Commentary—Redefining HER2-Equivocal Breast Cancers: Lessons Learned from Genomic Pathology

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Abstract

In the era of precision medicine, human epidermal growth factor receptor 2 (*HER2)* is the most important predictive and prognostic biomarker in breast cancer. The HER2 status of a patient's tumor can be analyzed at the protein level by immunohistochemistry (IHC) and at the chromosome level by in situ hybridization (ISH) techniques to determine the average *HER2* gene copy number. Yet, despite these 2 complementary methods for HER2 testing, there remains a subset of high-risk breast cancer patients (>20%) whose HER2 status is reported as "equivocal," an assessment that provides no useful information about how to treat the patient. Given there are 2 FDA approved HER2 assays readily available in the clinical laboratory, the currently confused state of HER2 testing in breast cancer is perplexing and raises the following questions: are IHC and dual-probe ISH giving the wrong answer 20% of the time, or alternatively, could these tests be giving the correct answers and we are misinterpreting the data? For the past decade, genomic pathologists have used chromosomal microarrays (CMAs) as a DNA-based approach for obtaining high-resolution images of *HER2* gene status on chromosome 17. These studies provide confirmation that ISH is a reliable method for determining average *HER2* gene copy number, and it is the HER2 ratio denominators (cep17 or alternative probes) that can introduce instability into the final results. However, even though CMA provides more detailed information about chromosome 17 status in breast cancer than conventional cytogenetics or FISH, the complexity of the method and interpretation make it impractical for routine use by the clinical laboratory as a HER2 testing method. Thus, IHC and fluorescence in situ hybridization will remain for the foreseeable future, the mainstay of HER2 testing in breast cancer. The current challenge is thus not to introduce a new HER2 assay into the clinical laboratory but rather to develop a strategy for reporting unequivocal, biologically accurate results using existing FDA-approved testing methods.

AJHO®. 2017;13(5):4-7

Introduction

It has been 3 decades since the human epidermal growth factor receptor 2 (*HER2*) was identified as an oncogenic driver of human breast carcinomas.[1] Now, as medical oncology enters the era of precision medicine, *HER2* is still the most important predictive and prognostic biomarker in breast cancer.[2] The HER2 status of a patient's tumor can be analyzed at the protein level by immunohistochemistry (IHC) to visualize cell-surface receptor targets for anti-HER2 directed therapy. Additionally, HER2 status can be evaluated at the chromosome level by in situ hybridization (ISH) techniques to determine the *HER2* gene copy number within the cell nucleus. Yet despite these 2 FDA-approved methods for HER2 testing and the many biotechnological advances in clinical pathology laboratory medicine, there remains a subset of high-risk patients with breast cancer (>20%) whose HER2 status is reported (often after multiple rounds of testing) as "equivocal."

Classifying a patient's tumor as HER2 equivocal gives clinicians no insight into the tumor's HER2 biology, nor does the word provide any useful information about how to treat the patient. In the Merriam-Webster dictionary, the adjective "equivocal" is defined as "subject to 2 or more interpretations and is usually used to mislead or confuse." Certainly clinicians and patients who have received breast cancer prognostic marker reports with HER2 equivocal results would agree that this term is both misleading and confusing. In my pathology consultation practice, many such cases have been referred to me, including this recent example:

Case History

A 69-year-old woman presents with a grade 2, <2 cm, node negative, strongly estrogen receptor–positive (ER+)/progesterone receptor–positive (PR+) tumor with HER2 initially reported as negative based on IHC score of 1+. The case was reflexed to FISH [fluorescence ISH] due to "histopathologic discordance" and reported as equivocal. The HER2/D17Z1 (chromosome 17 centromere) FISH ratio was 1.4 (negative) "to be interpreted with caution" due to the average copy number signals per cell of *HER2* 4.6 and D17Z1 3.2 (equivocal). The case was further reflexed for chromosome 17 "alternative probe" FISH where the results were reported as positive based on the HER2/TP53 ratio of 2.3 and HER2/SMSCR ratio of 2.0 [TP53 and SMSCR are chromosome 17 regions].

Is it any wonder that oncologists often feel misled and confused by pathology reports for HER2 status in breast cancer? The above case is just 1 example of why incalculable numbers of hours and healthcare dollars are continually spent on HER2 testing methods to "resolve" equivocal *HER2* breast cancer into clearly actionable HER2-positive or HER2-negative categories. The collective effort to create a binary, 2-tier framework around HER2 status in breast cancer is understandable given that oncology clinical practice guidelines have clearly actionable treatment directives only for unequivocally positive or negative HER2 results. High-risk tumors with a combination of low HER2 protein expression and nonamplified *HER2* gene copy number fit neither of these categories. Yet tumors with low HER2 protein expression represent a significant subset of breast cancer cases. Could these tumors be trying to announce their biological reality by consistently showing 1 to 2+ protein and <6 copy numbers after repeated rounds of testing?

Since the term "equivocal *HER2*" was introduced as part of the first College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guidelines published in 2007, the term has become synonymous with a third category of breast cancer.[3] Following implementation of updated CAP/ASCO guidelines in 2013, the number of breast cancer cases falling into the equivocal category has increased, along with the number of additional tests that must be performed to resolve equivocal results.[4, 5] Within this equivocal category, clinicians often end up with a collection of results from repeated and alternative testing methods used to attempt to resolve the equivocal HER2 status of the tumor. These test results often disagree as to whether the tumor is HER2-positive, HER2-negative, or something in between. The discordant test results may arise from IHC, ISH, alternative chromosome 17 probes, RNA multigene expression arrays, 21-gene recurrence score assays, DNA microarrays, and serum HER2 protein analysis, but only 2 of the aforementioned tests—IHC and FISH—are actually FDA approved for reporting HER2 status in breast cancer!

Given then that there are 2 excellent HER2 assays (IHC and ISH) readily available in the clinical laboratory, the currently confused state of HER2 testing in breast cancer is perplexing and raises some questions:

- Are IHC and 2-probe ISH giving wrong answers 20% of the time, consistently, requiring alternative testing methods to resolve discrepancies?
- Alternatively, could IHC and ISH be giving correct answers, but we misinterpret the data and thus miss the true HER2 biology of HER2 "equivocal" tumors?

Seeking answers to these questions, multiple genomic pathology groups have analyzed breast cancers that have been characterized by IHC and FISH using comparative genomic hybridization, also called chromosomal microarrays (CMAs).[6-10] CMAs provide a DNA-based approach to chromosome analysis with the capability of producing a high-resolution view of the *HER2* gene on chromosome 17. The chromosome "ratio plot" allows simulated visualization of the p arm, q arm, pericentromeric region, and *HER2* gene within the 17q12 amplicon. These high-resolution CMA images of *HER2* gene status on chromosome 17 in multiple types of breast cancer have revealed the following interesting findings:

- CMA studies provide confirmation that ISH is a reliable method for determining *HER2* gene copy number independent of a ratio as long as formalin-fixed paraffin-embedded tissue handling is within CAP/ASCO guidelines for formalin fixation times.
- CMAs have revealed that tumors with gains of entire copies of chromosome 17 (polysomy 17) occur in <10% of breast cancers even though the HER2/cep 17 ratio used in dual-probe FISH is intended to correct for this biological phenomenon (cep 17 is another area of chromosome 17 used as a denominator in ratios). Instead of polysomy, many tumors contain segmental gains on chromosome 17, particularly on the long arm.[11,12] A standard definition of HER2 "amplification" by genomic copy number analysis (including CMA) has not yet been established.
- CMA allows visualization of relative gains or losses of chromosome 17 regions used as the ratio denominator (cep17, *TP53*, SMSCR, *RARA*), causing the ratio to skew towards false negative or false positive.
- Although CMA provides more detailed information about chromosome 17 status in breast cancer than do conventional cytogenetics or FISH, the complexity of the method and interpretation make it impractical for routine use by the clinical laboratory. Thus IHC and FISH will remain, for the foreseeable future, the mainstay of testing for HER2 status in breast cancer.

The above observations from genomic pathology help explain many of the primary problems with current HER2 testing, and they suggest strategies that could potentially improve results reporting.

1. Is it time to move away from dual-probe testing and the HER2/cep17 ratio to a single-probe approach? Beginning with the first Southern blots used to identify *HER2* gene amplification in breast carcinomas, *HER2* gene testing has historically been reported as a ratio. In the initial studies, *HER2* gene DNA was compared with DNA of other genes such as *ARG1* as a nonamplified internal control.[1] In the era of FISH, a ratio of HER2 gene copy number per nucleus to chromosome 17 centromere copy number per nucleus is used as an internal control to "correct for" polysomy 17. However, from CMA studies we know that single-probe ISH is giving the correct answer, and it is the ratio

that can introduce instability by skewing the result toward a false positive or a false negative. This ratio skewing is a result of the segmental gains or losses within chromosome 17 that are more common than polysomy 17 in breast cancer.[7] In addition, the process of interchanging alternative denominator probes does not alter the gene copy number of the numerator. According to current CAP/ASCO guidelines, a tumor with 4 to 6 copies of the *HER2* gene will be called "HER2 equivocal" provided the denominator generates a ratio less than 2.0. Although reporting average *HER2* gene copy number would thus seem to be the most straightforward approach, substantial supporting data do not yet exist for making such a change. Therefore, pathologists will need to continue to critically evaluate FISH results based on the numerator, denominator, IHC findings, and the patient's clinical presentation.

2. How can we create an unequivocal reporting system? The current strategy of trying to fit all breast cancers into 2 HER2 categories for protein expression and *HER2* gene copy number may not be representative of the true biological spectrum of HER2 results. A 3-tier system including a borderline amplified group was described by Ross and colleagues in 1998 using data from multivariate analysis of a subset (n = 220) of node-negative breast cancers derived from 324 cases reported by Press and colleagues in 1997.[13-15]

A more recent retrospective study by Press et al re-interpreted enrollment and outcomes data from the Breast Cancer Research Group clinical trials using 2013 CAP/ASCO guidelines for FISH. Findings from more than 10,000 patients enrolled in the clinical trials support the original FDA-approved criteria (in which there is no equivocal category) to be strongly predictive of treatment response.[16] In this authors' opinion, and based on these previous studies, a 3-tier system for HER2 reporting, one that recognized 3 categories of HER2 biology, could be considered an unequivocal reporting strategy: tumors showing high-level gene amplification with high (3+) protein expression would be HER2-positive, tumors with borderline gene amplification (<6 copies) and low-level protein expression would be HER2-low; and tumors with no gene amplification and no protein expression would be HER2-negative. Response to Herceptin in the HER2-low category of tumors is currently being studied in the National Surgical Adjuvant Breast and Bowel Project (NSABP)-B47 trial. This randomized phase III trial is comparing chemotherapy alone with chemotherapy plus trastuzumab in more than 3000 women with node-positive or high-risk node-negative HER2-low invasive breast cancer.[17]

The specific aim is to determine whether the addition of trastuzumab improves invasive disease–free survival in women with high-risk breast cancer reported as HER2-low by IHC and FISH. Eligibility for the trial is determined by an IHC score of 1 to 2+ and by a HER2-to-chromosome enumeration probe ratio of <2.0, which, together, document the presence of HER2 target receptors on the tumor cell surface and lack of *HER2* gene amplification in the cell nucleus.

3. Are there currently any treatment recommendations for the HER2-low category of breast cancer? The NSABP-B47 trial began in January 2011, and its estimated primary completion date is in 2017. Although there are no current treatment recommendations for HER2-low tumors, identifying this subtype in high-risk patients will give clinicians insight into the HER2 biology of their patients' tumors and provide unequivocal categorization of the HER2 status.

In summary, it may be time to replace ratio reporting with single-probe ISH, and to categorize breast tumors with average *HER2* gene copy number <6 and 1-2+ protein expression as HER2-low. Recognizing this distinct genomic subtype on pathology reports will give a clinician critical information about a patient's HER2 biology, while saving time and healthcare dollars that are currently being spent trying to transform HER2-low tumors into those that can be definitively called HER2-positive or HER2-negative. We must await the results of the NSABP-B47 trial for guidance as to how to best treat this subset of high-risk patients, but to recognize this genomic subtype now would at least identify the HER2-low tumors and "give them their seat at the table," as one of my pathology colleagues has eloquently stated.

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Disclosures: Dr Gunn discloses that she has been a speaker for Roche/Genentech.

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