Trastuzumab is a humanized monoclonal antibody that is approved for the treatment of breast and gastric cancers that overexpress human growth factor receptor 2 (HER2). Since its approval, controversy has existed over whether immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) is the best method for assessing HER2 status. Proponents of IHC point out that trastuzumab targets the HER2 protein molecule, which is assessed directly by IHC, and that the original "clinical trial assay" [and the basis of US Food and Drug Administration (FDA) clearance for the drug] was IHC. Proponents of FISH argue that IHC is more prone to technical issues (fixation, subjective interpretation, lack of proper controls on the actual procedure) and that evaluation by FISH is more objective. Although it is clear that most cases of invasive breast cancer show a direct correlation between amplification of the ERBB2 gene [v-erb-b2 erythroblast leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian); also known as HER2] and overexpression of HER2 protein, IHC and FISH are complementary tests and examine different aspects of the biology of HER2-driven cancer.

The accuracy of HER2 testing has generated substantial interest, especially given the poor concordance of HER2 testing results in clinical trials (1, 2). For example, 2 of the adjuvant clinical trials for HER2-positive breast cancer [NCCTG (North Central Cancer Treatment Group) N9831 and NSABP (National Surgical Adjuvant Breast and Bowel Project) B31] enrolled patients on the basis of a positive HER2 test result from a local laboratory, but retesting by the central laboratory revealed a negative result by both IHC and FISH in approximately 20% of the patients. These patients were still enrolled in the adjuvant trials and surprisingly demonstrated hazard ratios similar to those of patients tested as positive by IHC and FISH in the central laboratory. These findings imply that any positive test result may be predictive of benefit from trastuzumab therapy in the adjuvant setting.

Contrary to widespread belief, discordance in FISH interpretations may be as common as for IHC. A recent report from the NCCTG N9831 trial (3) described a study in which 3 expert pathologists performed independent reads of 381 IHC samples and 373 FISH samples and obtained the same discordance rates (8%) for both IHC and FISH, even after technical standardization. These results highlight the inherent limitations of both techniques. The similar discordances between FISH and IHC are not surprising, because FISH is also subject to such preanalytical variables as cold ischemia time, type of fixative, and length of fixation, as well as analytical variables. Samples fixed in Prefer, HistoChoice, UNIFIX, and GTF, as well as delay in fixation, were associated with absent or technically compromised FISH staining (4, 5). Furthermore, FISH analysis is only as good as the cells that are analyzed, and identifying the invasive tumor cells can be challenging, given the presence of confounding elements (e.g., ductal carcinoma in situ, sclerosing adenosis), tumor heterogeneity, and the fact that FISH is performed in a dark room under ultraviolet light. Finally, even the objectivity of FISH has been questioned. Abnormalities of chromosome 17 are frequent in breast cancer, and the measurement of ERBB2 gene status depends on the criteria used, a ratio of the ERBB2 copy number to the chromosome 17 centromere enumerating probe number (i.e., the HER2/CEP17 ratio) ≥2.0, a ratio ≥2.2, or a mean ERBB2 copy number ≥6.0; however, some tumors that show ERBB2 gene amplification according to an ERBB2 gene copy number >6 do not exhibit amplification according to the ERBB2/CEP17 ratio (6). The rationale for assessing amplification status as a ratio to the centromeric region was to compensate for potential polysomy; however, detailed

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Received April 15, 2011; accepted April 26, 2011.

Previously published online at DOI: 10.1373/clinchem.2010.160853

Nonstandard abbreviations: HER2, human growth factor receptor 2; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; FDA, US Food and Drug Administration; NCCTG, North Central Cancer Treatment Group; NSABP, National Surgical Adjuvant Breast and Bowel Project; CEP17, chromosome 17 centromere enumerating probe.
chromosomal analysis by comparative genomic hybridization and single-nucleotide polymorphism arrays have demonstrated that chromosome 17 polymosity is a rare event in breast cancer (7, 8).

There is no doubt that HER2 assessment by IHC is subject to a number of pitfalls. Among the most important is the subjective nature of the interpretation of HER2 staining intensity. The FDA-cleared IHC assays for HER2 that were developed show a clear relationship between HER2 staining intensity and distribution and the number of HER2 molecules on the surface of a tumor cell. The proper interpretation of a HER2 IHC assay mandates that the pathologist (a) review appropriate cell line controls to be confident that the formulation of the HER2 kit is acceptable and (b) has knowledge that the tissue was obtained and processed in a manner that was validated to maintain the relationship between the staining intensity and number of HER2 molecules. The criteria for interpreting HER2 staining intensity relies on the pathologist’s subjective visual assessment, and it is clear that this subjectivity is a substantial source of discrepancy (9, 10). Although the human eye is excellent at distinguishing morphologic characteristics, it is not adept at distinguishing shades of color; however, this drawback of IHC has been well addressed. The use of a validated image-analysis system has been shown to substantially reduce interpathologist discordance in interpreting IHC assay results (9).

The considerations for evaluating HER2 in gastric cancer are similar to those in breast cancer, and both IHC and FISH can be used to determine a patient’s eligibility for trastuzumab therapy. Heterogeneity in HER2 expression is common in gastric cancer and is substantially higher in areas of tumor showing intestinal differentiation than in areas showing a diffuse histology. In fact, the presence of heterogeneity suggests an advantage of IHC over FISH in HER2 evaluation of gastric cancer, because IHC is an easier screening method and is more likely to detect small focal areas of HER2 overexpression.

Little information exists on heterogeneity in breast cancer, but one study demonstrated that of 30 tumors showing ERBB2 gene amplification, 13% revealed a nonamplified pattern in other parts of the tumor (11). This finding in turn indicates that proper selection of the material to be analyzed may also be important. In fact, 2 separate blocks were tested by both FISH and IHC in 123 cases from the N9831 trial (3). Three pathologists agreed across both blocks in 90% of the IHC assessments and in 95% of the FISH assessments. This variation in test results would prompt a change of therapy in 5%–10% of cases on the basis of the block submitted for testing. Currently, there are no standard guidelines describing how to select a tumor block that optimizes the detection of HER2 overexpression. Selecting the block with the most poorly differentiated area of tumor seems reasonable, however, because HER2 overexpression is strongly correlated with a high tumor grade.

The good news is that both IHC and FISH are excellent methods for assessing HER2 overexpression and predicting the response to trastuzumab therapy. These methods are complementary, and each is affected by preanalytical and analytical variables to varying degrees. It is therefore not surprising that some discordance is noted when tumors are analyzed by both methods. The trastuzumab package insert even warns that limitations in assay precision make it inadvisable to rely on a single detection method for assessing HER2 status. Simply put, a negative or equivocal result on one test does not completely rule out ERBB2 gene amplification or HER2 protein overexpression. It is becoming increasingly clear that there are many instances in which both IHC and FISH testing should be applied. The responses to trastuzumab are similar no matter what assay is used, and even patients who show HER2 overexpression by IHC but are negative by FISH may benefit from trastuzumab therapy. It is currently unclear which cases will most benefit from additional testing, but current guidelines recommend FISH testing if the IHC result is 2+. Other indicators may help guide the pathologist to retest. For example, features such as a large tumor size, high-grade lesions, a young age, and progesterone receptor–negative tumors are most likely to demonstrate HER2 overexpression. In these cases, a negative HER2 result by one method could reasonably lead to retesting by another method and/or retesting of a different block.

When it comes to breast cancer, the job of the pathologist has evolved beyond the evaluation of tumor size, tumor grade, and lymph node status. Pathologists now must help determine the most suitable therapies for these patients. Determining eligibility for trastuzumab therapy is more complicated than simply selecting the testing method and reporting a result. Because trastuzumab provides an enormous clinical benefit to appropriately targeted patients, it is imperative that the pathologist not overlook HER2 overexpression, even if it is present only focally. Selecting another block for testing, testing by a second method, and even retesting a previously negative tumor may be reasonable, depending on the clinical circumstances. It is time to put emotions over HER2 testing aside and do what is best for patients by providing the greatest chance to identify the patients who may benefit from this powerful treatment.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design,
acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: K.J. Bloom, Clarient.
Consultant or Advisory Role: K.J. Bloom, Genentech; R.J. Cote, Clarient.
Stock Ownership: None declared.
Honoraria: K.J. Bloom, Genentech and Dako.
Research Funding: None declared.
Other Remuneration: R.J. Cote, Genentech and Clarient.

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Clinical Chemistry 57:7 (2011) 985