Researchers Find Discordance Between Standard Human Epidermal Growth Factor Receptor 2 (HER2) Testing and HER2 Status Reported on Oncotype DX

Researchers at the University of Pittsburgh conducted an assessment of concordance between human epidermal growth factor receptor 2 (HER2) status found by the Oncotype DX test and HER2 status as determined by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) (J Clin Oncol. 2011;29:4279-4285). Their data suggest that the Oncotype DX test may be falsely negative for HER2 status in a substantial number of breast cancer cases found to be positive by a guideline-specified combination of IHC and FISH assays.

“Clinicians and laboratory directors have to be vigilant for discordance between Oncotype DX results and routinely assessed histologic parameters and IHC markers,” says corresponding author Rohit Bhargava, MD, codirector of surgical pathology at Magee Women’s Hospital (MWH) in Pittsburgh, Pennsylvania. “Any significantly discordant results should be investigated as it may directly affect patient care.”

The Oncotype DX test (Genomic Health, Redwood City, Calif) is increasingly being used to help in decisions regarding the use of chemotherapy in the adjuvant treatment of patients with estrogen receptor-positive breast cancer. The recurrence score, as reported in the Oncotype DX test, represents the 10-year risk of recurrence in patients with lymph node-negative, estrogen receptor-positive breast cancer who have been treated with 5 years of tamoxifen. Levels of expression of 16 genes, measured by reverse transcriptase-polymerase chain reaction (RT-PCR), are used to determine the Recurrence Score. Along with an overall recurrence score, scores for expression of 3 of the 16 genes, HER2, estrogen receptor, and progesterone receptor, are listed separately in the Oncotype DX report.

According to the study authors, this is the first independent study of concordance between the HER2 status reported on the Oncotype DX RT-PCR assay and IHC/FISH testing in a high-volume laboratory.

Study Findings

The database was comprised of all the cases sent for Oncotype DX testing from July 2008 through June 2010 at MWH. Two other laboratories also participated: the Cleveland Clinic in Cleveland, Ohio, and Riverside Methodist Hospital in Columbus, Ohio. The focus of the study was to compare the HER2 results found using combined IHC and FISH with the HER2 results included in the Oncotype DX report.

After excluding the equivocal results from all tests, the percent positive and percent negative agreements were calculated including the cases from all 3 institutions. The overall percent agreement was 98%; however, the percent positive agreement was only 42% (10 of 24), while the percent negative agreement was 100% (779 of 779).

Considering only the 507 cases studied at MWH, 468 (92%) were negative, 26 (5%) were positive, and 13 (3%) were equivocal by IHC/FISH. Of the 468 negative cases, 464 (99%) were also negative by RT-PCR on the Oncotype DX test, with the remaining 4 cases reported as equivocal. All 13 cases found to be equivocal on IHC/FISH were negative by RT-PCR; of the 26 IHC/FISH-positive cases,
8 (31%) were reported as positive on the Oncotype DX report. Excluding equivocal cases, the percent positive agreement was 47% and the percent negative agreement was 100%.

At MWH, initial IHC and FISH assays for HER2 were performed on core needle biopsy samples while a tumor block from the resection specimen was sent for Oncotype DX testing. Therefore, to exclude tumor heterogeneity as a possible cause for disagreement, IHC and FISH were repeated on the same tumor block that was sent for Oncotype DX testing in the cases that were found to be positive at MWH but equivocal or negative by Oncotype DX. All 9 positive patients who were found to be equivocal on Oncotype DX but positive by IHC/FISH remained positive on repeat IHC/FISH. Of the 9 cases found negative on Oncotype DX but positive by IHC/FISH initially, 5 were still positive on repeat IHC/FISH, with the other 4 being equivocal.

Similar results were found in the 236 cases from the Cleveland Clinic, where a 25% positive agreement and a 100% negative agreement were reported, and in the 100 cases from Riverside Methodist Hospital, where a 33% positive agreement and 100% negative agreement were reported.

**Clinical Implications**

A previous study showed a percent positive agreement of 98% between FISH- and Oncotype DX-determined HER2 status (J Clin Oncol. 2010;28: 4300-4306). The authors of the current report found the high discordance rate in the current study surprising and were not sure of the explanation. They hypothesized that the specimens of tumor for testing may have included nonneoplastic tissue, thus potentially diluting the amount of HER2 mRNA and producing a false-negative result. They reviewed the tissue blocks sent to Genomic Health and almost all contained no more than 50% invasive tumor, thus making this explanation plausible. However, microdissection of specimens by Genomic Health should be able to mitigate this issue by removing most of the extraneous tissue.

“Although one can attribute this discordance to many issues such as RT-PCR process, primer design, or probes, I believe dilution of tumor mRNA with nontumor RNA to be the most likely cause,” Dr. Bhargava says.

Daniel Hayes, MD, professor of medicine and clinical director of the Breast Oncology Program at the University of Michigan in Ann Arbor, Michigan, said the study was done by superb pathologists but the group of patients appears to be highly selective, as evidenced by only a 4% rate of HER2 positivity, potentially affecting the findings. “This may be due to the fact that only ER-positive patients have Oncotype DX done so all of the ER-negative, HER2-positive patients were automatically excluded. Further, most clinicians do not send an ER-positive, HER2-positive tumor for Oncotype, excluding most of this subset as well,” he says.

Steven Shak, MD, chief medical officer at Genomic Health, says the company did perform manual microdissection on all the cases that were found to be HER2-negative by Oncotype DX and positive by IHC or FISH in the current study to ensure tumor tissue dilution did not affect the outcome. He says he would welcome independent testing of the cases in question to help clarify the issue.

In the current guidelines from the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), the recommendation for determining HER2 status may start with IHC (J Clin Oncol. 2006;25:118-145). A result of 0 or 1+ is considered negative and a result of 3+ is considered positive. For cases that are 2+, further testing with FISH is recommended. A second option noted in the guidelines is to test all samples initially by FISH.

No guidelines state that HER2 status as reported by RT-PCR should be used to determine HER2 positivity, nor does Genomic Health state or promote the use of their results to determine if a patient should receive anti-HER2 therapy. It is feasible, however, that clinicians may consider the HER2 results from the Oncotype DX in their clinical decisions regarding anti-HER2 therapy. The findings of this study support the ASCO/CAP recommendation that clinicians should use IHC/FISH or FISH alone to determine HER2 status.

“Our study on HER2 discordance highlights the fact that one test cannot provide all the answers [regarding optimal treatment for women with breast cancer]. The oncology community needs to continue using the validated HER2 assays in clinical treatment decisions and reexamine their overreliance on the Oncotype DX test,” Dr. Bhargava says.

Dr. Hayes agrees, noting that the Oncotype DX HER2 assay was not developed, validated, or approved as a predictive test to determine which patients should receive anti-HER2 therapies.