



## **HER2 Testing: Summary for Breast Cancer Patients™**

**Based on the NCCN Task Force Report: HER2 Testing in Breast Cancer™**

***Developed by Research Advocacy Network and NCCN***

Breast cancers can be categorized as being HER2 positive or HER2 negative. HER2-positive breast cancer is faster growing and considered more aggressive. Studies<sup>1, 2</sup> indicate that the drug trastuzumab (Herceptin) is effective in the treatment of HER2-positive early stage breast cancer and HER2-positive metastatic breast cancer (cancer that has spread to other parts of the body). Trastuzumab is not effective in the treatment of HER2-negative breast cancers. Trastuzumab, like any drug, can have serious side effects<sup>3</sup> and should only be given to patients likely to benefit from it. Because of the effectiveness, side effects, and cost of trastuzumab in treating HER2-positive early stage and metastatic breast cancer, it is very important to have tests that accurately determine HER2 tumor status. HER2 tumor status is used in determining a patient's treatment plan, and an incorrect test result can have serious consequences. If a HER2 test report indicates that a patient's tumor is HER2 positive when it is actually HER2 negative (false positive), the patient may be given trastuzumab, which can have serious side effects and is unlikely to be effective in treating her disease. If a HER2 test report indicates that a patient's tumor is HER2 negative when it is actually HER2 positive (false negative), her treatment plan will not include trastuzumab and the treatment may not be as effective.

The National Comprehensive Cancer Network (NCCN) convened an expert task force after reports of HER2 testing problems encountered in clinical studies involving adjuvant breast cancer therapies.<sup>4</sup> The NCCN Task Force concluded that "accurate assignment of the HER2 status of invasive breast cancer is essential to clinical decision making in the treatment of breast cancer in both adjuvant (early stage) and metastatic settings."

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### **HER2 TUMOR STATUS**

About 15 to 20% of women with breast cancer have HER2-positive tumors. The HER2 gene resides on chromosome 17 and carries the blueprint for the cell to manufacture the HER2 protein. The HER2 protein is a receptor on the surface of the cell and sends messages to the cell to grow and divide more frequently. In normal, resting (nondividing) cells, there are two copies of the HER2 gene, one on each of two copies of chromosome 17. In HER2-positive breast cancer:

- the cell has more than the normal number of copies of the HER2 gene; the gene is "amplified"
- the amount of HER2 protein in the cell increases or is "overexpressed"
- the increased number of HER2 receptors on the surface of the cell send more messages for the cell to grow and divide
- tumor growth can be very fast and the breast cancer is considered to be aggressive

### **TREATMENT**

Trastuzumab is an effective treatment for women with early stage or advanced (metastatic) HER2-positive breast cancer. Trastuzumab:

- specifically targets and binds to the HER2 receptors on the tumor cell surface
- may decrease the number of messages sent to the cell that tell it to grow and divide
- may also signal the body's immune system to destroy the cancer cell and may work with chemotherapy (e.g., paclitaxel) to destroy HER2-positive cancer cells
- is not known to be effective with HER2-negative breast cancers

### **TESTING**

There are two methods of testing for HER2 tumor status in women with breast cancer: immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). Results from both tests are used in the clinical setting and the results of the tests influence treatment choices for women with breast cancer. The pathology laboratory where the HER2 testing is done should be accredited to perform such testing. It should have quality control procedures in place to ensure that the test is done correctly, and a quality assurance plan to validate (i.e. determine the accuracy of) the HER2 test results.

#### **Immunohistochemistry (immuno-histo-chemistry IHC)**

IHC is a protein-based test that is used to provide an assessment of the amount of HER2 protein receptors on the surface of the cancer cells. In HER2-positive tumors there is more than a normal amount of HER2 protein (i.e. HER2 protein "overexpression") on the cell surface.

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The IHC test is done by a pathologist in a laboratory on a sample of a tumor removed during a biopsy, a lumpectomy or a mastectomy. The steps in the procedure are:

- breast cancer tissue is prepared for testing
- the sample of tumor (a thin slice) is exposed to an antibody which attaches to the HER2 receptors
- the antibody attached to the HER2 protein receptors reacts with other substances that cause a color change in the tissue sample
- a pathologist must judge the degree of color change in the cells of the sample; the more HER2 protein the darker the staining: evaluation of the number of cells with color and the color intensity of the cells may be performed with computer imaging methods
- the percentage of cells in the sample with color and the intensity of the color of the cells on the slide determines the score for the test

The scoring for an IHC test is from 0 to 3+.

- Zero is HER2 negative
- 1+ is considered HER2 negative
- 2+ is considered a borderline or equivocal result
- 3+ is HER2 positive

Problems encountered with IHC testing:

- The protein being measured can be damaged during certain preparations of the tissue sample causing variability in test outcome.
- Evaluation of the sample often requires the pathologist to subjectively judge the degree of color (of the HER2 protein on the cell).
- False positive or false negative HER2 test results can occur

### **Fluorescence in situ Hybridization (FISH)**

FISH is a gene-based test used to determine the number of HER2 genes in the cells of the tumor. In HER2-positive breast cancer there are too many copies of the HER2 gene, the gene is "amplified".

The FISH test is done by a pathologist in a laboratory on a sample of a tumor removed during a biopsy, a lumpectomy or a mastectomy. The steps in the most commonly used procedure (Pathvysion) are:

- breast cancer tissue is prepared for testing
- the sample of tumor (a thin slice) is exposed to fluorescent compounds (will glow under certain light)
- one fluorescent compound adheres (sticks) to the HER2 genes in a cell and another adheres to chromosome 17 in a cell

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- when the sample is exposed to a special light the HER2 genes and chromosome 17 light up with different colors and can be counted; a pathologist or computer then reads the prepared slide
- the proportion or ratio of genes to chromosomes 17 in 60 cells is determined and the average number of HER2 genes/chromosome 17 per cell is then reported
- The HER2 gene/chromosome 17 ratio in a normal, nondividing cell should be 1 to 1- 1 gene on each of the 2 copies of chromosome 17; the HER2 gene/chromosome 17 ratio can increase up to 2 to 1 in cells during certain stages of normal cell division; an average HER2 gene/chromosome 17 ratio of less than 2 to 1 is reported as HER2 negative
- tumor samples with an average HER2 gene/chromosome 17 ratio of greater than or equal to 2 to 1 (2.5 to 1, 3.2 to 1, etc.) are reported as HER2 positive
- Another FISH procedure (INFORM) uses a similar procedure but measures only the average number of HER2 gene copies/cell; tumor samples with an average of greater than 4 HER2 gene copies/cell are considered HER2 positive.

### Problems encountered in FISH testing:

- Scoring problems can occur when the HER2 gene/chromosome 17 ratios are in the 1.8 to 2.2 range (Pathvysion test) or when the average number of HER2 gene copies/cell are in the range of greater than 4 to less than 6 (INFORM test); these are considered “borderline amplified”.
- At this time no high-level evidence or agreement is available on how results in the borderline range should be interpreted or confirmed.
- Scoring difficulties found with FISH testing may be associated with the specific set of cells chosen to include in the determination or tissue processing.
- False positive or false negative HER2 test results can occur

## NCCN RECOMMENDATIONS

If appropriate quality control/assurance procedures are in place for a laboratory<sup>5</sup>, either IHC or FISH methods may be used to determine HER2 tumor status. If the laboratory does not have control/assurance procedures in place, the sample should be sent to a reference laboratory<sup>6</sup> that does meet quality control/assurance procedures.

- HER2 positive status is IHC 3+ or FISH positive
- HER2 negative status is IHC 0, 1+ or FISH negative
- A borderline IHC result of 2+ should be followed by performing a FISH test.
- A borderline FISH result of an average HER2 gene/chromosome 17 ratio of 1.8 to 2.2 (or an average of greater than 4 to less than 6 HER2 gene copies/cell) should be followed by one of the following:
  - Counting additional cells in the tissue sample
  - Retesting with FISH
  - Performing an IHC test

### Questions To Ask About Testing

- **What tests will be done on the sample of my breast cancer tumor?**  
One test done on a sample determines whether the tumor is estrogen/progesterone positive or negative. This will help determine if treatment will include hormone therapy. Another test will determine if your tumor is HER2 positive or negative. The report from the pathologist will also contain information about how the sample looked to the naked eye and then under a microscope.<sup>7, 8</sup>
- **Is the laboratory that will test my tumor for HER2 status accredited to perform such testing?**  
Your doctor may or may not know the answer to this question. If your doctor does not know, it is important that she find out because treatment decisions will be made based on the results of the HER2 tumor testing. The information about the accreditation of the laboratory can be found by contacting the head of the laboratory (often a pathologist). If the laboratory is not accredited to perform HER2 testing, your doctor should have the test done at a reference laboratory that is. If the laboratory is connected with a large hospital and does many HER2 tests, it is more likely to meet the standards for accreditation.<sup>5</sup>
- **Has the laboratory properly validated the HER2 test(s) it uses?**  
Your doctor may or may not know the answer to this question. If your doctor does not know, it is important that she find out because treatment decisions will be made based on the results of the HER2 tumor test. The information about the validation of the HER2 test(s) can be found by contacting the head of the laboratory (often a pathologist). If the laboratory has not validated the HER2 test(s) they use, your doctor should have the test done in a reference laboratory that has validated HER2 testing.<sup>5</sup>
- **Which test will be used to determine my HER2 status?**  
Either IHC or FISH testing is acceptable.
- **What will happen if the IHC test comes back 2+?**  
This is considered a borderline result and it should be followed by doing a FISH test.
- **What will happen if the FISH test comes back with a borderline result?**  
There are three possible things that could be done: The pathologist can look at the original slide and count more cells to determine the gene/chromosome ratio (or the number of gene copies/cell); the sample can be retested using FISH; or an IHC test can be done.

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<sup>1</sup>Romond EH, Perez EA, Bryant J, et al. *Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer*. N Engl J Med. 2005;353:1673-1684.

<sup>2</sup>Slamon DJ, Leyland-Jones B, Shak S, et al. *Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2*. N Engl J Med. 2001;344:783-792.

<sup>3</sup>breastcancer.org. *Herceptin Side Effects*, 19 Sep 2006. [http://www.breastcancer.org/herceptin\\_side\\_effects.html](http://www.breastcancer.org/herceptin_side_effects.html)

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<sup>4</sup> Carlson RW, Moench SJ, Hammond MEH et al. *HER2 testing in breast cancer: NCCN Task Force report and recommendations*. J Natl Compr Canc Netw.. 2006;4 suppl 3:S1-S-22. (url to be added when available online)

<sup>5</sup> *Standards for Laboratory Accreditation* . College of American Pathologists. 2000. 19 Sep 2006.  
[http://www.cap.org/apps/cap.portal?\\_nfpb=true&cntvwrPtlActionOverride=%2Fportlets%2FcontentViewer%2Fshow&\\_windowLabel=cntvwrPtl&cntvwrPtl%7BactionForm.contentReference%7D=laboratory\\_accreditation%2Fstandards%2Fstandards.html&\\_state=maximized&\\_pageLabel=cntvwr](http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlActionOverride=%2Fportlets%2FcontentViewer%2Fshow&_windowLabel=cntvwrPtl&cntvwrPtl%7BactionForm.contentReference%7D=laboratory_accreditation%2Fstandards%2Fstandards.html&_state=maximized&_pageLabel=cntvwr)

<sup>6</sup> "Reference Laboratory." Encyclopedia of Public Health. Ed Lester Breslow, Thomson Gale, 2002. enotes.com. 2006. 19 Sep 2006. <http://health.enotes.com/public-health-encyclopedia/reference-laboratory>

<sup>7</sup> *Your Pathology Report*. breastcancer.org. [http://www.breastcancer.org/pathology\\_intro.html](http://www.breastcancer.org/pathology_intro.html) (Free brochure)

<sup>8</sup> *Breast Cancer Work-Up*. National Comprehensive Cancer Network, American Cancer Society. 19 Sep 2006.  
[http://www.nccn.org/patients/patient\\_gls/\\_english/\\_breast/3\\_work-up.asp](http://www.nccn.org/patients/patient_gls/_english/_breast/3_work-up.asp)

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