

# How Do You Tell Whether a Breast Cancer is HER2 Positive? Ongoing Studies Keep Debate in High Gear

By Charlie Schmidt

More than a decade after the drug trastuzumab (Herceptin) won approval in 1998, scientists still disagree over how to identify breast cancer patients for treatment. Trastuzumab targets human HER2, which contributes to poor outcomes in breast cancer when amplified. HER2-positive cases account for up to 20% of all newly diagnosed breast cancers, and there is no argument about trastuzumab's efficacy in these cases.

The debate is over the assays used to determine HER2 positivity: Which is more accurate? Even more controversial, how should their results be interpreted? Breast cancer strikes more than 200,000 women in the United States every year, so even small discordance rates between HER2 assays can mean thousands of potentially inaccurate findings. That's worrisome not just because women misclassified as false negative are denied trastuzumab but also because false-positive results needlessly saddle HER2-negative patients with the drug's \$100,000 annual price tag—not to mention its side effects, which can include heart failure.

Michael Press, M.D., a pathologist at the University of California, Los Angeles, said these issues highlight a central challenge in HER2 testing. “We have to move from really good testing to perfect testing,” he said. “And that's where people are in conflict; everyone's looking for the best way to do that.”

HER2 misclassification is a long-standing problem. In 2002, Edith Perez, M.D., from the Mayo Clinic in Jacksonville, Fla., and colleagues reported that only 74% of 119 HER2-positive tumor specimens identified by local laboratories could be confirmed by centralized laboratories that process samples

for clinical trials (*see* J. Natl. Cancer Inst. 2004;94:855–857). Whether the discordance between local and centralized laboratories has improved since remains open to debate.

Meanwhile, discordance between the two tests that account for almost all HER2 assessments has also raised concerns. The most common test, accounting for 80% of HER2 assessments in the United States, is the HercepTest, manufactured by Dako, in Glostrup, Denmark. Approved concurrently with trastuzumab, the HercepTest is based on immunohistochemical (IHC) methods—clinicians using it evaluate staining reactions between HER2 proteins and an antibody on slides of breast tissue. A positive finding is contingent on staining intensity, which ranges from 0 (negative) to 1+ (weakly positive) to 2+ (moderately positive) and finally to 3+ (strongly positive).

The second test relies on fluorescence in situ hybridization (FISH). Unlike IHC, which checks for overexpressed HER2 protein, FISH checks for excessive HER2 DNA. Normal cells each have two copies of the HER2 gene on chromosome 17—one inherited from the mother and one from the father. In HER2-positive cancer cells, the gene is amplified—each cell has more

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than two copies. To assess HER2 status, those running the test look for binding reactions (or signals) between the genes and a red fluorescent tag. Elevated HER2—more than two binding signals—suggests positivity, but cancer cells can also have aneuploid chromosomes, meaning that all their DNA, including their HER2 genes, are amplified. The test controls for this confounding variable with a green fluorescent tag that binds to a different region, called a centromere, on chromosome 17.

HER2-positive cells will always have more HER2 signals than they do centromere signals.

## Rift With ASCO and CAP

The interpretation for both tests is now hotly debated. Their approval by the U.S. Food and Drug Administration was based on clinical data showing that the drug is effective among women with either IHC 3+ findings on at least 10% of breast tissue specimens or FISH ratios of at least 2.0 (twice as much HER2 DNA as centromere DNA). Test kit package inserts from Dako and other manufacturers now advise pathologists to classify cells as HER2 positive according to these thresholds.

But in 2007, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) called for more stringent thresholds. Aiming to minimize false-positive results, ASCO and CAP together released guidelines stating that HER2-positive status should be contingent on IHC 3+ staining intensity over at least 30% of breast tissue specimens or on FISH ratios of more than 2.2.

Elizabeth Hammond, M.D., a professor at the University of Utah School of Medicine who led the CAP team that devised the guidelines, said the changes were necessary to address variability around the 2.0 threshold. Interpreted by different analysts, the same sample might yield a FISH ratio of 1.9, which is negative, or 2.1, which is clearly positive, she explained. But a higher threshold of 2.2 accounts for that variability. Results between 2.0 and 2.2 are considered equivocal and still warrant trastuzumab treatment, Hammond said. “The change was made merely to acknowledge



Michael Press, M.D.

statistical variability near the clinical threshold of 2.0.”

Perez, who was on the ASCO–CAP guideline committee, said she’s contacted several times a week by pathologists who don’t understand how to interpret the newer threshold. The problem, she said, is that the FISH manufacturer’s package inserts and the guidelines both still state that a FISH ratio of 2.0 warrants trastuzumab treatment. But clinicians are aware of the ASCO–CAP 2.2 cutoff and do not realize scores between 2.0 and 2.2 also fall in the trastuzumab treatment range.

According to the 2007 guidelines, equivocal findings on both FISH and IHC justify trastuzumab treatment. But Press said that clinicians who aren’t aware of this technicality might base treatment decisions on the higher ASCO–CAP cutoffs instead of what the package inserts call for. And that’s a concern because up to 3,000 women fall between FISH ratios of 2.0 and 2.2. The newer IHC cutoff isn’t as problematic, he added, because 3+ findings, defined as 3+ staining on between 10% and 30% of a given sample, will almost invariably cover at least 30% of a breast tissue slide.

Press, Perez, and other experts interviewed for this article agreed that the new thresholds can be challenging to interpret and that pathologists should base treatment decisions on earlier thresholds—particularly FISH ratios of 2.0 or greater—linked to trastuzumab benefits in clinical data. “If the FISH is done properly, a ratio of greater than 2.0 is, by definition, amplification,” said John Glaspy, M.D., an oncologist at the University of California, Los Angeles, Jonsson Comprehensive Cancer Center. Asked about reimbursement worries when pathologists rely on test manufacturers’ less stringent criteria, Glaspy answered that they had never had problems getting Herceptin or lapatinib (indicated for HER2-positive patients with metastatic disease) covered in patients with ratios at the old threshold. Hammond concurred:

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“A finding of 2.0 does not interfere with trastuzumab reimbursement,” she said.

Still, should clinical laboratories eschew the guidelines, they might jeopardize their CAP accreditation, Press warned. “I have a clinical lab that is routinely inspected by CAP,” he said. “And CAP mandates that if we don’t follow the guidelines, the lab can be given a deficiency (or a citation for departing from recommended procedures). And that puts me in a bind because a whole body of accumulated clinical data supports the 2.0 ratio cutoff.”

To that, Hammond responded, “These measurement artifacts would have no bearing on laboratory inspection criteria. It’s not a clinically relevant problem that would lead to a loss of accreditation.

#### **IHC versus FISH**

Meanwhile, clinicians still disagree over whether FISH or IHC is the better test; both have their pros and cons. Pathologists have more experience with IHC, which also has a lower cost—roughly \$150, compared with \$300 for FISH. But IHC also relies on subjective interpretations; those who run the tests must qualify shades of brown stain, and observers can reach different conclusions. Moreover, sample results can be influenced by the time to and duration of fixation. FISH, however, generates quantitative ratios that Press, for one, prefers, but its results can also be influenced by fixatives, chemicals, or heat.

At first, the two tests suffered from poor concordance—their results didn’t always match up. But now that’s a lesser problem. In a recent editorial appearing in the *Journal of Clinical Oncology*, Pradip De, an oncologist at Emory University’s Winship Cancer Center, wrote that the initial concordance rate of 82%, measured in 2000, had improved to 96% by 2007. Attributed to standardized methods in the ASCO–CAP guidelines, that better concordance rate still applies today, Ross said.

In a newly published study, Perez found that IHC and FISH work equally well in the hands of a trained pathologist. She bases her results, which appeared in the *Journal of Clinical Oncology* in October, on a review of 1,888 HER2-positive women enrolled in the National Cancer Institute’s N9831 adjuvant trastuzumab phase III clinical trial. “There was a general opinion in the field that FISH was the better predictor,” Perez said. “We found that either one is as good as the other.” The finding has two implications, Perez said: first, that Doctors don’t need to



Edith Perez, M.D.

verify positive results obtained with one test by running another, and second, that women deemed HER2 positive by either test should be treated with trastuzumab.

Negative results with either FISH or IHC should be treated cautiously, Perez said. According to her data, 8% of breast cancer tumors deemed to be FISH negative turned out to be IHC positive when retested or evaluated by a different laboratory. “So in the case of negative results, it’s advisable to repeat the test you started with or to run a different test,” she said.

#### **FISH Ratio Outmoded?**

In yet another discovery from the same study, Perez found that centromere copy numbers used to calculate FISH ratios are unnecessary, particularly when HER2 copy numbers are high. FISH manufacturer guidelines base positivity on the ratio, which is also what most pathologists also rely on. But even before trastuzumab came on the market in 1998, the FDA had approved a HER2 FISH test based only on copy number. According to that test, known as the INFORM assay and manufactured by Ventana Medical Systems in Tucson, Ariz., positive results were based on 4.0 HER2 copy numbers per cell. The 2007 ASCO–CAP guidelines acknowledge this approach as valid (in addition to the ratio), but they raise the threshold from 4.0 to 6.0 HER2 copy numbers per cell.

Ross describes the centromere copy number as a gimmick and advocates abandoning the ratio approach. “It doesn’t add any value,” he said. Hammond emphasizes that either approach can be used but that the ratio allows pathologists to control for aneuploidy, which is a common finding. Scientists also disagree over how many HER2 copy numbers denote positivity—both Perez and Ross say that the ASCO–CAP 6.0 cutoff is too high, given clinical data that link trastuzumab benefits to lower copy numbers.

Scientists are reluctant to speculate on how few HER2 copies might be linked to these benefits (and Perez doesn’t offer a number), in part because of controversial studies suggesting that even HER2-negative patients do better on trastuzumab. Perez and other sources interviewed firmly reject that possibility, claiming that the populations on which those findings are based were later found to include some HER2-positive patients.

“I would not recommend trastuzumab for HER2-negative women today based on the available data,” Perez said. “It doesn’t make any biological sense.

Could a different HER2 test improve patient classification? No—at least not any of the tests in development now. Genomic Health, which is on the leading edge of efforts to develop an alternative, recently began including HER2 test results as part of its 21-gene Oncotype DX assay for estrogen receptor-positive breast cancer. In a recent study, also in the *Journal of Clinical Oncology* last October, this test—which measures HER2 mRNA—achieved 97% concordance with FISH. That finding is similar to the best concordance rates between IHC and FISH that other laboratories have achieved. Genomic Health offers HER2 testing as a free supplement to Oncotype DX’s \$3,795 test.

But Press questions whether it will ever be possible for HER2 testing to achieve 100% accuracy. “This is biology, not chemistry or physics, so there’s going to be some variation and cases where you’re on the margins with respect to making a decision,” he said. “It’s up to clinicians and pathologists to interpret these cases as accurately as possible, but there will always be some judgment involved.”