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Research Article

Clinicopathologic Characteristics and Follow-Up Outcomes of Invasive Breast Carcinoma With Different Positive *HER2* Fluorescence In Situ Hybridization Patterns: Experience From a Single Academic Institution

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ABSTRACT

Human epidermal growth factor receptor 2 (HER2)-positive breast carcinoma (BC) encompasses a spectrum of molecular subtypes, characterized by varying *HER2/CEP17* ratios and *HER2* copy numbers, influencing responses to anti-HER2 therapy. This study stratified *HER2* fluorescence in situ hybridization (FISH)-positive patients into 3 distinct groups—group 1 with high copy number (G1-HC: ratio ≥2, copy number ≥6), group 1 with low copy number (G1-LC: ratio ≥2, copy number ≥4 and <6), and group 3 (G3: ratio <2.0, copy number ≥6.0)—and evaluated their clinicopathologic features, response to anti-HER2 therapy, and outcomes. In a cohort of 2702 continuous primary BCs, G1-HC BCs accounted for 304 cases (11.3%), G1-LC for 37 cases (1.4%), and G3 for 75 cases (2.8%). G1-HC BCs were associated with younger age, higher tumor grade, and estrogen receptor negativity compared with G1-LC BCs. Furthermore, G1-HC BCs exhibited increased progesterone receptor negativity and HER2 immunohistochemistry 3+ compared with G1-LC and G3 BCs. Analysis of the subgroup of HER2 immunohistochemistry 2+-only cases (n = 166) showed similar results. Notably, G1-HC patients exhibited significantly enhanced responses to anti-HER2 neoadjuvant chemotherapy compared with G1-LC and G3 patients. Conversely, G1-LC patients displayed a lower likelihood of disease-free status compared with G1-HC and G3 patients, albeit with no significant differences in overall survival, distant metastasis, or local recurrence among the groups. These findings offer valuable clinicopathologic insights into different *HER2* FISH positive subgroups, potentially informing future criteria for interpreting *HER2* FISH results.

Introduction

The oncogenic human epidermal growth factor receptor-2 (HER2), a receptor tyrosine-protein kinase, is encoded by the *ERBB2* gene, also known as *HER2*, situated at 17q12 on chromosome 17s long arm. Studies consistently demonstrate HER2 gene amplification in 15% to 20% of breast carcinoma (BC), underscoring its clinical significance. Remarkably, HER2-targeted therapies, notably the monoclonal antibody trastuzumab, have revolutionized outcomes for HER2-positive BC patients.[1,2] Despite the efficacy of anti-HER2 treatment, variations in HER2 expression or amplification levels across patients yield divergent responses to therapy.[3-7] Consequently, understanding the interplay between distinct HER2-positive statuses and responses to anti-HER2 therapy assumes paramount importance.

The standardized methods and testing guidelines for determining the HER2 status in BC were initially developed in 2007 and subsequently updated in 2013 and 2018 by the American Society

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Table 1
Different groups of *HER2* in situ hybridization

Group	FISH results	Interpretations
Group 1 (G1)	*HER2/CEP17* ratio of ≥2.0, with an average *HER2* copy number ≥4.0 signals/cell	Positive for gene amplification
Group 1-high copy # (G1-HC)	*HER2/CEP17* ratio of ≥2.0, with an average *HER2* copy number ≥6.0 signals/cell	
Group 1-low copy # (G1-HC)	*HER2/CEP17* ratio of ≥2.0, with an average *HER2* copy number ≥4.0 and <6.0 signals/cell	
Group 2 (G2)	*HER2/CEP17* ratio of ≥2.0 with an average *HER2* copy number <4.0 signals/cell	• If IHC is 3+, diagnosis is HER2 positive. • If IHC result is 2+ and the *HER2* ratio and signal remain the same, diagnosis is HER2 negative. • If the IHC result is 0 or 1+, diagnosis is HER2 negative.
Group 3 (G3)	*HER2/CEP17* ratio of <2.0, with an average *HER2* copy number ≥6.0 signals/cell	• If IHC is 3+, diagnosis is HER2 positive. • If IHC result is 2+ and the *HER2* ratio and signal remain the same, diagnosis is HER2 positive. • If the IHC result is 0 or 1+, diagnosis is HER2 negative.
Group 4 (G4)	*HER2/CEP17* ratio of <2.0, with an average *HER2* copy number ≥4.0 and <6.0 signals/cell	• If IHC is 3+, diagnosis is HER2 positive. • If IHC result is 2+ and the *HER2* ratio and signal remain the same, diagnosis is HER2 negative. • If the IHC result is 0 or 1+, diagnosis is HER2 negative.
Group 5 (G5)	*HER2/CEP17* ratio of <2.0, with an average *HER2* copy number <4.0 signals/cell	Negative for gene amplification

of Clinical Oncology and the College of American Pathologists (ASCO/CAP).[8,9] The primary aim of these guidelines is to accurately identify patients suitable for HER2-targeted therapies while minimizing the occurrence of false-positive or false-negative results. Presently, the established standard testing methods encompass immunohistochemistry (IHC) for assessing protein expression and in situ hybridization, particularly fluorescence in situ hybridization (FISH), for evaluating *HER2* gene amplification (copy number and/or *HER2/CEP17* ratio). HER2 IHC results have been categorized as positive (3+ staining), equivocal (2+ staining), or negative (1+ or zero staining) based on the completeness of membranous staining, staining intensity, and the percentage of tumor cells displaying staining. Equivocal HER2 specimens undergo further ISH testing to definitively determine HER2 status as either positive or negative. Through the integration of both IHC and FISH results, HER-positive status is presently defined as either IHC 3+ or IHC 2+/ISH+, whereas HER-negative status is delineated by IHC zero, IHC 1+, or IHC 2+/ISH−. Patients were divided into 5 groups according to *HER2* FISH results in the 2018 ASCO/CAP guidelines.[9] Patients in group 1 (G1: *HER2/CEP17* ratio of ≥2.0, with an average *HER2* copy number ≥4.0 signals/cell), group 3 (G3: *HER2/CEP17* ratio of <2.0, with an average *HER2* copy number ≥6.0 signals/cell) with HER2 IHC 2/3+, and in other groups with HER2 IHC 3+ were considered HER2-positive patients.[9]

Nevertheless, the effectiveness of anti-HER2 therapy for HER2-positive BCs, as defined by the 2018 version of ASCO/CAP, with varying FISH patterns, remains inadequately explored, with most studies failing to capture real-world data. Recent research indicates that a subset of patients in the *HER2* FISH G1, characterized by *HER2/CEP17* ratios ≥2.0 and *HER2* copy numbers ≥4.0 and <6.0, may not derive the same level of benefit from current anti-HER2 therapies as patients exhibiting other FISH positive patterns.[10,11] In the current study, we further subcategorized *HER2* FISH G1 into 2 distinct subgroups: G1 with high copy number (G1-HC: ratio ≥2, and ≥6 signals/cell) and G1 with low copy number (G1-LC: ratio ≥2, and ≥4 and <6 signals/cell; Table 1).

Utilizing a large real-world cohort from a single institution, our objective was to delineate distinct categories of *HER2* FISH positivity (G1-HC, G1-LC, and G3) by examining clinicopathologic characteristics, responses to anti-HER2 therapy (both neoadjuvant and adjuvant), and clinical outcomes. The findings from our study provided important insights into the utilization of anti-HER2 therapy among BC patients exhibiting varied FISH results, potentially leading to a re-evaluation of the criteria utilized for assessing *HER2* FISH results.

Material and Methods

Patients and Specimens

The study cohort comprised 2702 consecutive cases of invasive BC diagnosed between 2018 and 2022 at The Ohio State University Wexner Medical Center. Clinicopathological features, including age, histologic type, grade, estrogen receptor (ER) IHC, progesterone receptor (PR) IHC, HER2 IHC, and FISH results, were meticulously collected. Approval for the study was obtained from the institutional review board of The Ohio State University.

Immunohistochemistry

HER2 protein expression was assessed through manual quantitative IHC on formalin-fixed (for >6 and <72 hours whenever feasible), paraffin-embedded tissue, employing the US Food and Drug Administration-approved clone 4B5 (rabbit monoclonal, Ventana Medical System) on a Ventana auto-stainer. The interpretation of HER2 IHC results was conducted by specialized breast pathologists in accordance with the ASCO/CAP HER2 guidelines.

ER and PR evaluation entailed manual quantitative IHC on formalin-fixed (for >6 and <72 hours if possible), paraffin-embedded tissue, utilizing clone SP1 (Spring Bioscience) for ER, clone PgR 636 (DAKO) for PR, and the Leica/Bond polymer detection system on either a Leica/Bond or DAKO auto-stainer. For ER, the percentage of positive tumor cell nuclei was scored as <1% negative, 1% to 10% low positive, or >10% positive, with the overall staining intensity categorized as weak, moderate, or strong. For PR, the percentage of positive tumor cell nuclei was scored as <1% negative or >/=1% positive, and the overall staining intensity was categorized as weak, moderate, or strong.

HER2 Fluorescence In Situ Hybridization

At our institution, *HER2* FISH was conducted concurrently with HER2 IHC for all primary invasive BCs.[12] *HER2* FISH analysis utilized the dual-color Vysis FDA-approved PathVysion HER2 DNA Probe Kit (Abbott Molecular). Computer-assisted scoring (BioView; Abbott Molecular) was employed to evaluate 100 tumor cells, enumerating Spectrum Orange (O) *HER2* and Spectrum Green (G) *CEP17* signals. The mean *HER2/CEP17* ratio was computed by BioView across all selected cells, with results verified through fluorescence microscopy. Cells lacking signals or exhibiting signals of only 1 color (eg, 1G and 1O) were excluded from scoring. *HER2* FISH interpretations were performed by specialized molecular pathologists in accordance with the 2018 ASCO/CAP HER2 testing guideline.

Statistical Analysis

Statistical analyses were conducted utilizing GraphPad Prism (GraphPad Software, Inc) and SAS version 9.4 for Windows (SAS Institute, Inc). Descriptive statistics were employed to summarize patients' clinical and pathological characteristics. Categorical data (such as grade, HER2 IHC score, ER/PR positivity, tumor stage, lymph node stage, response to chemotherapy, etc) were presented as frequencies and percentages, whereas continuous variables (including ER/PR percentage, *HER2* FISH ratio, and copy number) were expressed as medians and ranges. Fisher exact test was utilized to compare each variable across different groups, whereas an unpaired *t* test was employed for analyzing continuous variables. Kaplan-Meier survival analysis, implemented using GraphPad Prism, was employed to analyze survival data. A significance threshold of adjusted *P* value $\leq$ .05 was applied.

Results

Distribution of Invasive Breast Carcinoma With Different HER2 Fluorescence In Situ Hybridization Patterns

The distribution of invasive BCs with different *HER2* FISH patterns encompassed a comprehensive analysis of 2702 continuous primary cases spanning from January 2018 to December 2022. Each case underwent evaluation for both HER2 IHC and *HER2* FISH. The breakdown revealed distinct proportions within various *HER2* FISH groups: 341 cases (12.7%) fell under G1 including 304 cases (11.3%) with high copy number (G1-HC: $\geq$6 signals/cell) and 37 cases (1.4%) with low copy number (G1-LC: $\geq$4 and <6 signals/cell). Group 2 accounted for 5 cases (0.2%), G3 for 75 cases (2.8%), group 4 for 229 cases (8.5%), and the predominant group 5 for 2052 cases (75.9%). This comprehensive assessment, detailed in Table 2, sheds light on the heterogeneous distribution of *HER2* FISH patterns within the cohort.

Comparison of Clinicopathologic Characteristics and Clinical Outcomes Among All Group 1-High Copy Number, Group 1-Low Copy Number, and Group 3 Breast Carcinomas (n = 416)

The comparison of clinicopathologic characteristics among G1-HC, G1-LC, and G3 BCs provided crucial insights into the landscape of HER2-positive BC. Notably, G1-HC patients exhibited a distinct age profile, typically younger than their G1-LC counterparts. Although no significant differences emerged in histologic type among the 3 groups, G1-LC tumors presented a lesser prevalence of grade 3 tumors compared with G1-HC tumors, indicative of potential variations in tumor aggressiveness. Furthermore, G1-HC tumors showcased a higher frequency of ER negativity than G1-LC tumors, suggesting differences in hormone receptor status between these subtypes. Additionally, G1-HC tumors displayed elevated levels of PR negativity compared with G1-LC or G3 tumors. Particularly noteworthy was the significantly higher incidence of HER2 IHC 3+ observed in G1-HC tumors relative to G1-LC and G3 tumors, highlighting the varying levels of HER2 expression among different *HER2* FISH groups. These findings, elaborated upon in Table 3, contribute valuable insights into the clinicopathologic characteristics of HER2-positive BCs.

Table 2
Distribution of different *HER2* FISH groups and HER2 IHC results

HER2 FISH group	#	%
Group 1	341	12.7%
Group 1—high copy # (G1-HC)	304	11.3%
Group 1—low copy # (G1-LC)	37	1.4%
Group 2	5	0.2%
Group 3	75	2.8%
Group 4	229	8.5%
Group 5	2052	75.9%
Total	2702	100.0%

FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

A total of 11 cases with HER2 IHC and FISH discordant results were identified, including 7 G1-HC and 4 G1-LC cases. Notably, the incidence of discordant cases was very high among G1-LC cases (10.8%). Additionally, 3 G3 cases showed HER2 IHC 0/1+ results.

We proceeded to analyze cases with clinical follow-up results and correlated them with clinicopathologic features. All patients included in this subset (n = 324) received HER2-targeted therapy (adjuvant and/or neoadjuvant). G1-LC patients displayed a lower probability of being free of disease (disease-free survival) than G1-HC and G3 patients. Nevertheless, there was no statistically significant difference observed in overall survival, distant metastasis, and local recurrence among the 3 groups (Table 3 and Fig. 1).

Comparison of Clinicopathologic Characteristics and Clinical Outcomes Among HER2 IHC2+ Breast Carcinomas (n = 166)

Next, we divided HER2 IHC 2+ cases into G1-HC, G1-LC, and G3 subgroups (65 G1-HC, 31 G1-LC, and 70 G3) and analyzed their clinicopathologic features and clinical outcomes accordingly. Notably, HER2 IHC 2+ G1-LC tumors presented a lesser prevalence of grade 3 tumors compared with IHC 2+ G1-HC or G3 tumors. Additionally, IHC 2+ G1-LC tumors displayed less PR negativity compared with G1-HC tumors. We further analyzed cases with clinical follow-up results and correlated them with clinicopathologic features. IHC 2+ G1-LC patients displayed a lower probability of being free of disease (disease-free survival) than G1-HC patients with statistical significance, or G3 patients without statistical significance, which may be due to limited case numbers. Like the results analyzed in all HER2 IHC cases, there was no statistically significant difference observed in overall survival, distant metastasis, and local recurrence among the HER2 IHC 2+ cases. Fourteen

Table 3
Comparison of clinicopathologic features and clinical outcomes in all patients with *HER2* FISH positive results (n = 416)

		Group 1-high copy # (G1-HC)		Group 1-low copy # (G1-LC)		Group 3		*P* value (G1-HC vs G1-LC)	*P* value (G1-HC vs G3)	*P* value (G1-LC vs G3)
No. of total cases		304		37		75				
Age		57.0	26-93	62.4	29-87	59.5	30-95	.0270	NS	NS
Histologic type	IDC	257	84.3%	34	91.9%	60	80.0%	NS	NS	NS
	ILC	18	5.9%	2	5.4%	6	8.0%			
	Mixed	27	8.9%	1	2.7%	8	10.7%			
	Metaplastic	2	0.7%	0	0.0%	1	1.3%			
Grade	1	9	3.0%	1	2.9%	1	1.4%	.0130	NS	NS
	2	128	43.2%	22	64.7%	30	41.7%			
	3	159	53.7%	11	32.4%	41	56.9%			
	NA	8		3		3				
ER	Positive	209	70.4%	26	83.9%	54	78.3%	.0080	NS	NS
	Negative	88	29.6%	5	16.1%	15	21.7%			
	NA	7		6		6				
	%	55.7%	0%-100%	76.9%	0%-100%	74.2%	0%-100%	.0078	.0015	NS
PR	Positive	135	45.5%	24	77.4%	48	69.6%	.0006	<.0001	NS
	Negative	162	54.5%	7	22.6%	21	30.4%			
	NA	7		6		6				
	%	20.6%	0%-100%	56.1%	0%-100%	42.9%	0%-100%	<.0001	<.0001	NS
HER2 IHC	0	0	0.0%	1	2.7%	1	1.3%	<.0001	<.0001	NS
	1+	7	2.3%	3	8.1%	2	2.7%			
	2+	65	21.3%	31	83.8%	70	93.3%			
	3+	232	76.1%	2	5.4%	2	2.7%			
No. of cases with follow up		229		30		65				
No evidence of disease		172	75.1%	18	60.0%	51	78.5%	.046	NS	.036
Death due to disease		23	10.0%	4	13.3%	6	9.2%	NS	NS	NS
Distant metastasis		45	19.7%	8	26.7%	8	12.3%	NS	NS	NS
Local recurrence		4	1.7%	1	3.3%	1	1.5%	NS	NS	NS

There are 11 *HER2* FISH and IHC discordant cases [10 cases with FISH+ (7 G1-HC and 3 G1-LC) and IHC 1+, 1 case with FISH+ (G1-LC) and IHC 0].
ER, estrogen receptor; FISH, fluorescence in situ hybridization; IDC, invasive ductal carcinoma; IHC, immunohistochemistry; ILC, invasive lobular carcinoma; NA, not available; PR, progesterone receptor.

G1-HC/IHC 2+ cases received neoadjuvant chemotherapy with 4 (28.6%) showing pathological complete response (PCR) (3 PCR cases were negative for ER/PR). Eight G1-LC/IHC 2+ received neoadjuvant chemotherapy, but none showed PCR. Twenty-six G3/IHC 2+ cases received neoadjuvant chemotherapy with 4 (15.4%) showing PCR (3 PCR cases were negative for ER/PR). Statistical analysis did not reveal any significant difference in PCR or residual cancer burden among the 3 groups due to the limited sample size (Table 4).

Comparison of Clinicopathologic Characteristics Among Group 1-High Copy Number, Group 1-Low Copy Number, and Group 3 Breast Carcinoma Patients With Anti-HER2 Neoadjuvant Chemotherapy (n = 158)

Next, we analyzed all cases with anti-HER2 neoadjuvant chemotherapy, correlating them with clinicopathologic features. G1-HC patients demonstrated a significantly heightened response to anti-HER2 neoadjuvant chemotherapy, characterized by more

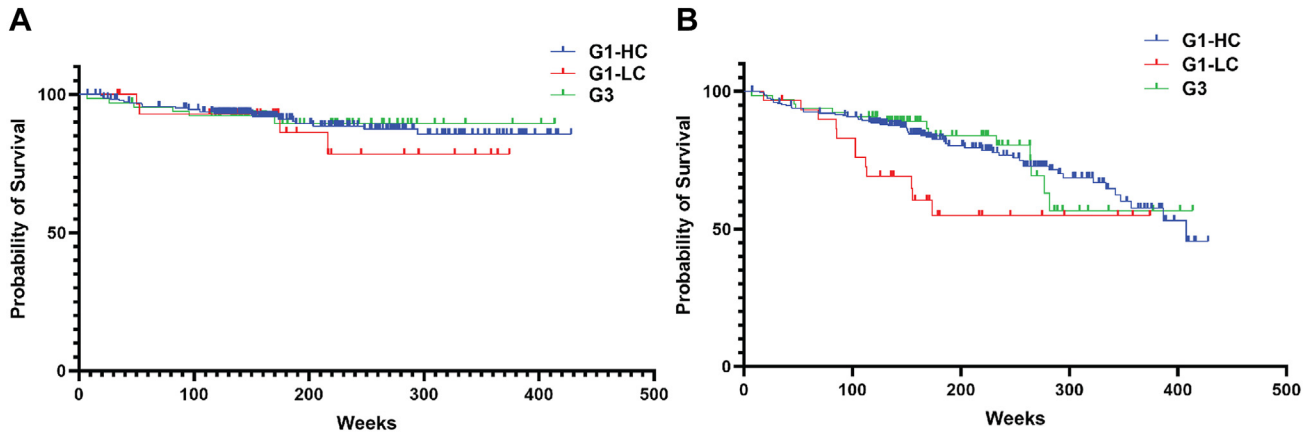

Figure 1.
Comparison of overall survival and disease-free survival in 3 *HER2* FISH positive groups. (A) Overall survival in 3 *HER2* FISH positive groups. No statistical significance was found. *P* values are *P* = .5457 (G1-HC vs G1-LC), *P* = .8849 (G1-HC vs G3) and *P* = .5357 (G1-LC vs G3) by log-rank (Mantel-Cox) test. (B) Disease-free survival in 3 *HER2* FISH positive groups. No statistical significance was found. *P* values are *P* = .0208 (G1-HC vs G1-LC), *P* = .9109 (G1-HC vs G3) and *P* = .0454 (G1-LC vs G3) by log-rank (Mantel-Cox) test.

Table 4
Comparison of clinicopathologic features and clinical outcomes in patients with HER2 IHC 2+ and *HER2* FISH positive results (n = 166)

		Group 1-high copy #		Group 1-low copy #		Group 3		*P* value (G1H vs G1L)	*P* value (G1H vs G3)	*P* value (G1L vs G3)
No. of cases	n (%)	65		31		70				
Age		59.6	34-86	62.1	29-87	59.8	30-95	NS	NS	NS
Histologic type	IDC	50	76.9%	28	90.3%	55	78.6%	NS	NS	NS
	ILC	7	10.8%	2	6.5%	6	8.6%			
	Mixed	7	10.8%	1	3.2%	8	11.4%			
	Metaplastic	1	1.5%	0	0.0%	1	1.4%			
Grade	1	7	10.9%	1	3.6%	1	1.5%	NS (G3 vs others)	0.0159 (G3 vs others)	0.0028 (G3 vs others)
	2	31	48.4%	20	71.4%	27	40.3%			
	3	23	35.9%	7	25.0%	39	58.2%			
	NA	1		3		3				
ER	Positive	51	78.5%	24	77.4%	50	71.4%	NS	NS	NS
	Negative	10	15.4%	2	6.5%	14	20.0%			
	NA	4		5		6				
PR	Positive	39	63.9%	22	84.6%	45	70.3%	.0437	NS	NS
	Negative	22	36.1%	4	15.4%	19	29.7%			
	NA	4		5		6				
HER2 FISH	Ratio	3.46	2-12	2.23	2-3.2	1.43	0.8-1.9	<.0001	<.0001	<.0001
	Copy	9.33	6-24.2	5.14	4.2-5.9	6.62	6-9.1	.002	<.0001	<.0001
No. of cases with follow up		41		25		61				
No evidence of disease		34	82.9%	16	64.0%	48	78.7%	.0486	NS	NS
Death due to disease		4	9.8%	2	8.0%	6	9.8%	NS	NS	NS
Distant metastasis		6	14.6%	6	24.0%	8	13.1%	NS	NS	NS
Local recurrence		0	0.0%	0	0.0%	1	1.6%	NS	NS	NS
Response to NAC	Case #	14	34.1%	8	32.0%	26	42.6%			
	PCR	4	28.6%	0	0	4	15.4%	NS	NS	NS
	Residual tumor	10	71.4%	8	100%	22	84.6%			
RCB	0	4	28.6%	0	0.0%	4	15.4%	NS	NS	NS
	1	1	7.1%	2	25.0%	5	19.2%			
	2	8	57.1%	5	62.5%	14	53.8%			
	3	1	7.1%	1	12.5%	3	11.5%			

ER, estrogen receptor; FISH, fluorescence in situ hybridization; IDC, invasive ductal carcinoma; IHC, immunohistochemistry; ILC, invasive lobular carcinoma; NA, not available; NAC, neoadjuvant chemotherapy; PCR, pathologic complete response; PR, progesterone receptor.

PCR patients and lower residual cancer burden values, compared with G1-LC or G3 patients. Moreover, G1-HC patients presented with more yT0 tumors (after neoadjuvant chemotherapy) than G3 patients, but not G1-LC patients, due to the limited number. Moreover, G1-HC patients presented with more yN0 tumors than G1-LC patients. There were no discernible differences in age, histologic type, tumor grade, or estrogen ER positivity among the 3 groups. Similarly, G1-HC tumors exhibited a higher incidence of PR negativity and HER2 IHC 3+ positivity compared with G1-LC or G3 tumors (Table 5).

Discussion

The 2018 ASCO/CAP guidelines delineate *HER2* FISH results into 5 groups, designating G1 and G3 as positive.[9] In our study, we further stratified G1 into 2 subcategories: G1 with high copy number (G1-HC: ratio ≥2, copy number ≥6) and G1 with low copy number (G1-LC: ratio ≥2, copy number ≥4 and <6). This refinement was prompted by recent findings suggesting that patients classified as G1-LC may not derive comparable benefits from anti-HER2 neoadjuvant chemotherapy when compared with other FISH positive patients.[10,11] In the current study, involving a substantial real-world cohort from a single institution, we observed that G1-LC and G3 constituted a minor fraction of cases (1.4% and 2.8%, respectively). The incidence of G3 tumors in our study aligns with that of previous research, demonstrating a range from 0.4% to 3.0%.[13-15]

In many published studies, *HER2* FISH was typically ordered as a reflex test for HER2 IHC 2+ cases, resulting in outcomes that might not fully represent the underlying biology of distinct *HER2* FISH groups. However, at our institution, *HER2* FISH has been systematically performed alongside HER2 IHC for all primary invasive BCs including HER2 IHC 0/1+/3+ cases.[12] Although performing FISH in HER2 IHC 0/1+/3+ cases is generally unnecessary and is not a recommended practice, this unique testing approach has enabled us to comprehensively explore the clinicopathologic characteristics across different *HER2* FISH groups, including HER2 IHC results. Our data unveiled notable distinctions between G1-HC tumors and G1-LC tumors, with G1-HC tumors demonstrating a younger age profile, a higher prevalence of tumor grade 3, and a greater frequency of ER negativity. Moreover, G1-HC tumors exhibited a higher incidence of PR negativity and HER2 IHC 3+ staining compared with both G1-LC and G3 tumors. These findings underscore the importance of evaluating *HER2* FISH and IHC results in tandem for a more nuanced understanding of BC subtypes and their associated clinicopathologic features.

In a study by Wilcock et al,[16] a cohort of 142 G3 cases revealed that HER2 IHC was negative (0/1+) in 36.6% of cases, IHC 2+ in 60.6%, and IHC 3+ in 2.8%. Another investigation, focusing on only 6 G3 cases, found 2 cases with IHC 0/1+, 3 with IHC 2+, and 1 with IHC 3+.[17] In our cohort comprising 75 G3 cases, HER2 IHC 2+ predominated in 93.3% of G3 tumors, with only 4% exhibiting HER2 IHC 0/1+ and 3% displaying IHC 3+. Similar trends were observed in Wilcock et al[16] study, where 42.3% of G3 tumors were of grade 3, and 86.6% were hormone receptor-positive (ER and/or

Table 5
Comparison of clinicopathologic features in patients with neoadjuvant chemotherapy among 3 *HER2* FISH positive groups

		Group 1-high copy #		Group 1-low copy #		Group 3		*P* value (G1-HC vs G1-LC)	*P* value (G1-HC vs G3)	*P* value (G1-LC vs G3)
No. of cases	n (%)	120		11		27				
Age		51.7	26-83	57.6	38-87	56.7	30-78	NS	NS	NS
Histologic type	IDC	105	87.5%	10	90.9%	20	74.1%	NS	NS	NS
	ILC	4	3.3%	1	9.1%	2	7.4%			
	Mixed	10	8.3%	0	0.0%	4	14.8%			
	Metaplastic	1	0.8%	0	0.0%	1	3.7%			
Grade	1	3	2.5%	1	9.1%	0	0.0%	NS	NS	NS
	2	33	27.5%	4	36.4%	10	37.0%			
	3	82	68.3%	6	54.5%	16	59.3%			
	NA	1	0.8%	0	0.0%	1	3.7%			
ER	Positive	81	67.5%	8	72.7%	21	77.8%	NS	NS	NS
	Negative	39	32.5%	3	27.3%	6	22.2%			
PR	Positive	49	40.8%	8	72.7%	19	70.4%	.0411	.0054	NS
	Negative	71	59.2%	3	27.3%	8	29.6%			
HER2 IHC	1+	1	0.8%	2	18.2%	0	0.0%	<.0001	<.0001	NS
	2+	14	11.7%	8	72.7%	26	96.3%			
	3+	105	87.5%	1	9.1%	1	3.7%			
HER2 FISH	Ratio	6.00	2-21	2.22	2.0-2.7	1.39	0.96-1.9	<.0001	<.0001	<.0001
	Copy	18.04	6.1-67.8	5.21	4.3-5.9	6.97	6-16.8	<.0001	<.0001	<.0001
Response to NAC	PCR	51	42.5%	2	18.2%	5	18.5%	NS	.0204	NS
	Residual tumor	69	57.5%	9	81.8%	22	81.5%			
RCB	0	51	42.5%	2	18.2%	5	18.5%	.0449	.0043	NS
	1	29	24.2%	2	18.2%	5	18.5%			
	2	34	28.3%	6	54.5%	14	51.9%			
	3	6	5.0%	1	9.1%	3	11.1%			
T stage	yT0	54	45.0%	3	27.3%	5	18.5%	NS (yT0 vs others)	0.0052 (yT0 vs others)	NS (yT0 vs others)
	yT1	45	37.5%	4	36.4%	14	51.9%			
	yT2	12	10.0%	3	27.3%	6	22.2%			
	yT3	7	5.8%	1	9.1%	1	3.7%			
	yT4	2	1.7%	0	0.0%	1	3.7%			
	NA	0	0.0%	0	0.0%	0	0.0%			
N stage	yN0	86	71.7%	5	45.5%	14	51.9%	0.0322 (yN0 vs others)	NS (yN0 vs others)	NS (yN0 vs others)
	yN1	21	17.5%	6	54.5%	8	29.6%			
	yN2	6	5.0%	0	0.0%	2	7.4%			
	yN3	3	2.5%	0	0.0%	1	3.7%			
	NA	4	3.3%	0	0.0%	2	7.4%			

Three yT0 G1-HC cases and 1 yT0 G1-LC case had residual tumor in lymph node.
ER, estrogen receptor; FISH, fluorescence in situ hybridization; IDC, invasive ductal carcinoma; IHC, immunohistochemistry; ILC, invasive lobular carcinoma; NA, not available; NAC, neoadjuvant chemotherapy; PCR, pathologic complete response; PR, progesterone receptor; RCB, residual carcinoma burden.

PR positive). Our findings echoed these results, with 57% of G3 tumors being grade 3 and 78.3% being ER-positive. The efficacy of anti-HER2-targeted therapy in HER2 G3 BC patients remains uncertain. Several studies have indicated relatively low PCR rates (ranging from 10% to 33%) in G3 BC patients following anti-HER2 neoadjuvant chemotherapy.[10,18] This contrasts with a PCR rate of 65% observed in the 161 tumors classified as HER2 molecular type (G1) from the Neoadjuvant Breast Registry Symphony Trial.[19] In our current study, G3 BC patients exhibited a significantly lower response to anti-HER2 neoadjuvant chemotherapy compared with G1-HC BC patients (18.5% vs 42.5%), indicating a notably diminished benefit from anti-HER2-targeted therapy in this subgroup.

Compared with G3 BCs, G1-LC BCs have been even less frequently studied. The prevalence of G1-LC tumors remains uncertain. In our investigation, G1-LC tumors were exceptionally rare, constituting only 1.4% of all BCs. A previous study examining 5 G1-LC BCs reported a prevailing HER2 IHC 2+ pattern in these cases (4 of 5, 80%).[17] In alignment with the findings of this study, our data revealed that HER2 IHC 2+ accounted for 83.8% of G1-LC BCs. However, a notable proportion (up to 11%) of G1-LC cases exhibited HER2 IHC 0/1+ results, categorizing them as discordant HER2 IHC and FISH results according to current HER2 guidelines. Similar to G3 BC patients, G1-LC patients also exhibited a significantly lower response to anti-HER2 neoadjuvant chemotherapy compared with G1-HC BC patients (18.2% vs 42.5%). This observation underscores a significantly reduced benefit from anti-HER2-targeted therapy in G1-LC BC patients. The recent study by Lv et al[10] reported a PCR rate of 5% in G1-LC BC patients, which is lower than the PCR rate observed in our cohort. Additionally, Alhamar et al[20] investigated clinical outcomes in 3 subgroups of G1 BCs (low amplified [*HER2/CEP17* ratio $\geq$ 2.0-2.99, mean *HER2*/cell 4.0-5.9], amplified [*HER2/CEP17* ratio $\geq$ 2.0-2.99, mean *HER2*/cell $\geq$ 6], and excessive amplification [*HER2/CEP17* ratio $\geq$ 3.0, mean *HER2*/cell $\geq$ 4.0]) and found that high *HER2* amplification in G1 BCs was significantly associated with longer overall survival and disease-free survival. Consistent with Alhamar's findings, our data also demonstrated that G1-LC patients had a lower probability of being free of disease compared with G1-HC and G3 patients. This suggests that the level of *HER2* amplification in G1 BCs may play a crucial role in determining clinical outcomes, with higher levels of amplification associated with improved overall survival and disease-free survival.

The most notable contribution of the current study lies in its large cohort size, comprising continuous primary BC cases (n = 2702) from a single institution, where parallel HER2 IHC and FISH testing was conducted. This allowed for a comprehensive investigation into the detailed clinicopathologic characteristics and follow-up outcomes among BCs with different *HER2* FISH positive patterns. Our data unveiled that G1-LC and G3 BCs are biologically and clinically different from G1-HC BCs, highlighting their notably reduced benefit from anti-HER2-targeted therapy. Notably, the anti-HER2-targeted therapies employed in our study cohort predominantly consisted of trastuzumab, either alone or in combination with pertuzumab. With recent advancements in HER2-targeting antibody-drug conjugates such as trastuzumab deruxtecan, there has been a transformative shift in the clinical treatment landscape for BCs, including HER2-low BCs.[21-23] It would indeed be intriguing to explore whether HER2-targeting antibody-drug conjugates could offer significant benefits to patients with G1-LC and G3 BCs, potentially opening new avenues for more effective therapeutic interventions in these subgroups.

Our study is subject to several limitations inherent to its retrospective design and the relatively small sample size of G1-LC and G3 BCs. Recognizing G1-LC and G3 BCs as rare entities, future multi-institutional studies with larger cohorts are warranted to validate our current findings. Additionally, the treatment decisions in our study were not randomized, and the follow-up period was relatively short. However, despite these limitations, our study stands out as the largest cohort from a single institution comprising continuous primary BC cases (n = 2702) with parallel HER2 IHC and FISH testing. This allowed for an in-depth investigation into the detailed clinical, pathological, and follow-up outcome information for G1-HC, G1-LC, and G3 BCs. Although our findings offer valuable insights, further research efforts with larger cohorts and longer follow-up durations are essential to corroborate and expand upon our observations. Additionally, clinical trials examining if HER2-targeted therapies are effective in these uncommon groups are necessary.

In summary, our study underscores the distinct biological and clinical profiles of G1-LC and G3 BCs compared with G1-HC BCs, highlighting their markedly reduced benefits from anti-HER2-targeted therapy. These findings contribute significant insights into the understanding of these rare *HER2* FISH groups and advocate for their consideration in defining the criteria for assessing *HER2* FISH results in the future.

Author Contributions

Z.L. and Y.H. designed the study and obtained the study cohort, analyzed data, prepared the figures and tables, and wrote the manuscript. G.T. also participated in obtaining the study cohort. D.J., W.Z., and A.V.P. participated in the study design, edited the manuscript, and prepared for submission.

Data Availability

Original data used in this study can be requested by emailing to the corresponding author Dr Zaibo Li at Zaibo.Li@osumc.edu.

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Declaration of Competing Interest

All authors have no conflict of interest.

Ethics Approval and Consent to Participate

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 was obtained from all individual patients included in the study.

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