Boxed WARNINGS and Additional Important Safety Information

Herceptin administration can result in left ventricular dysfunction and congestive heart failure (CHF). The incidence and severity of left ventricular cardiac dysfunction/CHF were highest in patients who received Herceptin concurrently with anthracycline-containing chemotherapy regimens. Discontinue Herceptin treatment in patients receiving adjuvant therapy for breast cancer and strongly consider discontinuation of Herceptin in patients with metastatic breast cancer who develop a clinically significant decrease in left ventricular function.

Patients receiving Herceptin should undergo frequent monitoring for deteriorating left ventricular function. More frequent monitoring should be employed in patients with pre-existing cardiac dysfunction receiving Herceptin. Monitoring will not identify all patients who will develop cardiac dysfunction.

Serious infusion reactions and pulmonary toxicity have occurred; rarely, these have been fatal. In most cases, symptoms occurred during or within 24 hours of administration of Herceptin. Herceptin infusion should be interrupted for patients experiencing dyspnea or clinically significant hypotension. Patients should be monitored until signs and symptoms completely resolve. Discontinuation of Herceptin should be strongly considered for infusion reactions manifesting as anaphylaxis, angioedema, pneumonitis, or acute respiratory distress syndrome.

Exacerbation of chemotherapy-induced neutropenia has also occurred.

The most common adverse reactions associated with Herceptin use were fever, nausea, vomiting, infusion reactions, diarrhea, infections, increased cough, headache, fatigue, dyspnea, rash, neutropenia, anemia, and myalgia.

Please see enclosed full Prescribing Information, including **Boxed WARNINGS**, for additional important safety information.

References: 1. Paik S, Bryant J, Tan-Chiu E, et al. Real-world performance of HER2 testing–National Surgical Adjuvant Breast and Bowel Project experience. *J Natl Cancer Inst.* 2002;94:852-854. **2.** Roche PC, Suman VJ, Jenkins RB, et al. Concordance between local and central laboratory HER2 testing in the Breast Intergroup trial N9831. *J Natl Cancer Inst.* 2002;94:855-857. **3.** Wolff AC, Hammond EH, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *J Clin Oncol.* 2007;25:118-145. **4.** Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *Arch Pathol Lab Med.* 2007;131:18-43. **5.** Newman EA, Guest AB, Helvie MA, et al. Changes in surgical management resulting from case review at a breast cancer multidisciplinary tumor board. *Cancer.* 2006;107(10):2346-2351. **6.** Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science.* 1987;235:177-182. **7.** Ross JS, Fletcher JA. HER-2/*neu* (c-*erb*-B2) gene and protein in breast cancer. *Am J Clin Pathol.* 1999;112(suppl 1): S53-S67. **8.** Herceptin Prescribing Information. Genentech, Inc. November 2006. **9.** College of American Pathologists. HER2 testing guidelines and resources. Available at: http://www.cap.org/apps/cap.portal?_nfpb=true&cntvwrPtlt_actionOverride=%2Fportlets% 2FcontentViewer%2Fshow&_windowLabel=cntvwrPtlt&cntvwrPtlt%7BactionForm.contentReference%7D=diseases%2Fcancer%2Fher2_index.html&_state=maximized&_pageLabel=cntvwr. Accessed September 19, 2007.

www.herceptin.com

Herceptin® trastuzumab

The pathologist's guide to

Optimal HER2 testing in invasive breast cancer

2007 update to ASCO/CAP recommendations

Indications

Herceptin (Trastuzumab), as part of a treatment regimen containing doxorubicin, cyclophosphamide, and paclitaxel, is indicated for the adjuvant treatment of patients with HER2-overexpressing, node-positive breast cancer.

Herceptin in combination with paclitaxel is indicated for treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have not received chemotherapy for their metastatic disease.

Herceptin as a single agent is indicated for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have received one or more chemotherapy regimens for their metastatic disease.

Pathologists and HER2-testing accuracy

- **Pathologists play a critical role in ensuring that HER2 status is accurately determined and, as a result, that patients are appropriately treated**
  - An awareness of what constitutes an "equivocal" test result can help ensure that the result is confirmed appropriately
  - An understanding of what criteria may indicate inaccuracies is critical to knowing when the initial test should be rejected and another assay performed
  - Standardized reporting elements help ensure that the treating physicians have a complete and accurate understanding of the test results

- **HER2-testing inaccuracy is a significant issue, whether IHC or FISH is used**
  - In some analyses, about 20% of HER2 tests performed in the field* were discordant with the result of the same specimen tested in a high-volume lab[1,2]
  - A number of factors can influence the accuracy of results, including testing procedures, interpretation, and accurate and complete reporting

- **To address this problem, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) established an expert panel to develop recommendations for standardizing HER2 testing in breast cancer[3,4]**
  - A summary of recommendations are included in this booklet
  - Additional information and details can be found in the full recommendations, published in the *Journal of Clinical Oncology* and the *Archives of Pathology & Laboratory Medicine*

*At the primary treatment site's pathology department.

ASCO/CAP recommendations relevant across specialties[3,4]

- The following highlights ways in which various specialists may utilize relevant elements of the recommendations:

Relevance of HER2 testing guidelines by specialty

	Medical oncologist	Surgeon	Pathologist
Testing algorithms	May compare the testing algorithm used for a given specimen with the recommended algorithm		Should understand when and how it is recommended to retest samples with equivocal test results
Testing requirements	May compare testing practices used for a given sample with the recommended requirements	Should utilize recommended techniques for tissue acquisition and length of fixation	Should adopt recommendations for length of fixation, controls, and cell counting
Interpretation criteria	Understand the implications of "positive" or "negative" reported result		Should ensure that test result is captured and interpreted in a standardized and accurate way
Reporting elements	Can look for specific testing elements that should be standardly reported, and may be relevant for clinical decision making		Should report the recommended elements of testing
Validation/ accredititation	May check whether the laboratory used is accredited and has validation and QA procedures in place		Should adopt internal validation and QA procedures, and maintain standards for external laboratory accreditation

Cooperation is key among specialists

- Cooperation among specialists is critical to help ensure accurate interpretation of results and appropriate disease management
  - One retrospective study demonstrated that multidisciplinary review of breast cancer patients is likely to impact management recommendations[5]
  - Among 149 referrals to a multidisciplinary breast cancer clinic from an outside facility, more than half (77) resulted in changes in surgical management recommendations[5]

Accurate HER2 testing has critical implications in breast cancer

HER2 overexpression or amplification signals aggressive disease

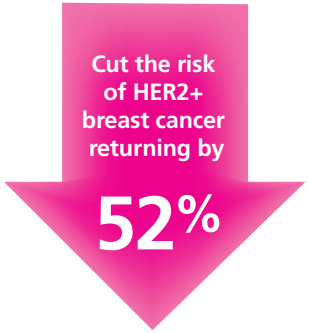
- HER2 positivity is associated with poor prognosis[6]
  - Higher rates of recurrence and mortality have been observed in patients newly diagnosed with HER2+ breast cancer
- HER2 positivity may impact response to endocrine therapies, certain chemotherapies, and HER2-targeted therapies[7]

Potential benefits and risks of Herceptin reinforce importance of accurate HER2 determination[8]

- Herceptin, a monoclonal antibody that specifically targets the HER2 receptor, is indicated only for patients with HER2+ breast cancer

In the adjuvant setting:

Reduced risk of recurrence[8]



- Adding 52 weeks of Herceptin to standard therapy reduced the risk of disease recurrence by 52% for patients with HER2+, node-positive disease (hazard ratio=0.48; 95% confidence interval=0.39-0.59; $P<0.0001$)
- Herceptin was also associated with an increased risk of cardiac dysfunction
  - 2% incidence of clinical cardiac events* in the Herceptin-containing arm versus 0.4% in the control arm

In the metastatic setting:

- Herceptin plus chemotherapy extended median survival and time to progression in patients with HER2+ breast cancer, compared with chemotherapy alone

In light of the potential benefits and the potential risks, only those patients with HER2+ disease are candidates for Herceptin therapy.

*Clinically symptomatic, laboratory-confirmed cardiomyopathy as determined by an external review committee (ACREC). Analysis includes all patients who completed AC chemotherapy and received at least 1 dose of paclitaxel.

Boxed WARNINGS and Additional Important Safety Information

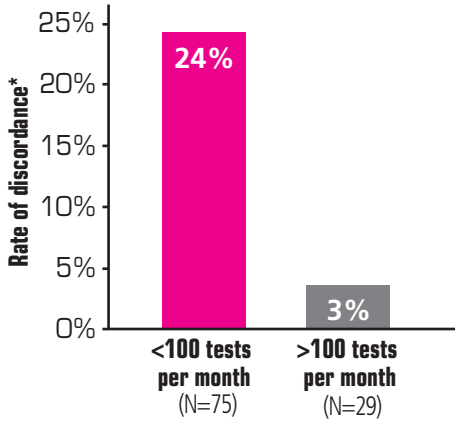
Herceptin administration can result in left ventricular dysfunction and congestive heart failure. Serious infusion reactions and pulmonary toxicity have occurred; rarely these have been fatal. Exacerbation of chemotherapy-induced neutropenia has also occurred. The most common adverse reactions associated with Herceptin use were fever, nausea, vomiting, infusion reactions, diarrhea, infections, increased cough, headache, fatigue, dyspnea, rash, neutropenia, anemia, and myalgia.

Please see enclosed full Prescribing Information, including **Boxed WARNINGS**, and back cover for additional important safety information.

Challenges in HER2 testing

- HER2 testing inaccuracy is a significant issue, whether IHC or FISH is used[3,4]
  - In some analyses, about 20% of HER2 tests performed in the field* were discordant with the results of the same specimen tested in a high-volume lab[1,2]
- Data suggest that greater lab experience is correlated with more accurate testing[1]
  - Labs performing many tests demonstrated lower rates of discordance with central testing facilities†

Discordance rates by lab experience[1]



ASCO/CAP released guidelines to standardize HER2 testing[3,4]

- To address the problem of inaccurate HER2 testing results, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) established an expert panel to develop recommendations for standardizing HER2 testing in breast cancer
- A summary of recommendations are included in this booklet
- Additional information and details can be found in the full recommendations, published in the *Journal of Clinical Oncology* and the *Archives of Pathology & Laboratory Medicine*[3,4]

*At the primary treatment site's pathology department.
†Discordance defined as differing HER2 result between initial local testing and follow-up central testing as part of quality assurance protocol. Central lab tested IHC by HercepTest and FISH by PathVysion.

Herceptin® trastuzumab

Optimal testing requirements[3,4]

- To help ensure accurate results, note the following requirements for IHC and FISH prior to, during, and following the assay

Optimal testing requirements		
	FISH	**IHC**
Fixation	Fixation for fewer than 6 hours or longer than 48 hours is not recommended prior to FISH (see page 7)	Fixation for fewer than 6 hours or longer than 48 hours is not recommended prior to IHC (see page 7)
Criteria to reject test	• Test is rejected and repeated if: – Controls are not as expected – Observer cannot find and count at least 2 areas of invasive tumor – >25% of signals are unscorable due to weak signals – >10% of signals occur over cytoplasm – Nuclear resolution is poor – Autofluorescence is strong	• Test is rejected and repeated or tested by FISH if: – Controls are not as expected – Artifacts involve most of sample – Sample has strong membrane staining of normal breast ducts (internal controls)
Interpretation	• Interpretation done by counting at least 20 cells – Pathologist must confirm that counting involved invasive tumor	• Interpretation follows guideline recommendations – Positive HER2 result requires homogeneous, dark circumferential (chicken wire) pattern in >30% of invasive tumors – Interpreters have method to maintain consistency and competency
Equivocal result	If result is equivocal, sample is subjected to increased counting and/or repeated (see page 8)	Sample is subjected to confirmatory FISH testing if initial result is equivocal (see page 8)
Reporting	Report must include guideline-detailed elements (see pages 10-11)	Report must include guideline-detailed elements (see pages 10-11)

Optimal tissue handling requirements[3,4]

- HER2 status should be determined for all cases of invasive breast cancer
- The following processes are recommended by ASCO/CAP to help ensure high-quality tissue samples for assays

Tissue acquisition

- Sections should ideally not be used for HER2 testing if cut >6 weeks earlier
  – This may vary with primary fixation or storage conditions
- Samples should be sliced at 5-10 mm intervals after appropriate gross inspection and margins designation

Tissue fixation

- Time from tissue acquisition to fixation should be as short as possible
- Samples should be placed in a sufficient volume of neutral buffered formalin
- Fixation for fewer than 6 hours or longer than 48 hours is not recommended prior to either FISH or IHC*
- Time to fixation and duration of fixation, if available, should be recorded for each sample

Needle core biopsies

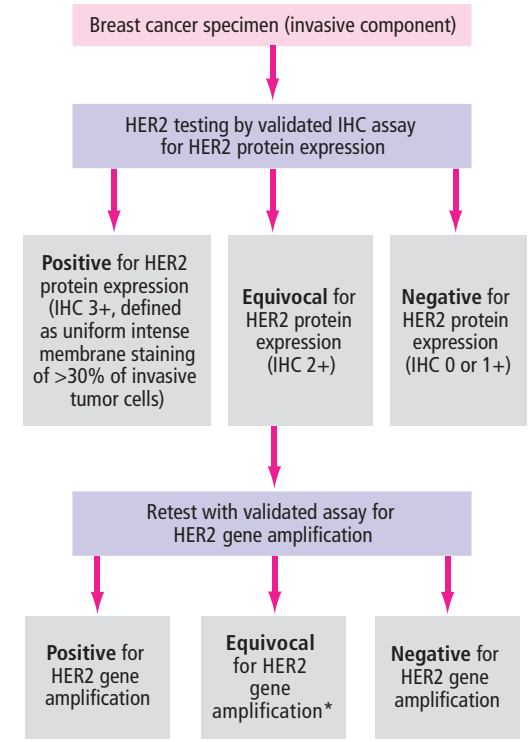
- Needle core biopsies should *not* be used for assays if they exhibit:
  – Edge or retraction artifact involving entire core
  – Crush artifact (thin gauge, vacuum extraction needle samples)

*Guidelines allow for needle core biopsies to be fixed for a minimum of 1 hour, but strongly suggest longer fixation times.

Algorithms for HER2 testing[3,4]

- It is recommended that a final positive or negative HER2 result be achieved using the testing algorithms for IHC and FISH

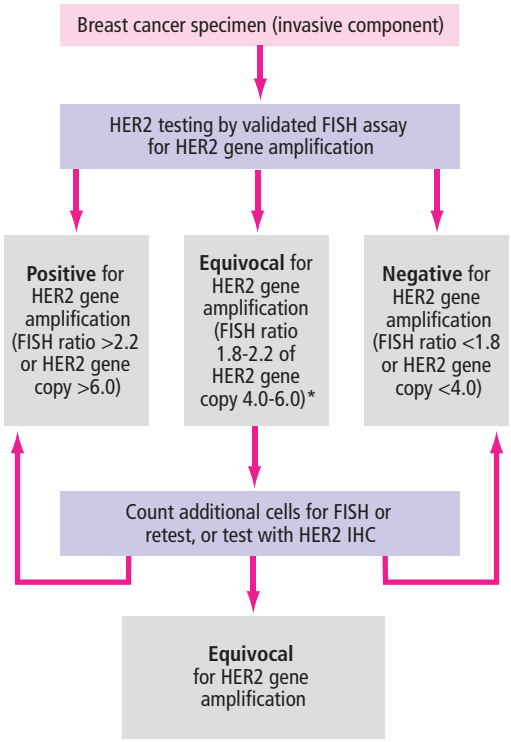
IHC testing algorithm



Adapted from Wolff AC et al. *J Clin Oncol*. 2007;25:118-145.

*Patients with HER2/CEP17 ratio ≥2.0 were eligible for the adjuvant trastuzumab trials.

FISH testing algorithm



Adapted from Wolff AC et al. *J Clin Oncol*. 2007;25:118-145.

*Patients with HER2/CEP17 ratio ≥2.0 were eligible for the adjuvant trastuzumab trials.

- The ASCO/CAP guidelines have redefined "equivocal" FISH results as a HER2/CEP17 FISH ratio between 1.8 and 2.2
- However, since patients with FISH scores of 2.0-2.2 were eligible for the adjuvant trastuzumab trials, the guidelines conclude that the available data **do not support excluding patients** with these results from receiving trastuzumab

Accreditation and quality assurance[3,4]

- According to the guidelines, HER2 testing should be conducted by a laboratory that:
  - Is accredited by CAP, **or**
  - Meets the accreditation and proficiency requirements detailed in the full guidelines
- Achievement or maintenance of CAP accreditation now requires laboratories to demonstrate proficiency in the specific type of testing being offered
- Details of accreditation and validation recommendations can be found in the full guidelines, published in the *Journal of Clinical Oncology* and the *Archives of Pathology & Laboratory Medicine*
- Compliance with the ASCO/CAP guidelines will be required as of December 31,2007[9]

Reporting elements[3,4]

- Clinicians rely upon pathology reports to accurately diagnose cancer and decide upon appropriate treatment
- The ASCO/CAP testing guidelines recommend standardized information that should be available to the clinician for these purposes

Standard elements[3,4]
• Patient identification information
• Physician identification
• Date of service
• Specimen information – Specimen identification (case and block number) – Specimen site and type
• Fixation information – Specimen fixative type – Time to fixation (if available) – Duration of fixation (if available)
• Method used (specifics of test/vendor and if FDA-approved)
• Adequacy of sample for evaluation (adequate number of invasive tumor cells present)
• **Comments** – If an FDA-approved method is used, it should be stated – If the FDA-approved method has been modified, a statement in the report should be included indicating what modifications were made and that the changes have been validated – If the test is not FDA-approved, or an FDA-approved test has been modified, a clear statement must be made that the lab that is reporting the results takes responsibility for the test performance

Assay-specific elements[3,4]	
Reporting elements for IHC	**Reporting elements for FISH**
• Antibody clone/vendor	• Probe(s) identification
• Image-analysis method (if used)	• Image-analysis method
• Controls (high protein expression, low-level protein expression, negative protein expression, internal)	• Controls (amplified, equivocal, and nonamplified, internal)
• Results – Percentage of invasive tumor cells exhibiting complete membrane staining – Uniformity of staining: present/absent – Homogeneous, dark circumferential pattern: present/absent	• Results – Number of invasive tumor cells counted – Number of observers – Average number of HER2 signals/nucleus or tile – Average number of CEP17 chromosome probes/nucleus or tile – Ratio of average HER2 signals/CEP17 probe signals **Note**: Tile is unit used for image system counting.
• Interpretation – Positive (for HER2-protein expression) – Equivocal (FISH will be done and reported) – Negative (for HER2-protein expression) – Not interpretable	• Interpretation – Positive (amplified), equivocal, negative (not amplified), not interpretable – Indicate if IHC is being done because of problems with assay or results

- Additional details of reporting recommendations can be found in the full guidelines, published in the *Journal of Clinical Oncology* and the *Archives of Pathology & Laboratory Medicine*[3,4]

Note: It is important to note that guidelines cannot always account for individual variation among patients. Guidelines are not intended to supplant physician judgment with respect to particular patients or special clinical situations and cannot be considered inclusive of all proper methods of care or exclusive of other treatments reasonably directed at obtaining the same result. Accordingly, ASCO considers adherence to these guidelines to be voluntary, with the ultimate determination regarding their application to be made by the physician in light of each patient's individual circumstances.