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HER2 Testing for Breast Cancer in the Genomics Laboratory

A Sea Change for FISH

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• ***Context.***—**Guidelines for HER2 testing in breast cancer have changed over time, from the US Food and Drug Administration guideline to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines published in 2007, 2013, and 2018.**

Objective.—**To investigate the change in assignment of HER2 status in breast cancers with equivocal (2+) immunohistochemistry (IHC) results by fluorescence in situ hybridization (FISH) following implementation of the ASCO/CAP 2018 guideline.**

Design.—**The study included 3556 invasive breast cancers that were HER2 equivocal (2+) by IHC and were submitted to our FISH laboratory after July 2018. Reflex testing (with repeat IHC staining) was performed on certain categories of FISH results known as groups 2, 3, and 4. Concomitant review of IHC and FISH was performed on these reflex cases per 2018 guideline recommendations. The FISH data were analyzed to compare US Food and Drug Administration and ASCO/CAP 2007, 2013, and 2018 interpretations.**

Results.—**Of 3548 invasive breast cancers with complete data available, the percentage agreement for FISH according to different guidelines was highest for ASCO/CAP 2018 versus US Food and Drug Administration (96.5%), followed by ASCO/CAP 2018 versus 2007 (93.8%), and lowest with ASCO/CAP 2018 versus 2013 (83.7%). Per the 2018 guideline, reflex IHC testing was performed on 633 breast cancers (17.8%); the majority of reflex testing results were negative (541 of 633; 85.5%). The overall distribution of HER2 FISH results (per the 2018 guideline) was 88.5% negative and 11.5% positive.**

Conclusions.—**By eliminating the equivocal FISH category, the 2018 ASCO/CAP guideline significantly reduced the HER2 FISH–positive rate in tumors with equivocal (2+) IHC results.**

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Targeted therapy has had a very favorable impact on survival for patients with HER2-positive breast cancer, and HER2-targeted therapy regimens continue to evolve. Detection of HER2 protein overexpression on the surface of tumor cells by immunohistochemistry (IHC) and detection of *HER2* (*ERBB2*) gene amplification in tumor nuclei by in situ hybridization are the standard methods for determining HER2 status in breast cancer. Accurate evaluation of HER2 status is critical for determining patient eligibility for HER2-targeted therapy. A revised guideline[1] for HER2 testing in breast cancer was published by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) in 2018. The updated guideline has altered the interpretation of certain categories of HER2 in situ hybridization results known as groups 2, 3, and 4, essentially eliminating the equivocal category. We reviewed the changes in reporting patterns for HER2 fluorescence in situ hybridization (FISH, the most commonly used in situ hybridization method) in our large national reference laboratory in the years following our implementation of the 2018 guideline.

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MATERIALS AND METHODS

This work was reviewed and approved by our institutional review board. The study included invasive breast carcinoma samples received for HER2 FISH testing in our laboratory between July 2018 and November 2019 (N = 6640). Only samples that had been previously tested by IHC and were reported as equivocal (2+) (n = 3556) were included in the study. Samples that lacked either adequate invasive tumor content (n = 120) or adequate FISH probe hybridization (n = 48) were excluded from this study. The samples were submitted as either unstained slides or paraffin blocks and were referred to our laboratory from approximately 130 different institutions. A pathology report that included prior IHC and/or FISH testing results was requested.

Fluorescence in situ hybridization testing was performed using differentially labeled fluorescent probes targeting the *ERBB2* (*HER2*) gene locus on the long arm of chromosome 17 at 17q12 and the centromere of chromosome 17 (chromosome enumeration probe CEP17; PathVysion, Abbott Molecular, Des Plaines, Illinois)

Table 1. HER2 Result Interpretations for Dual-Probe Fluorescence In Situ Hybridization Assays

Guideline	Group 1 (HER2:CEP17 Ratio ≥2.0, HER2/Cell ≥4.0)	Group 2 (HER2:CEP17 Ratio ≥2.0, HER2/Cell <4.0)	Group 3 (HER2:CEP17 Ratio <2.0, HER2/Cell ≥6.0)	Group 4 (HER2:CEP17 Ratio <2.0, HER2/Cell ≥4.0 and <6.0)	Group 5 (HER2:CEP17 Ratio <2.0, HER2/Cell <4.0)
FDA	Positive	Positive	Negative	Negative	Negative
ASCO/CAP 2007	Equivocal (ratio 2.0–2.2) or positive (ratio >2.2)	Equivocal (ratio 2.0–2.2) or positive (ratio >2.2)	Negative (ratio <1.8) or equivocal (ratio 1.8–<2.0)	Negative (ratio <1.8) or equivocal (ratio 1.8–<2.0)	Negative (ratio <1.8) or equivocal (ratio 1.8–<2.0)
ASCO/CAP 2013	Positive	Positive	Positive	Equivocal	Negative
ASCO/CAP 2018	Positive	Negative in the absence of IHC-positive (3+) results	Positive in the absence of IHC-negative (0 or 1+) results	Negative in the absence of IHC-positive (3+) results	Negative

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; D17Z1, chromosome 17 centromere; FDA, US Food and Drug Administration; HER2/cell, average HER2 signals per scored cell nucleus; IHC, immunohistochemistry.

Table 2. Final Results by Group According to the 2018 Updated Guideline for HER2 Testing in Breast Cancer From the American Society of Clinical Oncology and the College of American Pathologists

FISH Group	Negative	Positive	No.	Percentage
1	0	316	316	8.9
2	41 (9)[a]	8 (0)[b]	49	1.4
3	15 (4)[a]	47 (4)[b]	62	1.7
4	485 (102)[a]	37 (13)[b]	522	14.7
5	2599	0	2599	73.1
Uncertain			8	0.2
Total, No.	**3140**	**408**	**3556**	
Total, %	88.5	11.5		

Abbreviation: FISH, fluorescence in situ hybridization.

[a] Numbers in parentheses refer to cases that were negative (0–1+) by reflex immunohistochemistry performed within our laboratory.

[b] Numbers in parentheses refer to cases that were positive (3+) by reflex immunohistochemistry performed within our laboratory.

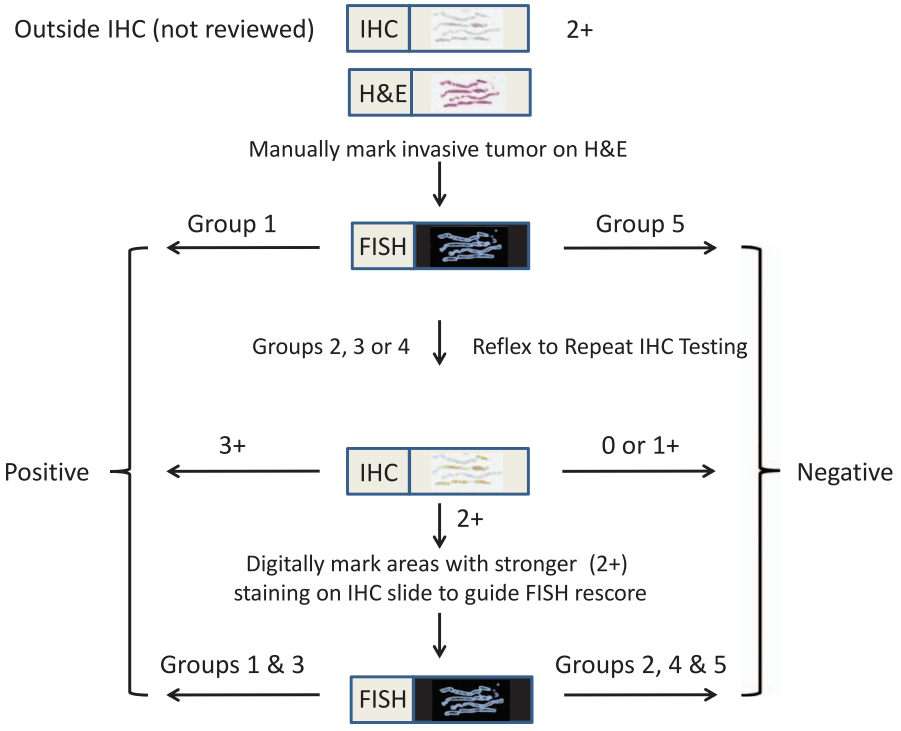
according to a validated laboratory protocol.[2] Two technologists manually scored 30 nuclei within areas of invasive tumor identified by a pathologist. Fluorescence in situ hybridization scoring was performed independently at the microscope. Raw FISH data were interpreted and reported according to ASCO/CAP 2018 but were reinterpreted according to ASCO/CAP 2013, ASCO/CAP 2007, and US Food and Drug Administration (FDA) criteria for the purposes of comparison (Table 1).[3,4]

Per the 2018 guideline, reflex IHC testing was performed on breast cancers with results in group 2, 3, or 4. HER2 IHC testing was repeated in the Mayo Clinic immunostains laboratory using an FDA-approved assay (Pathway [4B5], Ventana Inc, Tucson, Arizona), and results were interpreted with the aid of quantitative image analysis using an FDA 510(k)–cleared application. Immunohistochemistry image data and the corresponding glass slides were manually reviewed by a pathologist for the final interpretation. For those cases with equivocal (2+) results on the reflex IHC testing in our laboratory, areas of tumor with subjectively more intense membranous staining were circled on the IHC slides to guide targeted rescoring of the corresponding FISH slides. Reanalysis of FISH slides was performed independently by 2 technologists on 60 nuclei. The workflow for FISH testing under ASCO/CAP 2018 is illustrated in the Figure.

The submitted local IHC results were correlated with FISH interpretations and IHC results obtained in our laboratory according to the 4 HER2 FISH guidelines (FDA and ASCO/CAP 2007, 2013, and 2018).[1,3,4] Independent data cohorts (2013–2014 versus 2018–2019) were compared using the Pearson χ^2 statistic. The significance threshold (α) was set at 5% ($P = .05$).

RESULTS

This study encompassed 3556 invasive breast cancer samples with equivocal (2+) IHC results that were tested for *HER2* (*ERBB2*) amplification by FISH in our laboratory between July 2018 and November 2019. For FISH groups 1 and 5, results were reported as HER2 positive (group 1) or HER2 negative (group 5) without reflex IHC testing. The remainder (FISH groups 2, 3, and 4) were submitted for reflex IHC with or without FISH rescoring per 2018 guideline recommendations (Table 2). For reflexed samples, a final interpretation of either HER2 positive or HER2 negative was assigned based on combined review of IHC and FISH results. Initial FISH results were available on all 3556 samples, but FISH slides were uninterpretable on 7 after reflex testing, and 1 sample showed no hybridization for the chromosome 17 centromere control FISH probe



Workflow for fluorescence in situ hybridization (FISH) at the Mayo Clinic under the current American Society of Clinical Oncology/College of American Pathologists 2018 guideline. Abbreviations: H&E, hematoxylin-eosin–stained tissue slide; IHC, immunohistochemistry.

within the invasive tumor, precluding calculation of the *HER2*:centromere ratio; these 8 samples were excluded from the final data analysis. The overall HER2-positive rate was 11.5% and the overall HER2-negative rate was 88.5% for the 3548 samples with complete FISH data available. Of the 633 samples in FISH groups 2, 3, and 4 that underwent reflex IHC testing, 501 samples (79.1%) were also equivocal (2+) in our laboratory, concordant with the outside immunohistochemistry result (Table 2).

The available FISH data indicated that the HER2-negative rate was highest for the FDA guideline (89.7%) and lowest for the 2013 guideline (73.3%) in this study cohort. Concordance between interpretations of the raw FISH scores according to the different guidelines indicated the highest agreement between FDA and 2018 guidelines. The 2007 and 2013 guidelines categorized a subset of FISH results as equivocal (Tables 3 and 4).

Following implementation of the 2018 guideline, the HER2 FISH–positive rate among HER2-equivocal (2+) tumors declined to 11.5%, about half that reported on cases performed under the 2013 guideline (23.6%)[2] ($P < .001$). Most of our FISH-positive samples were in group 1, with an average *HER2* copy number of at least 4.0 and a positive *HER2* to control ratio of at least 2.0. Of the samples with FISH results in groups 2 through 4, most (85.5%) were negative with the combined IHC/FISH reflex testing approach recommended by the 2018 guideline.

DISCUSSION

The ASCO/CAP 2018 guideline for breast cancer has provided more specific guidance on the appropriate reflex testing for certain categories of FISH results in order to facilitate therapeutic decision-making. Most such cases would have been reported as HER2 positive under the 2013 guideline, including group 4 cases with positive reflex FISH testing and all cases in groups 2 and 3. Under the 2013 guideline, a variety of reflex FISH testing strategies were used for group 4 FISH results (previously referred to as FISH equivocal), and some group 4 cases remained equivocal even after reflex FISH testing. Under the 2018 guideline, repeat/reflex IHC testing resolves a substantial fraction of samples in groups 2 through 4, and the final HER2 status can be reported as either positive or negative after the combined IHC/FISH process.

Group 4 breast cancers are the most frequently encountered category of HER2 FISH results requiring reflex testing (groups 2–4); these breast cancers have moderately elevated *HER2* copy number (between 4 and 5.9 average *HER2* copies per nucleus), moderately elevated centromere copy number (polysomy), and a *HER2*:control ratio less than 2.0, and

Table 3. Comparison of HER2 Fluorescence In Situ Hybridization (FISH) Interpretations on 3548 Invasive Breast Cancers With Equivocal (2+) HER2 Immunohistochemistry Results Obtained at an External Laboratory

	FDA		ASCO/CAP 2007			ASCO/CAP 2013		
ASCO/CAP 2018	Negative	Positive	Negative	Positive	Equivocal	Negative	Positive	Equivocal
Negative, No.	3100	40	3048	14	78	2599	55	486
Positive, No.	84	324	71	281	56	0	371	37
Overall agreement, %	96.5		93.8			83.7		

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists. FDA, US Food and Drug Administration.

Table 4. HER2 Fluorescence In Situ Hybridization (FISH) Interpretations for 3548 Invasive Breast Cancers with Equivocal (2+) HER2 Immunohistochemistry Results Obtained at an External Laboratory

Guideline	Negative, No. (%)	Equivocal, No. (%)	Positive, No. (%)
FDA	3184 (89.7)		364 (10.3)
ASCO/CAP 2007	3119 (87.9)	134[a] (3.8)	295 (8.3)
ASCO/CAP 2013	2599 (73.3)	523[a] (14.7)	426 (12.0)
ASCO/CAP 2018	3140 (88.5)		408 (11.5)

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; FDA, US Food and Drug Administration.

[a] No reflex FISH testing data available.

typically have equivocal HER2 expression by IHC. Group 4 comprised 14.7% of the cases in our study and 82.5% of cases (in groups 2–4) submitted for IHC reflex testing; all were IHC equivocal at an external laboratory, and 78.0% were IHC equivocal within our laboratory. Two other studies published since the release of the 2018 ASCO/CAP guideline have also identified a frequent relationship between group 4 FISH results and equivocal HER2 expression by IHC. Zare et al[5] reported group 4 FISH results in 9.3% and equivocal IHC results in 27.2% of 1542 breast cancers tested for HER2 status by both methods; 56.9% of the breast cancers with group 4 FISH results were IHC equivocal. Similarly, Gordian-Arroyo et al[6] reported group 4 FISH results in 10% and equivocal IHC results in 18% of 1350 breast cancers tested by both methods; 60% of the breast cancers with group 4 FISH results were IHC equivocal. Our study included only breast cancers that were previously equivocal by IHC at an external laboratory; thus, we cannot directly compare our data with these previously published studies. However, our study suggests an important role for repeat IHC testing on cases referred from an external laboratory when FISH results are in groups 2 through 4. In our group 4 cases specifically, repeat IHC with image analysis was negative in 19.5% and positive in 2.5%, whereas all of these cases were previously interpreted as IHC equivocal at the external laboratory.

Although the HER2 FISH–positive rate has decreased significantly in the tumors with equivocal (2+) IHC results following implementation of the 2018 guideline, the overall impact of the guideline is predicted to be smaller. Based on unpublished data from our laboratory, of the breast cancers that are HER2 IHC (2+) equivocal (trending around 20%), only about 20% will yield FISH results in groups 2 through 4. Thus, if IHC is used as the initial strategy for determining HER2 status, about 3% to 5% of invasive breast cancers could be impacted by the change in FISH interpretations under the 2018 guideline.

This study did not include reflex FISH testing per 2007 or 2013 guidelines. However, we previously demonstrated[7] a major reduction in reflex positive HER2 results from 2013 to 2018 in 200 breast cancers with both IHC and FISH reflex testing methods performed. Many other laboratories have published their experiences with ASCO/CAP 2018 and have shown a reduction in the HER2 FISH–positive rate.[5,6,8–12] Our study supports these findings and provides additional data on the value of repeat IHC testing aided by digital image analysis in a high-volume reference laboratory environment.

CONCLUSIONS

For breast cancers with equivocal (2+) IHC results, the HER2 FISH–positive rate has declined significantly under the 2018 guideline. Most group 4 samples (previously called FISH equivocal under ASCO/CAP 2013) are reported as HER2 negative after the currently recommended reflex IHC/FISH testing is performed. Although groups 2, 3, and 4 accompanied by either positive reflex IHC (3+) or positive IHC-guided reflex FISH (ie, groups 1 and 3) are reassigned as HER2-positive status, the majority of reflexed samples (85.5% in our laboratory) are reported as HER2 negative, yielding an overall HER2 FISH–negative rate of 88.5% in our laboratory. The ultimate goal of HER2 testing is to select the best candidate patients who would respond to targeted anti-HER2 therapy while minimizing the risk of adverse effects of unnecessary therapy to patients who would not respond. HER2-targeted therapy has dramatically improved the outcome of patients with HER2-positive breast cancer, but such therapy also carries a risk for cardiotoxicity.[13] Whether the shift in HER2-status assignment will significantly alter the overall rates of patient responsiveness to HER2-targeted therapy remains to be determined.

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