mean (SD)	51 (12)		51 (13)		52 (12)		0.439
cT stage (pre-NST)							
cT1	89	16%	28	12%	61	18%	0.630[a]
cT2	315	56%	136	59%	179	54%	
cT3	90	16%	36	16%	54	16%	
cT4	63	11%	30	13%	33	10%	
cT0/is (occult)	3	0.5%	0	0	3	1%	
Clinical nodal status							
Negative	208	37%	88	38%	120	36%	0.658
Positive	352	62%	142	62%	210	64%	
Chemotherapy							
ddAC-T	462	82.5%	183	80%	279	85%	0.142
TC	56	10%	28	12%	28	9%	
Other	42	7.5%	19	8%	23	7%	
Anti-HER2 therapy							
H	51	9%	30	13%	21	6%	0.011
HP	509	91%	200	87%	309	94%	
Grade							
1	2	0.4%	1	0.4%	1	0%	<.0001[b]
2	122	22%	69	30%	53	16%	
2–3	119	21%	49	21%	70	22%	
3	311	56%	111	48%	200	62%	
ER							
Neg	224	40%	58	25%	166	50%	<.0001
Pos	336	60%	172	75%	164	50%	
PR							
Neg	277	49%	75	33%	202	61%	<.0001
Pos	283	51%	155	67%	128	39%	
HER2 IHC core							
1+	3	0.5%	3	1%	0	0%	
1+ −2+	12	2%	11	5%	1	0%	
2+	61	11%	49	21%	12	4%	
3+	455	81%	152	66%	303	92%	
NA	29	5%	15	7%	14	4%	N/A
HER2 IHC groups[c]							
1+/2+	76	14%	63	29%	13	4%	<.0001
3+	455	81%	152	71%	303	92%	
NA	29	5%					

ddAC-T dose dense doxorubicin and cyclophosphamide followed by paclitaxel, *TC* docetaxel and carboplatin, *H* herceptin (trastuzumab) alone; *HP* trastuzumab + pertuzumab

[a]Combine cT1-cT2 vs cT3-cT4 for analysis

[b]Combine grade 1 and 2 vs grade 2–3 and 3 for analysis (missing data for 6 cases)

[c]Combine IHC 1+, 1+ to 2+, and 2+ vs IHC 3+ for analysis

were slightly older (mean age 55 vs 51 years, $p=0.0056$), tended to have lower grade tumors ($p=0.0002$), and had more frequent ER-positive (83% vs 56%, $p<0.0001$) and PR-positive (76% vs 47%, $p<0.0001$) status (Table 2).

Clinically, all patients received standard cytotoxic chemotherapy agents plus HER2-targeted therapy. In terms of neoadjuvant chemotherapy in 560 patients, 462 (82.5%) patients received dose dense doxorubicin and cyclophosphamide

Table 2 Clinicopathologic characteristics by HER2 IHC status

	Overall ($n=531$)[a]		By HER2 IHC status				
			IHC 3+ ($n=455$)		IHC 1+, 1+ to 2+, 2+ ($n=76$)		p value
Age							
Mean (SD)	51 (12)		51 (12)		55 (13)		0.0056
Anti-Her2							
H	44	8%	40	9%	4	5%	
HP	487	92%	415	91%	72	95%	0.3745
Grade							
1	2	0.4%	1	0.2%	1	1%	
2	115	22%	86	19%	29	39%	
2–3	113	21%	97	22%	16	21%	
3	296	56%	267	59%	29	39%	0.0002[b]
ER							
Neg	213	40%	200	44%	13	17%	
Pos	318	60%	255	56%	63	83%	< .0001
PR							
Neg	258	49%	240	53%	18	24%	
Pos	273	51%	215	47%	58	76%	< .0001
pCR							
Yes	316	60%	303	67%	13	17%	< .0001
No	215	40%	152	33%	63	83%	

IHC immunohistochemistry

[a]HER2 IHC was not performed in 29 patients

[b]Combine grade 1 and 2 vs grade 2–3 and 3 for analysis (missing data for 5 cases)

followed by paclitaxel (ddAC-T), 56 (10%) received docetaxel and carboplatin (TC), and 42 (7.5%) received other chemotherapy combinations due to patient-related factors associated with chemotherapy intolerance. Most (509/560; 91%) patients received dual anti-HER2 regimens with HP. The overall pCR rate was 59% (330/560). At median follow-up of 28 months, 535 (96%) patients had no evidence of disease, 18 (3%) patients developed distant metastasis, 7 (1%) died of the disease.

Predictors of pathologic complete response

HER2 protein overexpression (IHC 3+) was a significant predictor of pCR. The pCR rate among patients with HER2 protein overexpression (IHC 3+) was 67% (303/455), compared to only 17% (13/76) among patients with HER2 amplification by FISH in the absence of HER2 overexpression (IHC equivocal or negative) ($p<0.0001$). Other factors significantly associated with higher rate of pCR in univariate analysis included grade 3 histology ($p<0.0001$), hormonal receptor negativity ($p<0.0001$), and dual anti-HER2 therapy ($p=0.011$) (Table 1). There was no association between patient age, pretreatment clinical T stage and nodal status, or chemotherapy regimens and pCR.

Among the 76 patients with HER2-positive breast cancer by FISH without HER2 protein overexpression, increased *HER2/CEP17* ratio and *HER2* copy number per cell were both associated with greater rate of pCR, with an average *HER2/CEP17* ratio of 3.8 and average *HER2* signals/cell of 10.2 in patients with pCR, compared to average *HER2/CEP17* ratio of 2.9 and *HER2* signals/cell of 6.8 in patients without pCR ($p=0.020$ and $p=0.001$, respectively).

On multivariate analysis, HER2 protein overexpression (IHC 3+) remained a significant predictor of pCR, with lower pCR rates in the absence of HER2 protein overexpression (OR = 0.12, 95% CI 0.06–0.23, $p<0.001$) (Table 3). Dual anti-HER2 therapy (HP), high-grade histology, and PR-negative status were also significantly associated with

Table 3 Multivariate analysis

Effect	Estimate	95% CI		p value
Anti_HER2_therapy H vs HP	0.31	0.16	0.62	0.001
Grade 1/2 vs 2–3/3	0.61	0.38	0.98	0.04
ER negative vs positive	1.64	0.94	2.84	0.08
PR negative vs positive	2.13	1.25	3.60	0.01
HER2 IHC_core 1+/2+ vs 3+	0.12	0.06	0.23	< .0001

H trastuzumab, *HP* trastuzumab + pertuzumab

greater pCR rates in multivariate analysis. ER-negative tumors had higher pCR rates compared to ER-positive tumors, which trended toward significance (OR = 1.64, $p = 0.08$).

Discussion

Neoadjuvant systemic therapy not only has the potential benefit of downstaging tumor size and axilla, but also identifies patients with a poor response who may benefit from additional therapy [7]. In this study, we found that patients with HER2 overexpression (IHC 3+) had significantly higher rates of pCR to standard chemotherapy plus HER2-targeted therapy than those with HER2 amplification by FISH in the absence of HER2 overexpression (FISH positive/IHC equivocal). The overall pCR rate in our study cohort was 59%, comparable to that in clinical trials with similar treatment regimens [8–10]. The pCR rate among breast cancer with HER2 amplification by FISH in the absence of HER2 overexpression was only 17%, compared to 66% in those with HER2 IHC 3+ ($p < 0.0001$). Other significant factors included dual anti-HER2 therapy (trastuzumab and pertuzumab), grade 3 histology, and hormone receptor-negative status, consistent with previously reported data [1, 3, 8–10].

HER2 is overexpressed and/or amplified in approximately 15–20% of breast cancers [11–13]. HER2 assessment is critical for selecting patients for HER2-targeted therapy. Both IHC and FISH are currently regarded as equivalent assays for the assessment of HER2 status, due to the high concordance rate. Standard HER2 testing algorithms consist of either IHC testing with reflex ISH if IHC is equivocal, or ISH testing alone to detect gene amplification with additional testing by IHC if ISH results fall into the uncommon categories, including *HER2/CEP17* ratio ≥ 2, but the average *HER2* signals/cell < 4.0 (ASCO/CAP group 2), or *HER2/CEP17* ratio < 2, but the average *HER2* signals/cell > 6.0 (ASCO/CAP group 3), or *HER2/CEP17* ratio < 2, but the average *HER2* signals/cell ≥ 4.0 but < 6.0 (ASCO/CAP group 4) [6]. Comparison of HER2 FISH and IHC for the evaluation of HER2 overexpression showed the concordance rate was over 80% [14–18]. The concordance rate was further increased after implementation of the ASCO/CAP guidelines and standardized tissue fixation and specimen processing [19]. There is an excellent correlation between HER2 IHC 3+ and gene amplification by FISH. The biggest discrepancy is among HER2 IHC 2+ tumors, only 24% of which had HER2 amplification by FISH [15]. There are limited data on the clinical benefit of HER2-targeted therapy in the subset of patients with HER2 amplification by FISH in the absence of HER2 protein overexpression. Given that the mechanism of action of trastuzumab and pertuzumab is by binding to HER2 receptor on the cell surface, we hypothesize that achievement of pCR is associated with HER2 IHC expression level (among other factors). Our results support this hypothesis.

In summary, our study demonstrated that HER2 protein expression level (IHC 3+) had significant impact on pCR, along with grade 3 histology, hormone receptor-negative status, and use of trastuzumab and pertuzumab. The excellent response to HER2-targeted therapies among HER2-positive breast cancer was mainly seen in patients with HER2 IHC 3+ tumors. The rate of pCR in the subset of patients with HER2 amplification by FISH in the absence of HER2 protein overexpression was significantly lower. We look forward to other studies to validate our findings and if confirmed, further work is needed to determine the degree of benefit provided by anti-HER2 therapy in patients with HER2 gene amplification without HER2 protein overexpression [7].

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to disclose.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Waiver of informed consent was approved by the institutional review board.

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