Impact of the 2018 ASCO/CAP HER2 Guideline Focused Update

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Key Words: ASCO/CAP HER2 guidelines; Breast cancer; HER2 FISH; HER2 IHC

Am J Clin Pathol July 2019;152:17-26

DOI: 10.1093/AJCP/AQZ012

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ABSTRACT

Objectives: *The 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) human epidermal growth factor receptor 2 (HER2) guideline focused update revises the HER2 scoring criteria. We evaluated the impact on HER2 rates in breast carcinoma diagnosed at our center.*

Methods: *In a retrospective series of breast core biopsies with invasive carcinoma diagnosed between 2014 and 2017 (n = 1,350), HER2 status was classified according to 2013 and 2018 ASCO/CAP guidelines and changes in HER2 status identified.*

Results: *The 2018 guidelines reclassified the HER2 status of 6% of patients. Most changed from HER2 equivocal status (equivocal by immunohistochemistry and fluorescence in situ hybridization under the 2013 guidelines) to HER2-negative status (2018 guidelines). The HER2-positive rate decreased by 0.4%.*

Conclusions: *The 2018 guidelines decrease the rate of HER2 equivocal and positive breast cancer and reduce repeat HER2 testing on excision specimens. Approximately 0.4% of patients will become newly ineligible for anti-HER2 therapy.*

Human epidermal growth factor receptor 2 (HER2) is a predictive and prognostic biomarker that is overexpressed in up to 15% to 20% of invasive breast carcinomas.[1] HER2 status is critically important in clinical decision making, as it informs the use of systemic chemotherapy and anti-HER2 targeted therapy. In 2018, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) revised the HER2 guideline recommendations and interpretation criteria for both immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) assays.[2] In comparison to the 2013 ASCO/CAP HER2 guideline (2013 guidelines),[3] the 2018 ASCO/CAP HER2 guideline focused update (2018 guidelines) further refines the HER2 evaluation algorithms by addressing tumors with uncommon HER2 in situ hybridization (ISH) amplification patterns that carry uncertain biological and clinical significance.[2] Additionally, the 2018 guidelines increase the emphasis on coordination between IHC and ISH results, soften the recommendations for repeat HER2 testing of grade 3 tumors initially found to be HER2 negative, discontinue the option of using alternate probe HER2 FISH for clinical HER2 assessment, and raise the thresholds for categorizing a patient as HER2 positive. As a result, the 2018 guidelines have the potential to recategorize the HER2 status of some patients by eliminating equivocal results, decreasing the rate of HER2-positive results, and reducing the numbers of cases subjected to repeat HER2 testing. While the 2018 guidelines[2] estimate that 5% of cases could have ISH results reclassified, the actual impact of the 2018 guidelines on HER2 rates is unknown.

Under the 2018 guidelines, tumors with uncommon HER2 ISH amplification patterns are subjected to a more

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complicated workup that requires concomitant review with the HER2 IHC. If the IHC result is equivocal, a blinded ISH recount is required utilizing the HER2 IHC slide to direct the selection of the region of the tumor to rescore HER2. Under the 2018 guidelines, both IHC and FISH should be conducted in the same institution to ensure parallel review and quality.[2] One main advantage of the integration of HER2 IHC and ISH is that this approach better addresses the tumors that demonstrate intratumoral heterogeneity for HER2 expression, which may potentially cause a false-negative result.

In our study we investigated the potential impact of the 2018 guidelines on the HER2 rates in primary invasive breast carcinomas diagnosed at our center. We conducted a retrospective search of breast core needle biopsy (CNB) specimens performed at our institution from 2014 to 2017 with a diagnosis of primary invasive breast carcinoma. The standard HER2 testing protocol that was used in our institution during this period closely approximates the testing algorithm provided in the 2018 guidelines in several key aspects. Simultaneous testing of HER2 using both IHC and dual-probe ISH was routinely performed and interpreted in parallel and within the same institution, with a minimum of 90 tumor cells assessed per case. The HER2 IHC slide was used to direct the selection of tumor region to score HER2 ISH in all cases showing intratumoral heterogeneity. As such, we were able to rescore all cases utilizing the 2018 guidelines and analyze the impact on HER2 rates in our institution.

Materials and Methods

Case Selection

After institutional review board approval, we conducted a retrospective search of our pathology database to identify all breast CNB diagnosed at The Ohio State University Comprehensive Cancer Center (OSUCCC) from January 1, 2014, to December 31, 2017, as primary invasive breast carcinoma. Outside CNB cases reviewed at our institution in which HER2 studies were performed at an outside laboratory were excluded. Outside CNB cases in which HER2 studies were performed at OSUCCC were included.

Data collected from the electronic medical record and pathology reports for each case included procedure date, patient age at the time of procedure, histologic subtype, tumor grade (1, 2, or 3), estrogen receptor (ER) and progesterone receptor (PR) status (positive or negative and percentage of tumor nuclei staining positive), HER2 IHC (score 0, 1+, 2+, 3+), and HER2 FISH (positive, negative, equivocal, indeterminate, ratio of HER2/centromere enumeration probe 17 [CEP17], and average HER2 copy number/nucleus). All cases were originally scored according to the 2013 guidelines.[3] Hormone receptor (HR) status for each tumor was considered positive if the tumor was either ER and/or PR positive (>1% nuclear reactivity), and HR negative if the tumors were clinically negative for both ER and PR (<1% nuclear reactivity).

Immunohistochemistry

ER and PR were assessed by IHC on formalin-fixed paraffin-embedded (FFPE) tissue utilizing SP1 clone (Spring Bioscience) for ER and PgR 636 clone (DAKO) for PR, and Leica/Bond polymer detection system on a Leica/Bond or DAKO autostainer. At our institution, all CNB with invasive carcinoma are simultaneously tested using both HER2 IHC (4B5, Ventana), on a Ventana autostainer, and HER2 FISH (PathVysion HER2 DNA Probe Kit). Interphase FISH HER2 gene amplification was collected with a FFPE tissue section containing invasive tumor, selected by the involved surgical pathologist. The FISH section/slide was analyzed by a licensed medical technologist who utilized a Food and Drug Administration-approved, validated semiautomated scanning imaging workstation (BioView Image Analysis System) and associated analysis software. This scanner identified a minimum of 90 interphase invasive carcinoma nonoverlapping nuclei from a minimum of three different fields at ×60 high-power magnification. The software later identified the number of HER2 and CEP17 signals in each cell with the technologist supervision to calculate the average HER2/CEP17 ratio. The interpreting pathologist later reviewed the FISH section/slide and compared the BioView-generated data to manually observed results under an Olympus fluorescence microscope. The pathologist further evaluated the section/slide for tumor adequacy, fluorescence staining quality, and verification of internal and external control tissue patterns. As a standard practice at our institution, all cases with intratumoral heterogeneity for HER2 IHC expression have the HER2 IHC stained slide and corresponding H&E stained slide reviewed in conjunction with the HER2 FISH and analysis is focused on the region of the tumor showing the most intense HER2 IHC staining. Alternative probe HER2 FISH data were not utilized in the analysis of this study.

Data Analysis

The overall HER2 status of each case was originally determined according to the 2013 guidelines,[3] and then rescored using the 2018 guidelines.[2] Cases of bilateral disease were recorded separately. In cases of multifocal ipsilateral disease, for which HER2 status differed between foci or HER2 intratumoral heterogeneity, the highest (most positive) HER2 status was recorded.

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2013 Overall HER2 Status Classification

1. Positive: any positive result (IHC and/or FISH)
2. Negative: negative results for both IHC and FISH or a single negative result when the second test was either not performed or equivocal
3. Equivocal: equivocal result for both IHC and FISH or a single equivocal result when the second test was not performed.

2018 Overall HER2 Status Classification

The 2018 guidelines were utilized to categorize each HER2 FISH result into appropriate ISH groups 1 to 5, and 2018 overall HER2 status in each case was determined utilizing both the HER2 IHC and FISH result, as follows:

ISH Group 1

Cases with an HER2/CEP17 ratio ≥2.0, and average HER2 signals/cell ≥4.0 copy number were considered HER2 FISH positive. The 2018 overall HER2 status was considered positive, regardless of the corresponding HER2 IHC result.

ISH Group 2

In cases with an HER2/CEP17 ratio ≥2.0 and average HER2 copy number <4.0, the 2018 overall HER2 status was determined according to the corresponding HER2 IHC result.

If the IHC result was positive (score 3+), the overall HER2 status was considered positive.

If the IHC result was equivocal (score 2+) the overall HER2 status was considered negative.

If the IHC result was negative (score 0 to 1+) the overall HER2 status was considered negative.

ISH Group 3

In cases with an HER2/CEP17 ratio <2.0 and average HER2 copy number ≥6.0, the 2018 overall HER2 status was determined according to the corresponding HER2 IHC result.

If the IHC result was positive (score 3+) the overall HER2 status was considered positive.

If the IHC result was equivocal (score 2+) the overall HER2 status was considered positive.

If the IHC result was negative (score 0 to 1+) the overall HER2 status was considered negative.

ISH Group 4

In cases with an HER2/CEP17 ratio <2.0 and average HER2 copy number ≥4.0 and <6.0, the 2018 overall HER2 status was determined according to the corresponding HER2 IHC result.

If the IHC result was positive (score 3+) the overall HER2 status was considered positive.

If the IHC result was equivocal (score 2+) the overall HER2 status was considered negative.

If the IHC result was negative (score 0 to 1+) the overall HER2 status was considered negative.

ISH Group 5

Cases with an HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals/cell were considered ISH negative. The 2018 overall HER2 status was determined according to the corresponding HER2 IHC result.

If the IHC result was positive (score 3+) the overall HER2 status was considered positive.

If the IHC result was equivocal (score 2+) the overall HER2 status was considered negative.

If the IHC result was negative (score 0 to 1+) the overall HER2 status was considered negative.

Statistical Analysis

The HER2 recategorization rate was determined by comparing the overall HER2 status of each case according to 2013 and 2018 guidelines. Concordant cases were defined as having the same overall HER2 status result under both sets of guidelines (positive/positive or negative/negative). Discordant cases were defined as having different overall HER2 status based on the guidelines used (positive/negative). Change in HER2 status was defined as having different overall HER2 status under each set of guidelines (positive/negative or equivocal/negative or equivocal/positive). Concordance was reported by percentage and evaluated by Cohen's κ coefficient. Statistical analysis was performed utilizing categorical variables and summarized using frequency. Significance of ISH group vs grade was assessed utilizing *t* test, and Pearson χ^2 test was used to assess the significance of ISH group vs HR status. Continuous variables were assessed utilizing mean and range, as applicable.

Results

Patient Demographics

We identified 1,350 CNB specimens with invasive breast carcinoma and HER2 testing performed at our institution from 2014 to 2017. The mean patient age at the time of biopsy was 59 years (range, 23-96 years). The histologic subtypes included ductal (84%), lobular (11%), mixed ductal/lobular (4%), metaplastic (1%), mucinous (<1%), low-grade adenosquamous (<1%), small cell (<1%), and invasive papillary (<1%). The majority (46%) of the tumors were grade 2, 28% were grade 3, and 26% were grade 1. The HR-positive rate was 82% Table 1.

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HER2 FISH Results

Based on the 2013 guidelines, 180/1,350 (13%) were HER2 FISH positive, 131/1,350 (10%) FISH equivocal, and 1,039/1,350 (77%) FISH negative. Based on the 2018 guidelines, 162/1,350 (12%) were in ISH group 1, 4/1,350 (0.3%) in ISH group 2, 14/1,350 (1%) in ISH group 3, 131/1,350 (10%) in ISH group 4, and 1,039/1,350 (77%) in ISH group 5. ISH groups 2 to 4 accounted for 11% of HER2 FISH results Table 2.

Overall HER2 Status

Under the 2013 guidelines, 180/1,350 (13%) were HER2 positive, 78/1,350 (6%) were HER2 equivocal, and 1,092/1,350 (81%) were HER2 negative. Under the 2018 guidelines, 174/1,350 (13%) were HER2 positive and 1,176/1,350 (87%) were HER2 negative Table 3. No cases from ISH groups 2 and 4 were HER2 IHC positive, and thus these were all categorized as overall HER2 status negative by the 2018 guidelines. The majority (86%) of ISH group 3 cases were HER2 score 2+ or 3+, and were therefore categorized as overall HER2 status positive based on the 2018 guidelines. No cases from ISH group 5 were HER2 IHC positive, and all were categorized as overall HER2 status negative under the 2018 guidelines. A total of 84/1,350 (6%) cases changed overall HER2 status, with the majority (78/1,350, 6%) due to shifts from overall HER2 status equivocal (under the 2013 guidelines) to overall HER2 status negative (under the 2018 guidelines). Of the 78 cases that changed HER2 status from HER2 equivocal to negative, all were "double equivocal" with FISH results that were equivocal (2013 guidelines)/ISH group 4 (2018 guidelines) and an HER2 IHC score of 2+. The discordance rate was 6/1,350 (0.4%), with all six cases changing from overall HER2 status positive (under the 2013 guidelines) to overall HER2 status negative (under the 2018 guidelines). Most (four) discordant cases were in ISH group 2 Image 1, Image 2, and Image 3, with two discordant cases in ISH group 3 with concomitant negative HER2 IHC Table 4 and Image 4.

Comparison of ISH Groups

ISH group and HER2 copy number correlated with histologic grade Table 5. The tumors from ISH group 1 had the highest mean grade of 2.5, significantly higher than ISH group 5 (mean grade of 1.9, $P < .00001$). The tumors from ISH group 3 and ISH group 4 had the second and third highest mean histologic grades of 2.5 ($P = .002$) and 2.3 ($P < .00001$), respectively (Table 5). HR status significantly correlated with ISH group ($P < .0001$). ISH group 1 showed a significantly lower rate of HR positivity of 61% ($P < .0001$) compared to ISH group 5, which had the highest HR-positive rate of 86% Table 6.

Discussion

To our knowledge, this is first study to analyze the potential impact on HER2 rates by the 2018 ASCO/CAP HER2 guideline focused update. In our analysis we observed that the 2018 guidelines will predominantly impact patients with ISH amplification patterns in ISH groups 2 to 4, as the new guidelines will recategorize the overall HER2 status of the majority of these patients. In our investigation we determined that ISH groups 2 to 4 accounted for 11% of ISH results, more than two times higher than the estimate anticipated in the 2018 guidelines.[2] For institutions that perform ISH first, this represents a relatively large number of cases that will require reflex HER2 IHC. More importantly, it suggests that the 2018 guidelines could recategorize the HER2 status of a higher percentage of patients than expected.

Table 1

Comparison of Numbers of Specimens of Each Histologic Subtype by Grade, Hormone Status, and HER2 IHC and FISH Result

	Grade			Hormone Status		HER2 IHC			HER2 FISH				
	1	2	3	Positive	Negative	0-1+	2+	3+	Group 1	Group 2	Group 3	Group 4	Group 5
Ductal NOS	276	507	352	908	226	793	209	133	153	4	12	123	843
Lobular	51	86	7	139	5	122	19	3	5	0	1	4	134
Mixed type	17	27	5	45	4	40	8	1	3	0	1	2	43
Metaplastic	0	1	11	1	11	10	2	0	1	0	0	2	9
Mucinous	2	5	0	7	0	6	1	0	0	0	0	0	7
LGAS	1	0	0	1	0	1	0	0	0	0	0	0	1
Small cell	0	0	1	0	1	1	0	0	0	0	0	0	1
Papillary	0	1	0	1	0	0	1	0	0	0	0	0	1
No. (%)	347 (26)	627 (46)	376 (28)	1,102 (82)	247 (18)	973 (72)	240 (18)	137 (10)	162 (12)	4 (0.3)	14 (1)	131 (10)	1,038 (77)

FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor; IHC, immunohistochemistry; LGAS, low-grade adenosquamous carcinoma; NOS, no special type.

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Table 2
Distribution of the HER2 FISH Result Utilizing the 2013 Guidelines and the 2018 Guidelines

	FISH Result				
	Group 1	Group 2	Group 3	Group 4	Group 5
HER2 status	Ratio ≥2.0, Copy No. ≥4.0	Ratio ≥2.0, Copy No. <4.0	Ratio <2.0, Copy No. ≥6.0	Ratio <2.0, Copy No. ≥4.0 and <6.0	Ratio <2.0, Copy No. <4.0
2013 guidelines	Positive	Positive	Positive	Equivocal	Negative
2018 guidelines	Positive	Additional workup	Additional workup	Additional workup	Negative
No. (%)	162 (12)	4 (0.3)	14 (1)	131 (10)	1,039 (77)

FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor.

Table 3
Comparison of the Overall HER2 Status Utilizing the 2013 Guidelines and the 2018 Guidelines

	HER2 Status		
	Negative	Equivocal	Positive
2013 guidelines, No. (%)	1,092 (81)	78 (6)	180 (13)
2018 guidelines, No. (%)	1,176 (87)	0 (0)	174 (13)
Difference	+6%	–6%	–0.4 %

HER2, human epidermal growth factor receptor.

The 2018 guidelines will have a net negative impact on HER2 status. This will occur by three principal means: (1) reduced need for repeat testing in patients in ISH group 4 (formerly classified as ISH equivocal under the 2013 guidelines); (2) reclassification of patients in ISH group 2 (high HER2/CEP17 ratio/low HER2 copy number) and a subset of ISH group 3 (low HER2/CEP17 ratio/high HER2 copy number) to HER2-negative status (formerly classified as HER2 positive under the 2013 guidelines); and (3) reduced mandatory repeat testing of patients who are initially HER2 negative and grade 3. In our series, we observed a total of 6% of cases that would change overall HER2 status, with the majority within ISH group 4 (low HER2/CEP17 ratio/borderline HER2 copy number) changing from equivocal (under the 2013 guidelines) to negative status (under the 2018 guidelines). Under the 2013 guidelines, this 6% of formally HER2 equivocal patients would have been retested for HER2 on the excision specimen, and a subset of these patients converted to HER2-positive status based on the repeat test results. We have previously reported a positive conversion rate of 6% from repeat testing on the excision specimens in patients who are initially HER2 IHC and ISH "double equivocal" on CNB under the 2013 guidelines.[4] Other published studies have reported even higher positive conversion rates from repeat HER2 testing, ranging from 23%[5] to 33%.[6] These patients will likely be missed under the 2018 guidelines, as all ISH group 4 cases in our series were classified as HER2 negative under the 2018 guidelines, with no mandatory requirement for additional repeat testing indicated.[2]

We estimate that the HER2-positive rate on CNB will decline under the 2018 guidelines by a minimum of 0.4% due to the direct reclassification of patients from HER2-positive status under the 2013 guidelines, to HER2-negative status under the 2018 guidelines. The majority of positive to negative recategorizations were in cases from ISH group 2, all of which were HER2 IHC negative or equivocal and therefore classified as HER2 final status negative under the 2018 guidelines. A small subset (15%) of patients within ISH group 3 would also be categorized to final HER2 status negative using 2018 guidelines. This 0.4% of patients would become newly ineligible for anti-HER2 therapy in the transition from the 2013 guidelines to the 2018 guidelines.

It is important to note that the 0.4% decrease in the HER2-positive rate we observed is mostly likely an underestimate, given that the softening of the 2018 guidelines requirement for mandatory retesting (eg, on grade 3 tumors, found to be HER2 negative on initial testing) will have a practical effect of further reducing the volume of repeat testing performed on excision specimens. Two studies[7,8] showed that mandatory retesting of grade 3 initially HER2-negative tumors identified 3% of cases with discordant (repeat positive) HER2 results on the excision. Several of these discordances were due to intratumoral heterogeneity for HER2 expression. While the prevalence of intratumoral heterogeneity for HER2 expression in primary breast cancer appears low, in one series it was estimated to be 6%.[9] If mandatory repeat testing is reduced under the 2018 guidelines, it is likely that fewer cases of intratumoral heterogeneity will be detected, and therefore additional patients (false-negative on CNB) who could potentially benefit from anti-HER2 targeted therapy could be missed.

While the 2018 guidelines increase the threshold to classify patients as HER2 positive and will have a net negative impact on HER2-positive rates, it is not fully understood if the revised cutoffs under the 2018 guidelines

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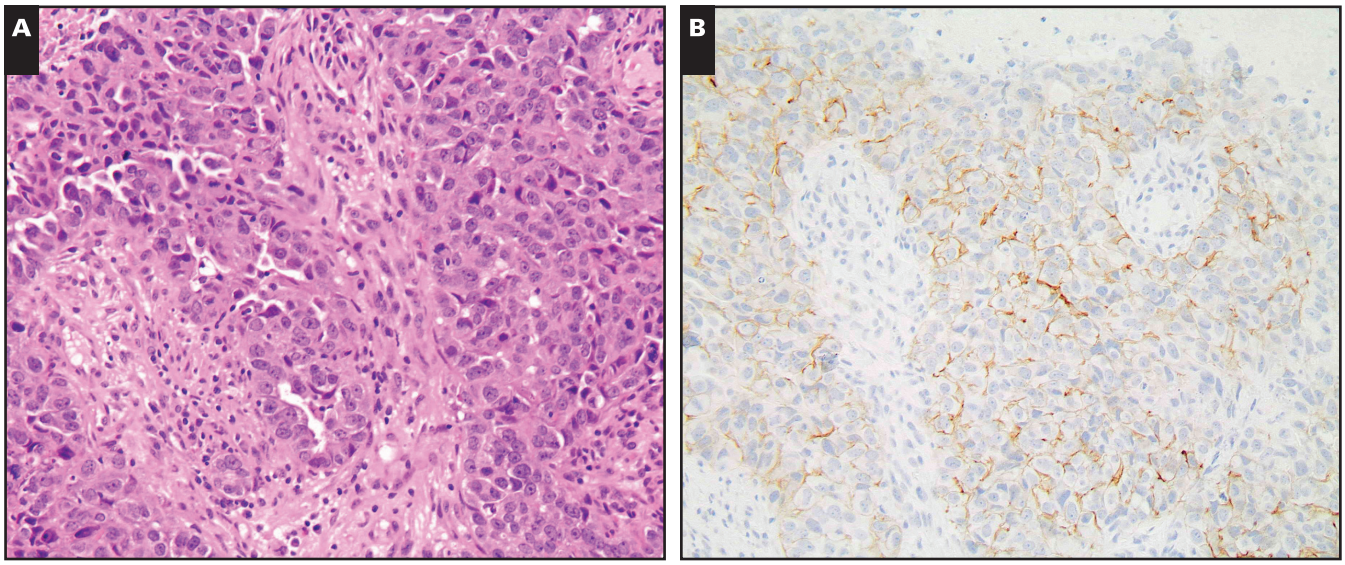

Image 1 **A**, A breast core needle biopsy from a 30-year-old woman with invasive ductal carcinoma, grade 3 (H&E, ×20). The tumor was estrogen receptor negative (0%) and progesterone receptor negative (0%). **B**, Human epidermal growth factor receptor 2 (HER2) immunohistochemistry at ×20 of the core needle biopsy was equivocal (score 2+). The HER2 fluorescence in situ hybridization (FISH) result was positive under the 2013 guidelines, with an HER2/CEP17 ratio of 2.30 and HER2 copy number of 3.60. Using the 2018 guidelines, this HER2 FISH result would fall under ISH group 2, and the tumor would be classified as HER2 negative.

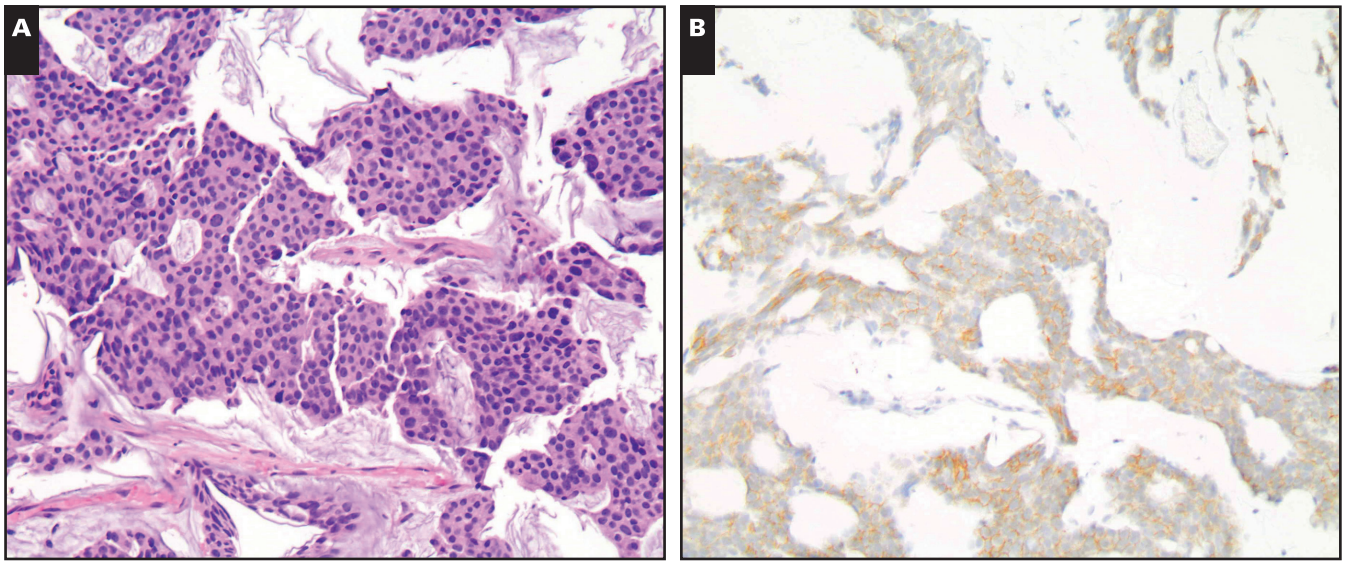

Image 2 **A**, A breast core needle biopsy from a 60-year-old woman with invasive ductal carcinoma with mucinous features, grade 2 (H&E ×20). The tumor was estrogen receptor positive (100%) and progesterone receptor positive (2%). **B**, Human epidermal growth factor receptor 2 (HER2) immunohistochemistry at ×20 of the core needle biopsy was equivocal (score 2+). The HER2 fluorescence in situ hybridization (FISH) result was positive under the 2013 guidelines with an HER2/CEP17 ratio of 2.47 and HER2 copy number of 2.95. Using the 2018 guidelines, this HER2 FISH result would fall under ISH group 2, and the tumor would be classified as HER2 negative.

are supported by clinical outcomes data. The findings from the recent NSABP-B47 trial do seem to suggest that patients with borderline HER2 overexpression who are not amplified by ISH (under the 2013 guidelines) do not benefit from HER2-targeted therapy.[10] Similarly, reanalysis of BCIRG 005 and 006[11] trials also lends clinical

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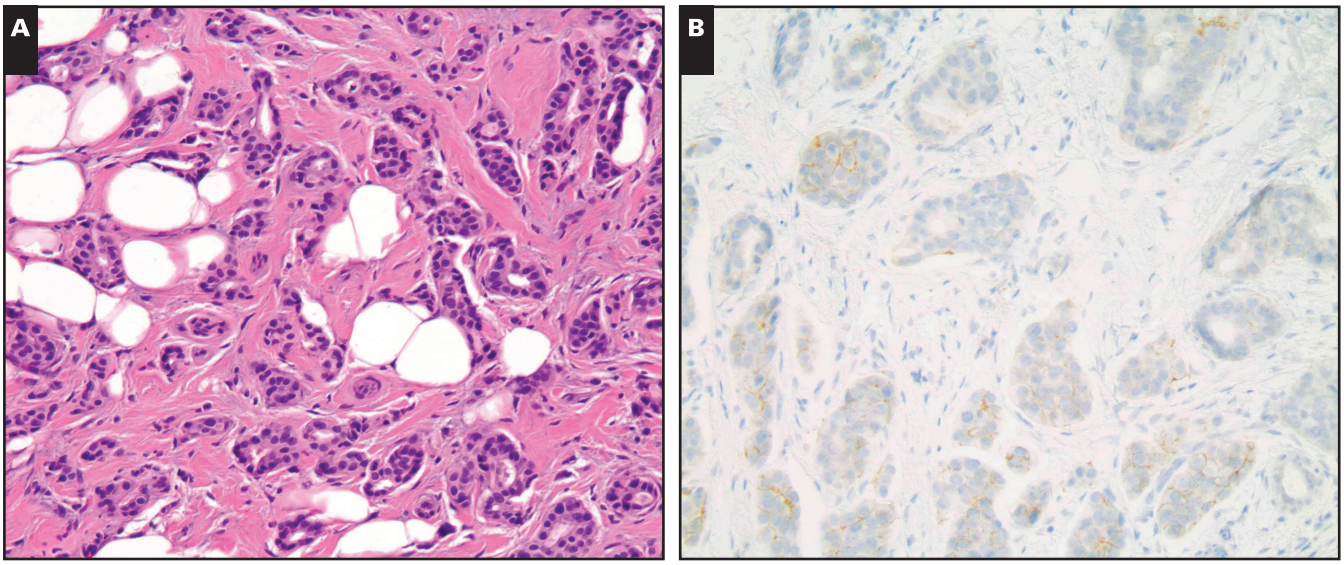

Image 3 **A**, A breast core needle biopsy from a 56-year-old woman with invasive ductal carcinoma, grade 1 (H&E ×20). The tumor was estrogen receptor positive (100%) and progesterone receptor positive (85%). **B**, Human epidermal growth factor receptor 2 (HER2) immunohistochemistry at ×20 of the core needle biopsy was negative (score 1+). The HER2 fluorescence in situ hybridization (FISH) result was positive under the 2013 guidelines with an HER2/CEP17 ratio of 2.20 and HER2 copy number of 3.80. Using the 2018 guidelines this HER2 FISH result would fall under ISH group 2, and the tumor would be classified as HER2 negative.

Table 4
Comparison of the HER2 IHC Result for Each ISH Group[a]

	FISH Result					
HER2 IHC Result	Group 1 Ratio ≥2.0, Copy No. ≥4.0	Group 2 Ratio ≥2.0, Copy No. <4.0	Group 3 Ratio <2.0, Copy No. ≥6.0	Group 4 Ratio <2.0, Copy No. ≥4.0 and <6.0	Group 5 Ratio <2.0, Copy No. <4.0	Total
0 to 1+, No. (%)	**4 (2)**	3 (75)	2 (14)	53 (40)	911 (88)	973 (72)
2+, No. (%)	**24 (15)**	1 (25)	**9 (64)**	78 (60)	128 (12)	240 (18)
3+, No. (%)	**134 (83)**	**0 (0)**	**3 (21)**	**0 (0)**	**0 (0)**	137 (10)
Total	162	4	14	131	1,039	1,350

FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor; IHC, immunohistochemistry.
[a]Bold indicates those cases categorized as positive Overall HER2 Status under the 2018 Guidelines.

evidence to support the new categorization algorithms, with the caveat that outcomes data were limited in some subsets (particularly ISH group 4). The study by Sneige et al[12] suggests that patients in ISH group 4 (HER2 equivocal under the 2013 guidelines) showed no survival disadvantage compared to HER2-negative patients. Overall, the available published data do indicate that the 2018 guidelines will prevent ineffective, costly, and potentially harmful treatment from being offered to patients who are unlikely to benefit from HER2-targeted therapy.

While the 2018 guidelines undoubtedly increase the complexity of HER2 testing interpretation for pathologists (breakup of HER2 ISH results into five different result groups, the requirement for concomitant review with IHC, and need for blinded ISH recounting of additional cells), the advantage of the revision is it will clearly simplify the management of patients. This will be achieved by the elimination of the HER2 equivocal category. In some series, the incidence of equivocal HER2 ISH results under the 2013 guidelines was as high as 10%.[4,5] This resulted in a large number of patients with unresolved HER2 status, the increased need for reflex and repeat testing, as well as the controversial use of alternative probe HER2 FISH testing.[13-15] In our series, we observed that all cases that were ISH equivocal under the 2013 guidelines (ISH group 4) became HER2 negative

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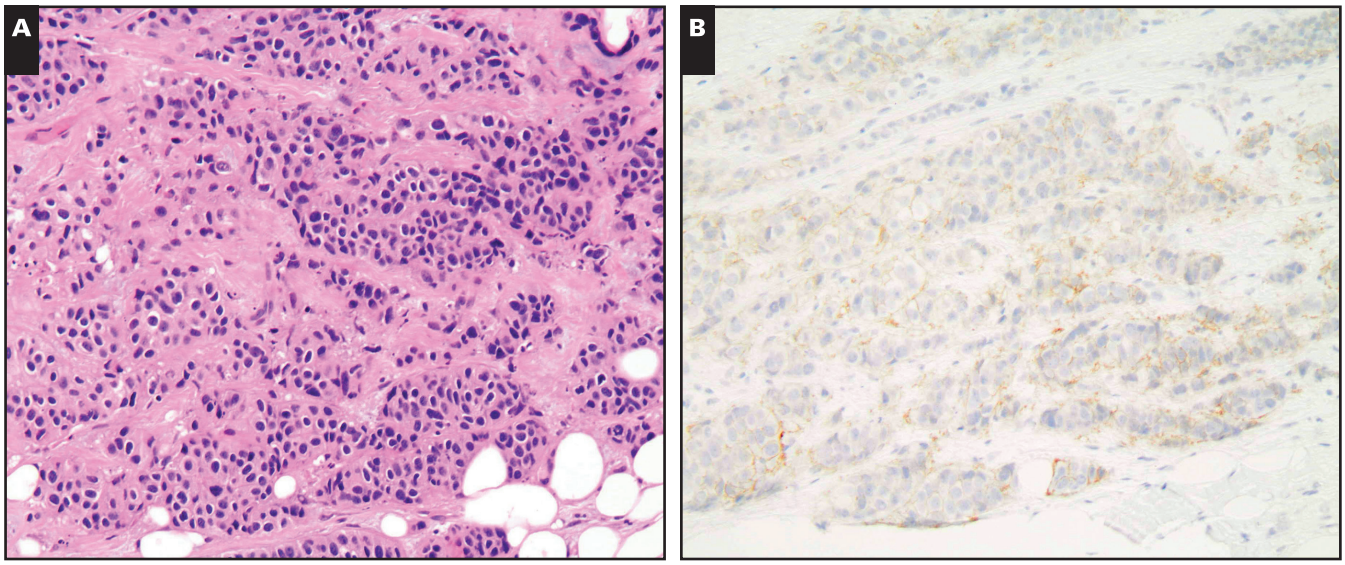

Image 4 **A**, A breast core needle biopsy from a 95-year-old woman with invasive ductal carcinoma, grade 2 (H&E ×20). The tumor was estrogen receptor positive (95%) and progesterone receptor positive (70%). **B**, Human epidermal growth factor receptor 2 (HER2) immunohistochemistry at ×20 of the core needle biopsy was negative (score 1+). The HER2 fluorescence in situ hybridization (FISH) result was positive under the 2013 guidelines with an HER2/CEP17 ratio of 1.29 and HER2 copy number of 6.40. Using the 2018 guidelines this HER2 FISH result would fall under ISH group 3, and the tumor would be classified as HER2 negative.

Table 5
Average Grade for Each In Situ Hybridization Group

	Group 1 Ratio ≥2.0, Copy No. ≥4.0	Group 2 Ratio ≥2.0, Copy No. <4.0	Group 3 Ratio <2.0, Copy No. ≥6.0	Group 4 Ratio <2.0, Copy No. ≥4.0 and <6.0	Group 5 Ratio <2.0, Copy No. <4.0
Average grade	2.5	1.7	2.5	2.3	1.9

under the 2018 guidelines. Therefore, we note that one of the greatest practical impacts of 2018 guidelines will be due to the elimination of the HER2 equivocal category, and the consequent significant reduction (6%) in the absolute volume of cases that would have required mandatory repeat HER2 testing under the 2013 guidelines. Additionally, we anticipate that the 2018 guidelines will reduce the overall volume of required HER2 testing that is needed to be performed on excision specimens, as many fewer patients will require additional testing to resolve (eg, HER2 double equivocal) or confirm HER2 status (initially HER2-negative/grade 3 tumors).

There were several strengths of our study. Our institution's standardized protocols to HER2 assessment effectively allowed for an assessment of the impact of the 2018 guidelines on HER2 rates. In our institution, routine dual testing by HER2 IHC and FISH enabled us to detect potential shifts in HER2 rates using the 2018 guidelines, and minimized potential selection bias that can occur when HER2 FISH is only performed as a reflex test. In addition, because all testing was completed in house this eliminated potential interlaboratory analytical variability. Only a single specimen type (CNB) was studied so there was minimal potential for preanalytic bias due to prolonged cold ischemia time or inadequate formalin fixation time (as is more frequently encountered in excision specimens). Our standard HER2 FISH protocol requires a minimum read of 90 cells, which is higher than the initial count of 20 cells required under both the 2013 and 2018 guidelines.[2,3] Concomitant review of the HER2 IHC and HER2 FISH is performed in all cases of intratumoral heterogeneity for HER2 expression. While we acknowledge that this approach is not identical to the 2018 guidelines requirement for separate blinded recount, the method of initial over-counting used in our study provides a reasonably accurate assessment of ISH grouping under the 2018 guidelines. Finally, our study covers a large consecutive series of CNB (n = 1,350), which provides adequate statistical power to our investigation.

In conclusion, the 2018 ASCO/CAP HER2 guideline focused update addresses cases with uncommon HER2

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Table 6
Comparison of Hormone-Positive Rate (HR+) for Each In Situ Hybridization Group

	Group 1 Ratio ≥2.0, Copy No. ≥4.0	Group 2 Ratio ≥2.0, Copy No. <4.0	Group 3 Ratio <2.0, Copy No. ≥6.0	Group 4 Ratio <2.0, Copy No. ≥4.0 and <6.0	Group 5 Ratio <2.0, Copy No. <4.0	Total
HR+, No. (%)	99 (61)	3 (75)	10 (71)	100 (77)	891 (86)	1,102 (82)
HR–, No. (%)	63 (39)	1 (25)	4 (29)	30 (23)	148 (14)	247 (18)
Total	162	4	14	130[a]	1,039	1,349

[a]Not performed in one case.

ISH amplification patterns that were difficult to classify under the 2013 guidelines, which had formerly represented a significant source of controversy and clinical uncertainty. We observed that the 2018 guidelines diagnostic approach to these borderline cases simplifies clinical decision making by recategorizing the majority of these cases as HER2 negative. However, the 2018 guidelines will also lower HER2-positive rates and impact therapeutic decision making for a subset of patients who will become newly ineligible for targeted therapy. The majority of patients (66%) previously classified as HER2 positive (2013 guidelines) and reclassified as HER2 negative (2018 guidelines) had an HER2 FISH result in ISH group 2 (HER2/CEP17 ratio > 2.0, HER2 copy number < 4.0) and a negative or equivocal HER2 IHC result, while a minority (33%) of positive (2013 guidelines) to negative (2018 guidelines) reclassifications occurred in patients with an HER2 FISH result in ISH group 3 (HER2/CEP17 ratio < 2.0, HER2 copy number > 6.0) and a negative HER2 IHC result. Continued studies are needed to confirm the clinical validity of this latest version of the ASCO/CAP HER2 guideline recommendations.

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This work was supported in part by The Ohio State University College of Medicine Roessler Research Scholarship to A.G.

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