Genetic Heterogeneity in HER2 Testing in Breast Cancer

Panel Summary and Guidelines

Gail H. Vance, MD; Todd S. Barry, MD, PhD; Kenneth J. Bloom, MD; Patrick L. Fitzgibbons, MD; David G. Hicks, MD; Robert B. Jenkins, MD, PhD; Diane L. Persons, MD; Raymond R. Tubbs, DO; M. Elizabeth H. Hammond, MD

Context.—Intratumoral heterogeneity of HER2 gene amplification has been well documented and represents subclonal diversity within the tumor. The reported incidence of intratumor HER2 amplification genetic heterogeneity ranges in the literature from approximately 5% to 30%. The presence of HER2 genetic heterogeneity may increase subjectivity in HER2 interpretation by the pathologist.

Objectives.—To define HER2 genetic heterogeneity and to provide practice guidelines for examining and reporting breast tumors with genetic heterogeneity for improvement of HER2 testing in breast cancer.

Design.—We convened an expert panel to discuss HER2 gene amplification testing by fluorescence in situ hybridization. Components addressed included a definition of HER2 amplification heterogeneity, practice guidelines for examination of the tissue, and reporting criteria for this analysis.

Results.—Genetic heterogeneity for amplification of HER2 gene status in invasive breast cancer is defined and guidelines established for assessing and reporting HER2 results in these cases. These guidelines are additive to and expand those published in 2007 by the American Society of Clinical Oncology and the College of American Pathologists.

Conclusion.—Standardized methods for analysis will improve the accuracy and consistency of interpretation of HER2 gene amplification status in breast cancer. (Arch Pathol Lab Med. 2009;133:611–612)

In 2007, the American Society of Clinical Oncology and the College of American Pathologists (CAP) published guidelines for HER2 testing in breast cancer to improve accuracy and reproducibility of the testing in the United States. At that time, it was clear that a subpopulation of breast cancers examined for HER2 gene amplification by fluorescence in situ hybridization (FISH) displayed intratumoral heterogeneity. It was also clear that such cases could give rise to discrepant results between immunohistochemistry and FISH assays for HER2. The CAP agreed to convene an expert panel to address this issue and publish separate recommendations. The CAP panel consisted of pathologists with special expertise in breast cancer, cytogenetics, clinical pathology, molecular pathology, image analysis, and immunohistochemistry. Members of the panel included individuals from large laboratories with extensive experience in HER2 FISH testing for breast cancer. These guidelines were created in 2008 during a group meeting held via conference call and vetted through the CAP/American College of Medical Genetics Cytogenetics Resource Committee. The final recommendations were reviewed and approved by the entire group.

DEFINITION

HER2 genetic heterogeneity (GH) exists if there are more than 5% but less than 50% of infiltrating tumor cells with a ratio higher than 2.2. For example, if 20 cells are counted and at least one cell is identified with a HER2/CEP17 signal ratio higher than 2.2, this specimen contains GH. Likewise, if 60 cells are examined and 3 or more cells have a ratio higher than 2.2, HER2 GH is present. This definition is based on published work that does not include outcome analysis of patients but appears to be reasonable to all committee members. If more than 50% of the infiltrating tumor cells have a ratio higher than 2.2, then the tumor is considered HER2 amplified.

For FISH methodologies using a probe for HER2 only without a control probe, HER2 GH exists if there are >5% but <50% of infiltrating tumor cells with >6 HER2 signals per cell. In such cases, practice guidelines and reporting criteria should be used that are comparable to those described in these guidelines for assays using HER2 ratios.

METHOD OF EXAMINATION TO DETERMINE WHETHER GH IS PRESENT

A hematoxylin-eosin–stained slide must first be examined by a pathologist to define regions of invasive tumor.
COMMENT IN SUCH CASES OF HER2 GH MUST INCLUDE THE FOLLOWING

- The percent of invasive tumor demonstrating HER2 amplification
- Whether amplified cells are present as scattered cells or in a specific cluster
- If a specific cluster of amplified cells is present, the ratio and number of CEP17 signals per cell or area and HER2 signals per cell or area should be provided, and comment included on whether the area is histologically distinctive

If the examination was performed on a needle core, the report should indicate that the evaluation may not be representative of the entire tumor and it might be desirable to perform FISH testing on a resection sample.

SUMMARY

This guideline provides specific guidance about the examination and reporting of those tumors with HER2 gene amplification status. It represents an extension of the 2007 American Society of Clinical Oncology/CAP HER2 guidelines. The American Society of Clinical Oncology/CAP guidelines are considered an “living document” that will be periodically reviewed and revised based on emerging scientific information and experience.

At present, the clinical significance of GH in terms of the potential benefit from trastuzumab therapy is unclear. However, an unambiguous, clear definition of HER2 genetic heterogeneity is the first step toward the development of clinical studies to help answer the important question as to the clinical significance of this finding and the suitability of these patients for trastuzumab treatment.

REFERENCES
