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Paik S et al. **Successful Quality Assurance Program for HER2 Testing in the NSABP Trial for Herceptin®.** *Breast Cancer Research and Treatment* 2002;76 (Suppl 1);Abstract 9.

Press MF et al. **Comparison of HER-2/Neu Status Determined by Fluorescence *In Situ* Hybridization (FISH) in the BCIRG Central Laboratories with HER-2/Neu Status Determined by Immunohistochemistry or FISH In Outside Laboratories.** *Breast Cancer Research and Treatment* 2002;76 (Suppl 1);Abstract 238.

## 16 Related Publications

## 17 Comparison of HER2 Assays

Perez EA et al. **HER2 Testing in Patients with Breast Cancer: Poor Correlation Between Weak Positivity by Immunohistochemistry and Gene Amplification by Fluorescence *In Situ* Hybridization.** *Mayo Clinic Proceedings* 2002;77:148-54.

Press MF et al. **Evaluation of HER-2/neu Gene Amplification and Overexpression: Comparison of Frequently Used Assay Methods in a Molecularly Characterized Cohort of Breast Cancer Specimens.** *Journal of Clinical Oncology* 2002;20:3095-105

Rhodes A et al. **Evaluation of HER-2/Neu Immunohistochemical Assay Sensitivity and Scoring on Formalin-Fixed and Paraffin-Processed Cell Lines and Breast Tumors: A Comparative Study Involving Results from Laboratories in 21 Countries.** *American Journal of Clinical Pathology* 2002;118(3):408-17.

Wang S et al. **Assessment of HER-2/Neu Status in Breast Cancer. Automated Cellular Imaging System (ACIS)-Assisted Quantitation of Immunohistochemical Assay Achieves High Accuracy in Comparison with Fluorescence *In Situ* Hybridization Assay As the Standard.** *American Journal of Clinical Pathology* 2001;116:495-503.

## 24 Related Publications

## 25 Concordance of HER2 Status Between Primary and Metastatic Lesions

Gancberg D et al. **Comparison of HER-2 Status Between Primary Breast Cancer and Corresponding Distant Metastatic Sites.** *Annals of Oncology* 2002;13:1036-43.

## 27 Related Publications

## 28 HER2 Status and Response to Trastuzumab

Slamon DJ et al. **Use of Chemotherapy Plus a Monoclonal Antibody Against HER2 for Metastatic Breast Cancer That Overexpresses HER2.** *New England Journal of Medicine* 2001;344:783-92.

Mass RD et al. **Improved Survival Benefit from Herceptin (Trastuzumab) in Patients Selected by Fluorescence *In Situ* Hybridization (FISH).** *Proceedings American Society of Clinical Oncology* 2001;Abstract 85.

Vogel CL et al. **Efficacy and Safety of Trastuzumab As a Single Agent in First-Line Treatment of HER2-Overexpressing Metastatic Breast Cancer.** *Journal of Clinical Oncology* 2002;20:719-26.

Esteva FJ et al. **Phase II Study of Weekly Docetaxel and Trastuzumab for Patients with HER2-Overexpressing Metastatic Breast Cancer.** *Journal of Clinical Oncology* 2002;20:1800-8.

Robert N et al. **Phase III Comparative Study of Trastuzumab and Paclitaxel with and without Carboplatin in Patients with HER-2/Neu-Positive Advanced Breast Cancer.** *Breast Cancer Research and Treatment* 2002;76 (Suppl 1);Abstract 35.

Burstein HJ et al. **Preoperative Therapy with Trastuzumab and Paclitaxel Followed by Sequential Adjuvant Doxorubicin/Cyclophosphamide for HER2-Overexpressing Stage II or III Breast Cancer: A Pilot Study.** *Journal of Clinical Oncology* 2003;21:46-53.

## 41 *Related Publications*

## 42 College of American Pathologists

Cell Markers and Cytogenetics Committees, College of American Pathologists. **Clinical Laboratory Assays for HER-2/Neu Amplification and Overexpression Quality Assurance, Standardization, and Proficiency Testing.** *Archives of Pathology and Laboratory Medicine* 2002;126:803-8.

Zarbo RJ, Hammond EH. **Conference Summary, Strategic Science Symposium. Her-2/Neu Testing of Breast Cancer Patients in Clinical Practice.** *Archives of Pathology and Laboratory Medicine* 2003;127:549-53.

## 45 *Related Publications*

## 46 Post-test

## 47 Evaluation

### Cover Image:

Fluorescence *in situ* hybridization (FISH) assay for HER2 gene amplification as seen by confocal laser microscopy. Multiple HER2 gene copies are represented by yellow dots in the red nuclei of the tumor cells.

Photo courtesy of Mehrdad Nadji, MD  
Professor of Pathology  
University of Miami School of Medicine

### Statement of Need/Target Audience

One of the most rapidly evolving areas of interest in breast cancer medicine involves the human epidermal growth factor receptor-2 (HER2). Published results from numerous studies lead to the continual emergence of new data and changes in the understanding of clinical assays of HER2. In order to offer optimal patient care, the practicing medical oncologist and pathologist must be well-informed of these advances. To bridge the gap between research and patient care, this HER2 journal club utilizes one-on-one discussions with leading oncology and pathology investigators. By providing highlights from recent publications about HER2 and the experts' perspectives, this CME program assists medical oncologists and pathologists in incorporating up-to-date information into their clinical practice.

### Learning Objectives

Upon completion of this activity, participants should be able to:

- Critically evaluate the clinical implications of the emerging data about the discordance between local and central laboratory HER2 testing
- Describe and implement an algorithm for HER2 testing
- Explain the components of a quality assurance program for HER2 testing
- Define clinical variables that should be considered when evaluating the accuracy of HER2 test results
- Assess the need to retest a patient's HER2 status

### Educational Method

To receive CME credit, the participant should listen to the CD or tape, review the monograph and complete the post-test and evaluation form.

### Accreditation Statement

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### Credit Designation Statement

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**Financial Disclosures**

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**Michael F Press, MD, PhD**

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**Ann D Thor, MD**

*Consultant:* Genentech Inc

**Pharmaceutical agents discussed in this program**

GENERIC	TRADE	MANUFACTURER
cyclophosphamide	Cytoxan®, Neosar®	Bristol-Myers Squibb Company, Pfizer Inc
doxorubicin hydrochloride	Adriamycin®	Pfizer Inc
epirubicin hydrochloride	Ellence®	Pfizer Inc
paclitaxel	Taxol®	Bristol-Myers Squibb Company
trastuzumab	Herceptin®	Genentech Inc

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*Grants/Research Support: Genentech Inc*

### Melody Cobleigh, MD

*Grants/Research Support: Genentech Inc, Agouron Pharmaceuticals Inc, Amgen Inc, Aventis Pharmaceuticals Inc, Bristol-Myers Squibb Company, Coley Pharmaceuticals, Genentech Inc, Genta Inc, Immunicon Corporation, Novartis Pharmaceuticals Corporation, SmithKline Beecham  
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Editor's Note

## Getting it Right

### CASE 1:

(Adapted from "Meet the Professor" session, Miami Breast Cancer Conference, February 28, 2003)

A 54-year-old woman presented with a 2.1-cm, infiltrating, ductal carcinoma of the right breast and two positive axillary lymph nodes. Assays for estrogen and progesterone receptor status were interpreted as strongly positive (greater than 60 percent of the cells staining positively). The tumor's HER2 status was assessed by immunohistochemistry (IHC) and scored 3+. The patient was believed to be a candidate for BCIRG-006, an adjuvant trial evaluating chemotherapy with or without trastuzumab. Central reference laboratory testing demonstrated that the tumor's HER2 status by fluorescence *in situ* hybridization (FISH) was negative and by repeat IHC was 2+. Subsequently, the patient was treated off protocol with an aromatase inhibitor and chemotherapy.

### CASE 2:

(Adapted from "Meet the Professor" session, American Society of Breast Disease Meeting, April 13, 2003)

A 65-year-old woman presented with rapidly progressing liver, lung and bone metastases two years after receiving adjuvant doxorubicin/cyclophosphamide for an estrogen receptor-negative breast cancer. The tumor's HER2 status at the time of diagnosis assessed by IHC revealed a score of 1+. At the time of recurrence, a supraclavicular node was biopsied and assessed for HER2 status by FISH, which revealed HER2 gene amplification. The patient was treated with trastuzumab and paclitaxel, with significant reduction in tumor volume (partial response) and complete symptom relief. After six cycles, paclitaxel was discontinued. The patient's cancer remains in partial remission on trastuzumab 18 months later.

These two cases provide examples of daily clinical scenarios in which HER2 testing can have an enormous impact on current and future breast cancer care. Four major cooperative group adjuvant trastuzumab trials (Table 1) are currently accruing patients, and many researchers are cautiously optimistic that these studies will be the first adjuvant trials in which a biologic approach may be beneficial. This crucial "proof of principle" is entirely dependent on accurately testing patients for the therapeutic target. As recounted on the enclosed audio program by Dr Edith Perez, the principal investigator of one of these groundbreaking studies, investigators have invested a great deal of effort to ensure that only women with truly HER2-positive tumors are enrolled.

The initial false-positive HER2 result in Case 1 illustrates a more immediate lesson for current clinical practice. Since trastuzumab is utilized selectively in the metastatic setting,

it is important to ensure that women with HER2-positive tumors are accurately identified and that women with HER2-negative tumors are not exposed to the expense and potential toxicity of an inappropriate therapy. Case 2 is a striking example of the potential human impact of imprecise HER2 testing. This woman may have been denied the prolonged clinical remission of her otherwise rapidly-progressing metastatic disease, had her treating physician not had the foresight to send her tissue for a confirmatory FISH test.

This CME monograph is intended to provide medical oncologists and pathologists a concise review of currently published medical journal articles and abstracts related to this critical issue. The audio program provides commentary from four research leaders who assisted in selecting these journal articles and abstracts, and their remarks focus on how these publications relate to clinical practice. Drs Perez, Thor, Pegram and Press note that there are a number of important analogies between HER2 and estrogen receptor testing, for which there have been longstanding efforts to implement quality control in terms of performance and interpretation. Most importantly, clinicians utilize these assays to determine whether to administer highly targeted, relatively nontoxic therapies that offer an excellent risk-to-benefit ratio in selected patients.

Undoubtedly, the articles selected for this monograph will be replaced by newer reports that will shed additional light on this critical topic. As noted by Dr Thor, until HER2 testing is more refined, the most valuable clinical asset for both pathologists and oncologists is the awareness that our current methodology is far from perfect.

Neil Love, MD

Table 1: Randomized Clinical Trials of Adjuvant Trastuzumab		
Trial (Target Accrual)	Eligibility	Randomization
NSABP B-31 (2,700 patients)	Node + IHC 3+ or FISH+	AC x 4 → paclitaxel x 4 AC x 4 → paclitaxel x 4 + H qw x 1 year
Intergroup N9831 (3,300 patients)	Node + IHC 3+ or FISH+	AC x 4 → paclitaxel qw x 12 AC x 4 → paclitaxel qw x 12 → H qw x 1 year AC x 4 → (paclitaxel + H) qw x 12 → H qw x 40
BCIRG-006 (3,150 patients)	Node + FISH+	AC x 4 → docetaxel x 4 AC x 4 → docetaxel x 4 + H (qw x 12 weeks) → H (qw x 40 weeks) (Docetaxel + C) x 6 + H (qw x 18 weeks) → H (qw x 34 weeks)
BIG-01-01 HERA (3,192 patients)	Node + and - IHC 3+ or FISH+	H q3w x 1 year H q3w x 2 years No H
H = trastuzumab; C = cisplatin or carboplatin; AC = doxorubicin + cyclophosphamide Sources: NCI Physician Data Query, May 2003; Piccart MJ et al. <b>Herceptin for the treatment of breast cancer: What we know — and what we have yet to learn.</b> <i>Cancer Futures</i> 2002;1:73-9.		

# Concordance Between Local and Central Laboratory HER2 Testing

Roche PC et al. **Concordance Between Local and Central Laboratory HER2 Testing in the Breast Intergroup Trial N9831.** *Journal of the National Cancer Institute* 2002;94:855-7.

- Objectives**
- To assess the concordance between local community and central HER2 testing
  - To assess the concordance between central assays of HER2 protein overexpression (measured by the HercepTest™) and central assays of HER2 gene amplification (measured by the PathVysion™ FISH assay)

- Methods**
- A central review of the first 119 tumor specimens from patients entered into the North Central Cancer Treatment Group (NCCTG) trial N9831 was conducted.
  - Eligibility for NCCTG-N9831 required a score of 3+ with the HercepTest™ [immunohistochemistry (IHC) assay], strong membrane staining (>33 percent of the tumor cells) with other IHC assays, or gene amplification with fluorescence *in situ* hybridization (FISH).
  - A central laboratory assayed the tumor blocks for these cases with both the HercepTest™ and the PathVysion™ FISH assay.

## Results

Comparison of Local HER2 Testing Performed for Study Entry onto N9831 and Central HercepTest™

	Central HercepTest™ score				Total
	0	1+	2+	3+	
Local HER2 testing					
IHC-positive (3+)	8	9	12	81	110
FISH-positive	1	1	0	7	9
Total	9	10	12	88	119



### Comparison of Local HER2 Testing Performed for Study Entry to N9831 and Central FISH

	Central FISH Result		
	Not amplified	Amplified	Total
Local HER2 testing			
IHC-positive (3+)	37	73	110
FISH-positive	3	6	9
Total	40	79	119

### Comparison of Central FISH and Central HercepTest™

	Central HercepTest™ score				Total
	0	1+	2+	3+	
Central Vysis FISH					
Not amplified	9	10	12	9	40
Amplified	0	0	0	79	79
Total	9	10	12	88	119

### Concordance for Central Testing

The concordance between central testing for FISH and HercepTest™ was 92 percent. The discordance occurred among 9 (8%) specimens classified as HER2/neu not amplified by FISH but as 3+ by HercepTest™.

### Authors' Conclusions

“Our results demonstrate that there is poor agreement between the results from local laboratory-based HER2 testing and those of central testing by experienced investigators.

“We have chosen to modify the HER2 testing requirement for our N9831 clinical trial... . Protocol eligibility was modified so that a woman can enroll in the trial if she has node-positive breast cancer that is found to strongly overexpress HER2, or has HER2/neu gene amplification by central testing or by a local laboratory. ... After central review, if the tumor specimen is found to strongly overexpress HER2 (3+ positivity by HercepTest™) or has HER2/neu amplification by FISH, then the patient will continue protocol treatment as randomly assigned.”

### Research Leader Commentary

We were surprised when we found poor concordance between community and central laboratory testing, in terms of both HER2 protein expression and gene amplification. Perhaps more unexpected, we found poor concordance in terms of FISH testing in a central


laboratory compared to the local laboratories. This last fact really came as a surprise, not only to us but also to many others, because the prevalent notion regarding FISH was that it was 100 percent accurate.

I've learned about these tests by spending time with our pathologists and looking at exactly what they see under the microscope with FISH. Although, theoretically, it is a matter of counting dots, it's not as simple as that — many tumors are aneuploid, some tumors have deletions of the chromosomes, and some tumors have clumping of dots in one spot. In other specimens it may be difficult to obtain the appropriate hybridization. There are some technical difficulties involved in FISH analysis.

The data from these 119 cases was so important that we actually changed the eligibility criteria for this large cooperative group trial (NCCTG-N9831). We modified the protocol so that physicians can still conduct HER2 testing based on any technology in their local laboratories. The patient is then enrolled in the study and starts the doxorubicin/cyclophosphamide (AC) portion of the chemotherapy.

During that time, we test the tumor specimens again by the HercepTest™ and the PathVysion™ FISH assay. If we find that neither of those two tests demonstrates HER2 positivity, we send the tumor specimen to another central laboratory to double-check our laboratory at the Mayo Clinic. If the other central laboratory also finds that the tumor is HER2-negative by both assays, then we notify the physician that the patient really should not participate in the trial.

*Edith A Perez, MD*



Paik S et al. **Real World Performance of HER2 Testing — National Surgical Adjuvant Breast and Bowel Project Experience.** *Journal of the National Cancer Institute* 2002;94:852-4.

- Objective**
- To determine the quality of HER2 assays performed in laboratories nationwide
- Methods**
- A central review of the first 104 cases entered into the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial was conducted.
  - Eligibility for NSABP-B-31 required a score of 3+ with the HercepTest™ [IHC assay], strong membrane staining (>33 percent of the tumor cells) with other IHC assays, or gene amplification with FISH.
  - A central laboratory assayed the tumor blocks for these cases with both the HercepTest™ and the PathVysion™ FISH assay.

Reproducibility of Community Laboratories' Results for HER2-Positive Tumor Specimens from NSABP-B-31	
Central Laboratory's Results	Percent of Cases (n=104)
Strongly positive (3+) by the HercepTest™ assay	79%
Positive for gene amplification by the PathVysion™ FISH assay	79%
Neither strongly positive (3+) by the HercepTest™ assay nor positive for gene amplification	18%

### Concordance for Central Testing

There was good concordance between central testing for FISH and HercepTest™, with agreement in 94 percent of the cases.

### Authors' Conclusions

"This brief communication provides a snapshot of the quality of HER2 assays nationwide. We found that an appreciable percentage of community-based assay results, which were used to establish the eligibility of patients to participate in NSABP-B-31, could not be confirmed when tested in a central facility.

"Our data suggest a need to improve quality control measures in laboratories that use IHC assays, including periodic testing for concordance with FISH.... Accordingly, the NSABP has amended eligibility criteria for B-31: only patients whose tumors score 3+ by IHC performed by NSABP-approved reference laboratories, or whose tumors demonstrate gene amplification by FISH from any laboratory, would be allowed entry."

### Research Leader Commentary

This was a wake-up call to clinicians in the community about how HER2 assays perform in the real world. Community laboratories don't have the same performance when compared to the "gold standard" of commercial reference laboratories. Therefore, it is important to find out who is doing the HER2 testing. Good clinicians can also take other clinical variables into account to decide about retesting.

There is a good deal of evidence showing a correlation between the number of cases one analyzes per week with IHC and assay performance, and that's where commercial laboratories win hands down. They do many more tests per week than a small hospital in rural North America. The bottom line is there is a learning curve with respect to reading IHC stains. To get to the top of the curve, you have to read a lot of them, and the only way to do that is to be in a big, busy center or in a commercial laboratory.

*Mark Pegram, MD*

There remains considerable controversy regarding the optimal method to routinely evaluate HER2 status. I won't treat a patient with metastatic breast cancer until I have a FISH assay. In the June 2002 issue of the *Journal of the National Cancer Institute*, the NSABP and the Intergroup published their experiences with HER2 assessment, and it really cast doubt about our quality control for immunohistochemistry. Until the College of American Pathologists does something to iron out this problem of quality control, I continue to use FISH.

*Charles Vogel, MD, FACP*

I assume that the tumors with a 3+ score on immunohistochemistry (IHC) are truly HER2-positive, and we do not test them further. An IHC score of 3+ is pretty reliable, as long as it is done at a laboratory that performs a lot of assays. If a tumor has a 2+ score on IHC, we test with fluorescence *in situ* hybridization (FISH). Even in patients with an IHC score of 0 or 1+ and other features of excessively aggressive disease, we may also do a FISH test.

Both the Intergroup and the NSABP study discovered that smaller community hospitals were overscoring tumors as 3+. Close to 20 percent of the 3+ scores were downstaged when they were reviewed centrally. The Intergroup protocol has now been amended to require that the patients wait for final randomization until there is a central review of their HER2 status.

I think the same things apply to FISH testing. Since FISH testing already tends to be done at more centralized laboratories, we have not yet explored the quality control issues. I suspect there will be a proliferation of FISH testing, and the reagents will go out to all the community hospitals. Even though there is probably less room for interobserver variability, the same issues will apply. I hope as the FISH technology disseminates, people will do these quality control-type studies.

At some point, it may be possible that the only test that will be done is FISH. I believe it to be more accurate and less subject to interobserver variability. I think the cost should be downplayed if it is only a difference of \$100 or \$200. However, when trastuzumab is given incorrectly for several months, that involves many thousands of dollars. It behooves us all — even from a cost standpoint — to have the most accurate test up and running.

*Debu Tripathy, MD*

Paik S et al. **Successful Quality Assurance Program for HER2 Testing in the NSABP Trial for Herceptin®.** *Breast Cancer Research and Treatment* 2002;76 (Suppl 1);Abstract 9. [San Antonio Breast Cancer Symposium Highlights Streaming Webcast. General Session I. ([www.smdn.net/smdn/sabcs/](http://www.smdn.net/smdn/sabcs/))]

- Objective**
- To assess the quality control of HER2 testing after amending the NSABP-B-31 trial to require HER2 testing from NSABP-approved laboratories. NSABP laboratory approval was based on the volume of HER2 testing (>100 HercepTest™ cases/month) or demonstration of high concordance between IHC and FISH.

- Methods**
- A central review of the first 240 cases enrolled after the protocol amendment was conducted.
  - Eligibility for NSABP-B-31 required a score of 3+ on IHC from an NSABP-approved laboratory or gene amplification with FISH from any laboratory.
  - A central laboratory assayed the tumor blocks for these cases with the PathVysion™ FISH assay.

## Results

False-Positive Rates for HER2 Tests Performed by NSABP-Approved Laboratories	
Original Assay Used By NSABP-Approved Laboratory	Central PathVysion™ FISH Assay Not Amplified
FISH (n=133)	4.5%
IHC (n=107)	2%
Total (n=240)	3%

## Authors' Conclusions

“We believe that the quality assurance program resulted in a dramatic improvement in the reliability of HER2 testing by IHC. The false-positive rate, as defined by FISH, decreased from 21 percent to 2 percent ( $p = 0.003$ ). The quality assurance program achieved the goal of reducing the false-positive rate below 10 percent, actually achieving a 3 percent overall false-positive rate.”


After laboratories underwent training from the NSABP and became certified, their accuracy went way up. Several things can be done to improve performance and reduce variability. One is to train the interpreter. Another is to have the laboratory be certified. It's very important that laboratories participate voluntarily in these quality control programs and that they use controls with every assay.

Oncologists need to be more aware of which laboratory performs the tests and who interprets the results, because it can make a huge difference. Whether it's a hospital-based laboratory or a reference laboratory, I think the oncologist should spend a lot of time getting to know their laboratories, which tests they're using, and how they read the results and interpret oncology and pathology guidelines.

*Ann D Thor, MD*

The NSABP found the discordance rate to be much lower when experienced or certified laboratories for HER2 testing are used. This is really good for clinical care, because HER2 testing is not only being done for patients potentially eligible for clinical protocols, but also in general clinical practice.

*Edith A Perez, MD*



Press MF et al. **Comparison of HER-2/Neu Status Determined by Fluorescence *In Situ* Hybridization (FISH) in the BCIRG Central Laboratories with HER-2/Neu Status Determined by Immunohistochemistry or FISH in Outside Laboratories.** *Breast Cancer Research and Treatment* 2002;76 (Suppl 1);Abstract 238.

- Objective**
- To compare the results of HER2 gene amplification determined by FISH at BCIRG central laboratories to the HER2 status determined by either IHC or FISH at outside laboratories
- Methods**
- 2,543 breast tumor specimens, submitted to either of two BCIRG central laboratories, were evaluated for HER2 gene amplification by FISH.
  - HER2 status determined by FISH in the BCIRG central laboratories was compared with the HER2 status determined in the outside laboratories.

## Results

### Agreement Rate with FISH Performed at BCIRG Central Laboratory

IHC at Outside Laboratory (n=1,608)

79%

FISH at Outside Laboratory (n=121)

92%

### Authors' Conclusions

"IHC assay methods used in outside referral laboratories have a relatively high rate of false-negative and false-positive results compare to FISH performed at centralized BCIRG reference laboratories. FISH performed at outside laboratories, on the other hand, showed a lower rate of both false-positive and false-negative results relative to FISH performed at centralized BCIRG reference laboratories."

### Research Leader Commentary

The data we presented in San Antonio described the initial results from patients screened for the Breast Cancer International Research Group clinical trials. In order to enter these clinical trials, the women needed to have tumors with HER2 gene amplification determined by FISH. Our laboratory and the laboratory of our collaborators in Switzerland are the central laboratories for screening all of the cases and determining HER2 gene amplification.

From the first 2,600 cases submitted, 2,543 had tissue samples that were acceptable for FISH characterization. We were able to obtain FISH results on 2,502 samples for a 98.4 percent success rate. Of those cases, 655 showed HER2 gene amplification, which was a 26 percent amplification rate.

Retrospectively, we requested the referring laboratories to indicate whether they had previously assessed the patients' HER2 status by any means. IHC had been performed on 1,608 cases at an outside laboratory. We were interested in how those outside laboratory determinations correlated with our central laboratory FISH analyses.

Of the cases that were classified by IHC at an outside laboratory as 0 or 1+, four percent to six percent had HER2 gene amplification as determined at our central laboratory. Approximately 79 percent of the cases that scored 3+ by IHC at an outside laboratory actually had HER2 gene amplification. Cases that scored 2+ by IHC at an outside laboratory had a HER2 gene amplification rate of about 17 percent.

There was about a 92 percent agreement between the results obtained by FISH at an

outside laboratory and FISH at our central laboratory. This was much higher than the agreement between IHC at an outside laboratory and FISH at our central laboratory.

*Michael F Press, MD, PhD*

IHC was all we initially had available for testing, but early on we saw that IHC was flawed. IHC has a false-negative rate of about 18 percent. In a good laboratory, the false-positive rate for IHC is probably a few percent; it goes up to 8 percent in general laboratories and was as high as 40 percent in some of the early reported trials.

Mike Press has data demonstrating a 52 percent concordance with the Dako HercepTest™ among Dako-approved pathologists. The College of American Pathologists has done its own study, evaluating the concordance between a central laboratory and pathologists in the community. They are seeing similar trends.

*Dennis Slamon, MD, PhD*

### **Related Publications:** *Concordance Between Local and Central Laboratory HER2 Testing*

Anderson S et al. **Concordance between local labs and a central lab using FISH and IHC for HER2 testing.** *Breast Cancer Res Treat* 2002;Abstract 235.

Cianciulli AM et al. **Contribution of fluorescence in situ hybridization to immunohistochemistry for the evaluation of HER-2 in breast cancer.** *Cancer Genet Cytogenet* 2002;133(1):66-71.

Ganberg D et al. **Evaluation of HER-2/NEU protein expression in breast cancer by immunohistochemistry: An interlaboratory study assessing the reproducibility of HER-2/NEU testing.** *Breast Cancer Res Treat* 2002;74(2):113-20

Hsu CY et al. **Interobserver reproducibility of Her-2/neu protein overexpression in invasive breast carcinoma using the DAKO HercepTest™.** *Am J Clin Pathol* 2002;118(5):693-8.

Larsimont D et al. **HER-2/neu evaluation by immunohistochemistry and fluorescence in situ hybridization in breast cancer: implications for daily laboratory practice.** *Anticancer Res* 2002;22(4):2485-90.

McCormick SR et al. **HER2 assessment by immunohistochemical analysis and fluorescence in situ hybridization: comparison of HercepTest™ and PathVysion commercial assays.** *Am J Clin Pathol* 2002;117(6):935-43.

Owens MA. **Correlation of HER-2/neu immunohistochemistry with fluorescence in situ hybridization in 3,568 breast cancers.** *Breast Cancer Res Treat* 2002;Abstract 236.

Persons DL et al. **Fluorescence in situ hybridization (FISH) for detection of HER-2/neu amplification in breast cancer: A multicenter portability study.** *Ann Clin Lab Sci* 2000;30(1):41-8.

Santinelli A et al. **Immunohistochemical evaluation of HER-2/neu expression in infiltrating breast carcinoma: a study of reproducibility.** *Anal Quant Cytol Histol* 2002;24(1):54-62.

Thomson TA et al. **HER-2/neu in breast cancer: Interobserver variability and performance of immunohistochemistry with 4 antibodies compared with fluorescent in situ hybridization.** *Mod Pathol* 2001;14(11):1079-86.

Zujewski JA. **"Build quality in" — HER2 testing in the real world.** *J Natl Cancer Inst* 2002;94(11):788-9.



# Comparison of HER2 Assays

Press MF et al. **Evaluation of HER-2/neu Gene Amplification and Overexpression: Comparison of Frequently Used Assay Methods in a Molecularly Characterized Cohort of Breast Cancer Specimens.** *Journal of Clinical Oncology* 2002;20:3095-105.

**Objective** • To compare the results from four FDA-approved assay methods and two other immunohistochemical assays used to test for HER2 status

**Methods** • 117 breast tumor specimens, previously molecularly characterized for HER2 gene amplification and protein overexpression, were evaluated for HER2 gene amplification by FISH with the PathVysion™ assay and Inform® HER2/neu assay.

• The same specimens were also evaluated for HER2 protein overexpression by IHC with the HercepTest™ assay, Pathway™ HER2 assay, R60 polyclonal antibody and 10H8 monoclonal antibody.

## Results

Accuracy of HER2 Results with Several FISH and IHC Assays of 117 Breast Tumor Specimens, Previously Molecularly Characterized for HER2 Gene Amplification and Protein Overexpression

FISH	Accuracy
PathVysion™	97.4%
Inform® HER2/neu	95.7%
IHC	
R60 polyclonal antibody	96.6%
10H8 monoclonal antibody	95.7%
Pathway™ HER2	89.7%
HercepTest™	88.9%

## Authors' Conclusions

“Our findings demonstrated that the FISH assays have higher sensitivity and higher accuracy and more often correctly identify altered HER-2/neu status (amplification/overexpression) in previously molecularly characterized specimens than did the FDA-approved immunohistochemistry assays interpreted manually.”

This analysis actually compared several different reagents. Press and colleagues used a polyclonal antibody and compared it to some monoclonal antibodies, including 10H8 (not yet widely used and not FDA-approved), Ventana's CB-11, and the Dako HercepTest™. I caution that one can alter the sensitivity or specificity of any reagent, so a ranking that's slightly (2% or 3%) better should be taken with a grain of salt. It sounds like the antibodies are pretty much in same ballpark.

Ann D Thor, MD

Perez EA et al. **HER2 Testing in Patients with Breast Cancer: Poor Correlation Between Weak Positivity by Immunohistochemistry and Gene Amplification by Fluorescence *In Situ* Hybridization.** *Mayo Clinic Proceedings* 2002;77:148-54.

**Objective** • To evaluate HER2 gene amplification by FISH in tumors with weak positive (2+) IHC staining

**Methods** • 1,556 breast tumor specimens were evaluated for HER2 status with the HercepTest™ assay at the Mayo Medical Laboratories in Rochester, Minnesota.

• Specimens scored as 2+ by IHC were routinely evaluated for HER2 gene amplification FISH with the PathVysion™ assay.

## Results

- 216 (14%) specimens were scored as 2+ by IHC and evaluated by FISH.
- 12 percent of the specimens that scored 2+ had HER2 gene amplification.

## Authors' Conclusions

"Our current recommendation is to use IHC as the initial screening test for HER2 and to perform reflex FISH testing when the tumor specimen has a 2+ IHC result. This recommendation uses the least expensive laboratory method (IHC) as a 'screening' tool and reflexes to the more expensive method for the appropriate subset of patients (those whose specimens have a 2+ IHC score). ...The decision to test by FISH in tumors scored 0, 1+, or 3+ by IHC or to use only FISH is one that will be answered after completion of additional research studies."

For practical and economic reasons, we initially tested all tumor specimens from patients with invasive breast cancer utilizing the HercepTest™. Then, if the tumors scored 2+, we automatically proceeded to testing by FISH based on the understanding that 12 percent of those specimens would be expected to have gene amplification.

One question that has been raised is, “Why not test everyone with FISH initially?” Because the great majority of tumor specimens are HER2-negative, we felt it was more practical to utilize the less expensive test initially. At the same time, in terms of the benefit from trastuzumab therapy, we realized that we don’t understand the real implications for patients with tumors that score 2+ on IHC and are FISH-positive.

*Edith A Perez, MD*

If one wants to know whether a patient has the HER2 alteration, one should do FISH testing. One should not do a default IHC and only if the tumor scores 2+, then do FISH. Using that algorithm, patients without the HER2 alteration will be treated with trastuzumab, and other patients with the HER2 alteration may not be treated.

The BCIRG trial we are conducting was designed with FISH as the only criteria for assessing HER2 status. I think the day when FISH testing is the only assay used in the community is coming, and I hope it will be sooner, rather than later.

*Dennis Slamon, MD, PhD*

Every patient with metastatic breast cancer in my practice has her tumor evaluated for HER2 gene amplification by FISH. Tumors with an IHC score of 3+ should be evaluated by FISH, because they may not have gene amplification. In those with an IHC score of 0 or 1+, 3 percent and 7 percent, respectively, will have HER2 gene amplification by FISH. We need to determine HER2 status accurately, because it is a matter of life or death.

*Melody Cobleigh, MD*


Tumors that score 2+ IHC are frequently found to be HER2-negative when tested by FISH. In those patients, I routinely have their tumors retested by FISH. On the other hand, I do not obtain a FISH analysis for tumors that score 3+ on IHC performed at a laboratory where I trust the pathologist.

Since HER2-positive breast cancer has a fairly specific phenotype (i.e., steroid receptor-negative, younger age, early relapse), I will retest those types of patients by FISH if I have a two- to three-year-old IHC score of 0 or 1+. If the patient’s tumor is IHC-negative and FISH-positive, I will treat them with trastuzumab despite the fact that we do not have clinical data for that group of patients. Tumors that are FISH-positive are likely to have

ample amounts of HER2 receptors on their cell surface.

We lack quality control for both IHC and FISH. This is analogous to the situation encountered with estrogen receptor testing in the mid- to late 1970s. One wonders how many patients died because they did not receive adjuvant tamoxifen as a result of inadequate estrogen receptor testing. If adjuvant trastuzumab provides a benefit like adjuvant tamoxifen, we may encounter the same problem.

*George Sledge, MD*



Rhodes A et al. **Evaluation of HER-2/neu Immunohistochemical Assay Sensitivity and Scoring on Formalin-Fixed and Paraffin-Processed Cell Lines and Breast Tumors: A Comparative Study Involving Results from Laboratories in 21 Countries.** *American Journal of Clinical Pathology* 2002; 118(3):408-17.

**Objective**

- To assess the accuracy of different IHC antibodies and methods to test for HER2 protein overexpression in various laboratories on two separate occasions

**Methods**

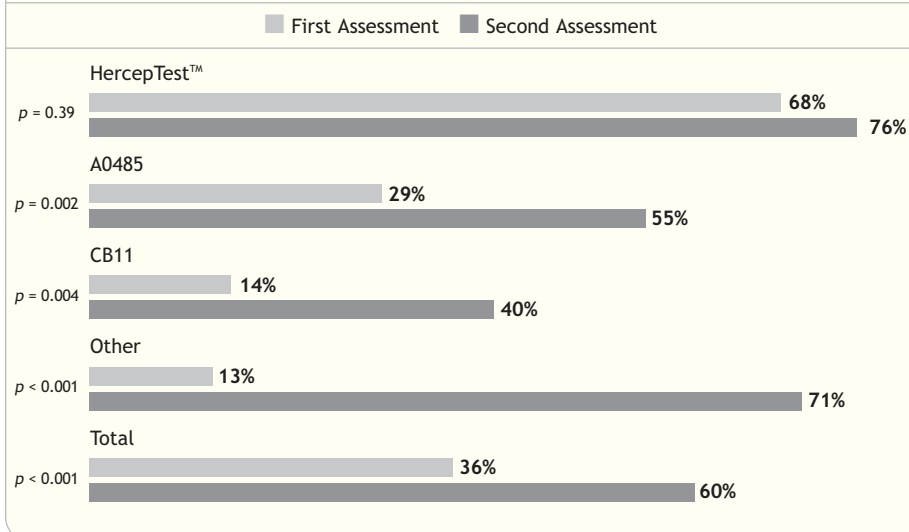
- Laboratories participating in the United Kingdom National External Quality Assessment Scheme for Immunohistochemistry were provided with three breast carcinoma cell lines (MDA-MB-453, BT-20, MCF-7) and an ovarian carcinoma cell line (SKOV-3) that had differing levels of HER2 protein expression and known HER2 gene amplification.
- Each laboratory evaluated the staining of each cell line for HER2 protein expression with the IHC assay they routinely used.

## Results

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- 94 laboratories from 21 countries participated in the first assessment
- 93 laboratories participated in the second assessment
- 78 laboratories participated in both assessments

## Proportion of the 78 Laboratories Participating in Both Assessments That Achieved Appropriate Results with Different IHC Assays



### Authors' Conclusions

"The proportion of laboratories achieving appropriate results with the HercepTest™ was significantly higher than for any other assay in both assessment runs... . The results also show a dramatic and significant increase in the proportion of laboratories achieving an appropriate result on the cell lines in the second assessment run. However, the antibodies used by laboratories participating in both assessment runs were very similar, indicating that that improvement is not due to laboratories switching to the HercepTest™ but due to laboratories improving their existing assays. Indeed, the only significant improvement seen between one assessment run and the next was noted with laboratories using assays other than the HercepTest™."

### Research Leader Commentary

This article looks at IHC sensitivity and scoring across laboratories in 21 countries. It highlights the need for standardization, stringent quality controls and ongoing quality assurance programs.

Reagents can lose reactivity when they sit around in a refrigerator. Low-volume laboratories may have a reagent sitting in a refrigerator for six months before it passes its end date. One doesn't know whether the reagent was left on the counter and lost some of its sensitivity.

The controls used in many laboratories are other tumors that were strongly positive. The problem with that is that strongly-positive tumors will stay strongly positive, or at least moderately positive, even if the reagent has lost some sensitivity. It's the weakly-positive tumors, or those 2+ control cell lines, that will lose their reactivity if the reagent goes bad.

*Ann D Thor, MD*


In two trials, the patients with IHC 3+ and FISH-negative disease had a response rate of zero to trastuzumab-based therapy. In one trial, two patients with IHC 3+ and FISH-negative disease responded; those cases need to be reanalyzed to make sure they are indeed FISH-negative. Since blocks were never kept, they had to use stained slides, take the cover slips off, unstain the slides and then do the FISH test.

Patients with IHC 0 or 1+ and FISH-positive disease are HER2-positive. By the traditional HercepTest™, those patients would have never received trastuzumab. This problem with IHC is a function of the fixation of the tumor when it goes into formalin.

If we had frozen material from all patients at the time of diagnosis, IHC would be just fine. The problem occurs because formalin works by cross-linking proteins. HER2 is a protein that is progressively cross-linked. The longer the tissue is in the formalin, the more epitope cross-linking and masking occurs.

Cross-linking to other proteins covers up the HER2 epitope that is detected by the antibody. Dako has tried to introduce antigen retrieval to make that better. Although one can put all kinds of fancy scanners onto the tissue, if one does not control the fixation of the tissue, there is no way one can control what is tested.

*Dennis Slamon, MD, PhD*



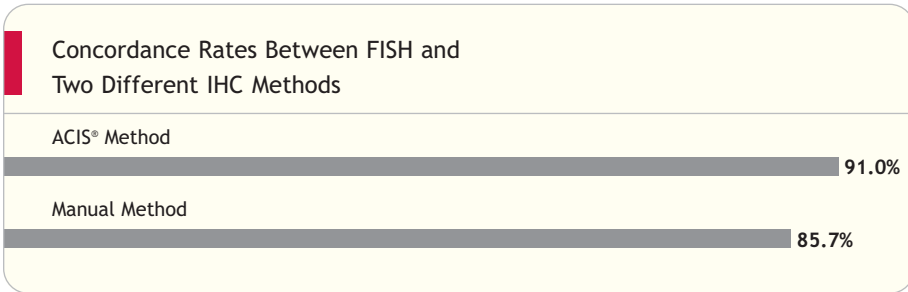
Wang S et al. **Assessment of HER-2/neu Status in Breast Cancer. Automated Cellular Imaging System (ACIS)-Assisted Quantitation of Immunohistochemical Assay Achieves High Accuracy in Comparison with Fluorescence *In Situ* Hybridization Assay As the Standard.** *American Journal of Clinical Pathology* 2001;116:495-503.

**Objective**

- Using the PathVysion™ FISH assay as the standard, compare the manual and the Automated Cellular Imaging System (ACIS®) IHC methods

**Methods**

- All infiltrating breast cancer specimens collected between August 1998 and March 2000 were evaluated by manual IHC with a polyclonal antibody (A0485).
- The PathVysion™ FISH assay was used to analyze 199 of those specimens.
- Quantitation of IHC staining by ACIS® was conducted on 189 of the 199 cases analyzed by FISH.



### Authors' Conclusions

"The correlation between FISH and the ACIS® method was statistically higher than that between FISH and the manual method."

"The present study demonstrates that an image analyzer, such as ACIS®, can be applied to quantitate protein on cell membrane. Compared with the manual immunohistochemical method, there are major benefits of using such an image analyzer to quantitate immunohistochemical staining."

### Research Leader Commentary

It really looks like IHC testing should remain in the purview of central reference laboratories. One of the reasons may be that a number of the large reference laboratories are now using digital image analysis for all of their IHC scoring. Digital image analysis takes some of the guesswork out of the interpretation of these IHC assays. There can be honest disagreement between good pathologists over the difference between a 2+ and a 3+, but a computer can actually read the same slide over and over again and give you the exact same result.

Pathologists actually call up the information on a digital screen to confirm and double check the assay performance. In most of the large studies in which head-to-head comparisons have been done with digital image analysis and FISH for HER2 testing, the concordance rate is about of 90 percent.

The remarkable feature of this type of assay is you can take a slide, score it using the digital assay system, and then read it a hundred years later and get the same answer. It's really highly reproducible in terms of run-to-run variability, much more than the manual read-out for IHC. Even if you give a good pathologist a test set of slides to read and ask them to re-read the same set some time later, there will be some variability that you can't control. This instrument removes that type of variability.

*Mark Pegram, MD*

## Related Publications: Comparison of HER2 Assays

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Couturier J et al. **Strong correlation between results of fluorescent in situ hybridization and immunohistochemistry for the assessment of the ERBB2 (HER-2/neu) gene status in breast carcinoma.** *Mod Pathol* 2000;13(11):1238-43.

Field AS et al. **Suggestions for HER-2/neu testing in breast carcinoma, based on a comparison of immunohistochemistry and fluorescence in situ hybridisation.** *Pathology* 2001;33(3):278-82.

Larsimont D et al. **HER-2/neu evaluation by immunohistochemistry and fluorescence in situ hybridization in breast cancer: Implications for daily laboratory practice.** *Anticancer Res* 2002;22(4):2485-90.

McCormick SR et al. **HER2 assessment by immunohistochemical analysis and fluorescence in situ hybridization: Comparison of HercepTest™ and PathVysion commercial assays.** *Am J Clin Pathol* 2002;117(6):935-43.

Ridolfi RL et al. **HER-2/neu testing in breast carcinoma: A combined immunohistochemical and fluorescence in situ hybridization approach.** *Mod Pathol* 2000;13(8):866-73.

Tubbs RR et al. **Discrepancies in clinical laboratory testing of eligibility for trastuzumab therapy: Apparent immunohistochemical false-positives do not get the message.** *J Clin Oncol* 2001;19(10):2714-21.

Bartlett J et al. **The clinical evaluation of HER-2 status: Which test to use?** *J Pathol* 2003;199(4):411-7.

Cianciulli AM et al. **Contribution of fluorescence in situ hybridization to immunohistochemistry for the evaluation of HER-2 in breast cancer.** *Cancer Genet Cytogenet* 2002;133(1):66-71.

Dowsett M et al. **Correlation between immunohistochemistry (HercepTest) and fluorescence in situ hybridization (FISH) for HER-2 in 426 breast carcinomas from 37 centres.** *J Pathol* 2003;199(4):418-23.

Gupta D et al. **Comparison of fluorescence and chromogenic in situ hybridization for detection of HER-2/neu oncogene in breast cancer.** *Am J Clin Pathol* 2003;119(3):381-7.

Konigshoff M et al. **HER-2/neu gene copy number quantified by real-time PCR: Comparison of gene amplification, heterozygosity, and immunohistochemical status in breast cancer tissue.** *Clin Chem* 2003;49(2):219-29.

Press MF et al. **Evaluation of HER-2/neu gene amplification and overexpression: Comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens.** *J Clin Oncol* 2002;20(14):3095-105.

Thomson TA et al. **HER-2/neu in breast cancer: Interobserver variability and performance of immunohistochemistry with 4 antibodies compared with fluorescent in situ hybridization.** *Mod Pathol* 2001;14(11):1079-86.

Yaziji H et al. **Testing for HER-2/neu in breast cancer: Is fluorescence in situ hybridization superior in predicting outcome?** *Adv Anat Pathol* 2002;9(6):338-44.



# Concordance of HER2 Status Between Primary and Metastatic Lesions

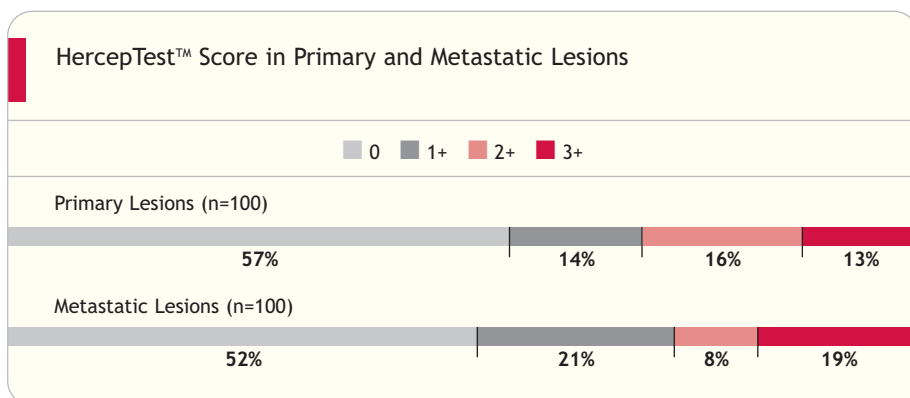
Gancberg D et al. **Comparison of HER-2 Status Between Primary Breast Cancer and Corresponding Distant Metastatic Sites.** *Annals of Oncology* 2002;13:1036-43.

- Objectives**
- To compare HER2 overexpression and amplification in primary tumors and their distant metastases
  - To evaluate the HER2 status in different metastatic sites from the same patient

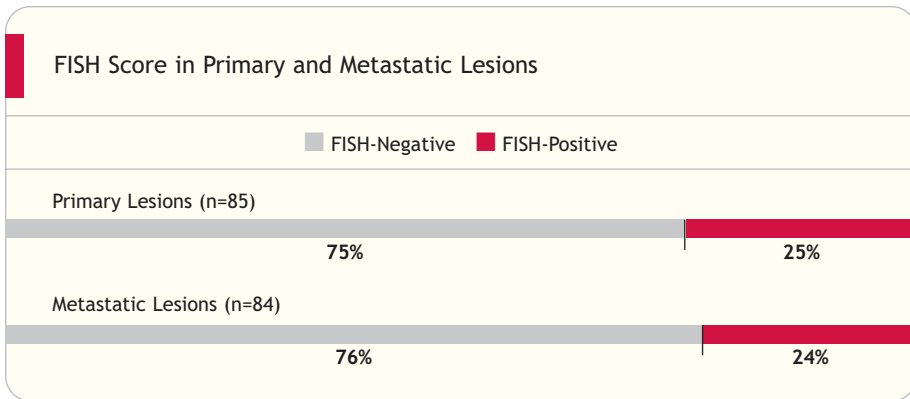
- Methods**
- 107 primary breast tumors and their corresponding distant metastases were analyzed by IHC, using the HercepTest™, and by FISH.
  - HER2 status was also evaluated in 17 patients with at least 2 samples from metastatic lesions.

## Results

- The time between the removal of the primary tumor and the biopsy of the metastatic lesion ranged from one month to 18 years.



- The discordance rate for HER2 overexpression between the primary and metastatic lesions was six percent. All six cases demonstrated greater HER2 overexpression in the metastatic lesion compared to the primary lesion.
- The discordance rate for HER2 overexpression between different metastatic lesions was 18 percent.



- The discordance rate with FISH between the primary and metastatic lesions was seven percent. Three cases were FISH-positive in the metastatic lesion but not in the primary lesion, and two cases were FISH-positive in the primary lesion but not in the metastatic lesion.
- The discordance rate with FISH between different metastatic lesions was 19 percent.

### Authors' Conclusions

“This study does not support the routine testing of metastases to confirm HER-2 positivity when detected in the primary tumour, particularly if results obtained by FISH are available. Assessment of HER-2 status in one of the metastatic sites may be worthwhile only in some patients with easily accessible metastases and for whom HER-2 evaluation by IHC, recently performed in a primary tumour sample collected many years before, shows a negative score. An alternative solution would be the determination of HER-2 amplification by FISH in the primary tumour sample.”

### Research Leader Commentary

The authors concluded that there’s no reason to retest metastases. For me, that would depend on the patient. If I had a patient with a HER2-negative primary tumor who had exhausted all avenues of treatment and wanted to try trastuzumab, I would try to biopsy a metastatic lesion to determine if it was positive because the tumor might respond to trastuzumab. Even if it were a 10 percent chance, I would take it in an individual patient who was motivated and was not responding to other therapies. Trastuzumab has relatively low toxicity and, in some cases, it has shown significant benefit.

*Ann D Thor, MD*

In our experience, it is highly unusual for the HER2 status to be altered during the development of the cancer. It is also very rare for us to find disagreement between the HER2 status of the invasive disease and the carcinoma *in situ* in the same patient. This is also true when we compare the primary tumor to the lymph-node metastasis.

In general, the HER2 status is quite similar or the same with only rare exceptions. In some of those exceptions, the morphologic appearance of the metastasis appears to be different, as if the tumor either developed new characteristics or was developed from an independent primary tumor.

Michael F Press, MD, PhD

**Related Publications:** *Concordance of HER2 Status Between Primary and Metastatic Lesions*

Cardoso F et al. **Evaluation of HER2, p53, bcl-2, topoisomerase II-alpha, heat shock proteins 27 and 70 in primary breast cancer and metastatic ipsilateral axillary lymph nodes.** *Ann Oncol* 2001;12(5):615-20.

Dittadi R et al. **Evaluation of HER-2/neu in serum and tissue of primary and metastatic breast cancer patients using an automated enzyme immunoassay.** *Int J Biol Markers* 2001;16(4):255-61.

Masood S, Bui MM. **Assessment of Her-2/neu overexpression in primary breast cancers and their metastatic lesions: An immunohistochemical study.** *Ann Clin Lab Sci* 2000;30(3):259-65.

Niehans GA et al. **Stability of HER-2/neu expression over time and at multiple metastatic sites.** *J Natl Cancer Inst* 1993;85(15):1230-5.

Sekido Y et al. **Heterogeneous gene alterations in primary breast cancer contribute to discordance between primary and asynchronous metastatic/recurrent sites: HER2 gene amplification and p53 mutation.** *Int J Oncol* 2003;22(6):1225-32.

Simon R et al. **Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer.** *J Natl Cancer Inst* 2001;93(15):1141-6.

Tanner M et al. **Amplification of HER-2/neu and topoisomerase IIalpha in primary and metastatic breast cancer.** *Cancer Res* 2001;61(14):5345-8.

Tuziak T et al. **Expression of HER2/neu in primary and metastatic breast cancer.** *Pol J Pathol* 2001;52(1-2):21-6.

Vincent-Salomon A et al. **HER2 status in patients with breast carcinoma is not modified selectively by preoperative chemotherapy and is stable during the metastatic process.** *Cancer* 2002;94(8):2169-73.

# HER2 Status and Response To Trastuzumab

Slamon DJ et al. **Use of Chemotherapy Plus a Monoclonal Antibody Against HER2 for Metastatic Breast Cancer that Overexpresses HER2.** *New England Journal of Medicine* 2001;344:783-92.

**Objective**

- To compare the time to disease progression, incidence of adverse effects, rates and duration of responses, time to treatment failure, and overall survival for chemotherapy plus trastuzumab and chemotherapy alone in women with metastatic breast cancer

**Eligibility**

- Metastatic breast cancer, previously untreated with chemotherapy
- HER2 2+ or 3+ measured by the Clinical Trial Assay (CTA)

**Schema**

ARM 1: Chemotherapy\* q 3 weeks x 6  
 ARM 2: Chemotherapy\* q 3 weeks x 6 plus trastuzumab q week

\*Chemotherapy = AC, if no prior adjuvant anthracycline, or paclitaxel if patient received prior adjuvant anthracycline-based chemotherapy

Trastuzumab was continued until disease progression. Upon disease progression, 66 percent of the women elected to receive trastuzumab alone or in combination with other therapies.

## Results

Time to Disease Progression, Survival Rate and Duration of Response for Trastuzumab Plus Chemotherapy Compared to Chemotherapy Alone						
	AC + trastuzumab (n=143)	AC (n=138)	p value	Paclitaxel + trastuzumab (n=92)	Paclitaxel (n=96)	p value
Median time to disease progression (months)	7.8	6.1	<0.001	6.9	3.0	<0.001
Median survival (months)	26.8	21.4	0.16	22.1	18.4	0.17
Complete and partial response rate	56%	42%	0.02	41%	17%	<0.001
Median duration of response (months)	9.1	6.7	<0.005	10.5	4.5	<0.01

AC=anthracycline plus cyclophosphamide

**Treatment Effect According to Level of HER2 Expression in the Pivotal Trial:  
Trastuzumab Plus Chemotherapy Compared to Chemotherapy Alone**

HER2 Assay Result	Number of Patients	Relative Risk** for Time to Disease Progression (95% CI)	Relative Risk** for Mortality (95% CI)
CTA 2+ or 3+	469	0.49 (0.40, 0.61)	0.80 (0.64, 1.00)
FISH (+)*	325	0.44 (0.34, 0.57)	0.70 (0.53, 0.91)
FISH (-)*	126	0.62 (0.42, 0.94)	1.06 (0.70, 1.63)
CTA 2+	120	0.76 (0.50, 1.15)	1.26 (0.82, 1.94)
FISH (+)	32	0.54 (0.21, 1.35)	1.31 (0.53, 3.27)
FISH (-)	83	0.77 (0.48, 1.25)	1.11 (0.68, 1.82)
CTA 3+	349	0.42 (0.33, 0.54)	0.70 (0.51, 0.90)
FISH (+)	293	0.42 (0.32, 0.55)	0.67 (0.51, 0.89)
FISH (-)	43	0.43 (0.20, 0.94)	0.88 (0.39, 1.98)

CTA= Clinical Trial Assay

\* FISH testing results were available for 451 of the 469 patients enrolled in the study.

\*\* The relative risk represents the risk of progression or death in the trastuzumab plus chemotherapy arm compared to the chemotherapy-alone arm.

Source: Herceptin® Package Insert

### Authors' Conclusions

“We found that trastuzumab-based combination therapy was effective in that it reduced the relative risk of death by 20 percent at a median follow-up of 30 months.... Particularly noteworthy is that two-thirds of patients who were initially assigned to receive chemotherapy alone began, after disease progression, to receive open-label trastuzumab alone or with chemotherapy. Such a crossover design would generally reduce the likelihood that a survival advantage would be found. Significant increases in the time to disease progression, the rates of response, the duration of responses and the time to treatment failure were observed in both subgroups that were given chemotherapy plus trastuzumab. These results increased survival, an end point free of ascertainment bias.”

### Research Leader Commentary

We used a combination of an anthracycline and cyclophosphamide, which was commonly used as first-line therapy in metastatic disease. For those patients who had received adjuvant doxorubicin, paclitaxel was utilized. Essentially, the patients were randomized to the best available standard chemotherapy plus or minus trastuzumab.

In the Phase III trial, the addition of trastuzumab led to a significant improvement in response rate, response duration and time to progression. A little-known fact from that trial is that the highest response rate was seen in the anthracycline/cyclophosphamide and trastuzumab arm. Paclitaxel/trastuzumab was ultimately included in the package label because of the toxicity encountered with the other arm.

We were very encouraged with the improvement in the median time to progression for the group receiving trastuzumab. Although it is only a three-month improvement, it translates, ultimately, into a survival advantage. At four years of follow-up, trastuzumab decreases the relative risk of death by 30 percent in women with truly HER2-positive breast cancer — those which are FISH-positive.

Dennis Slamon, MD, PhD

Mass RD et al. **Improved Survival Benefit from Herceptin (Trastuzumab) in Patients Selected by Fluorescence *In Situ* Hybridization (FISH).** *Proceedings American Society of Clinical Oncology* 2001;Abstract 85.

**Objective** • To determine the influence of HER2 gene amplification on the clinical benefit of trastuzumab in combination with chemotherapy by re-analyzing tissue from the pivotal trial by Slamon and colleagues

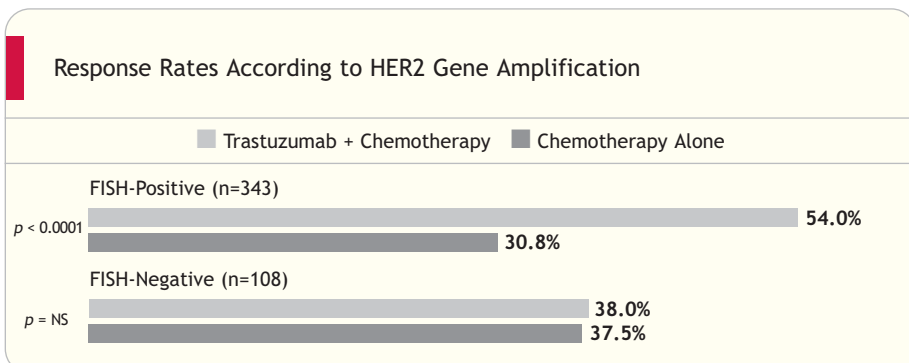
**Eligibility** • Metastatic breast cancer, previously untreated with chemotherapy  
• HER2 2+ or 3+ measured by the Clinical Trial Assay (CTA)  
• FISH testing for HER2 gene amplification

**Schema** ARM 1: Chemotherapy\* q 3 weeks x 6  
ARM 2: Chemotherapy\* q 3 weeks x 6 plus trastuzumab q week

\*Chemotherapy = AC, if no prior adjuvant anthracycline, or paclitaxel if patient received prior adjuvant anthracycline-based chemotherapy

Trastuzumab was continued until disease progression. Upon disease progression, 66 percent of the women elected to receive trastuzumab alone or in combination with other therapies.

## Results



## Authors' Conclusions

"Patient selection based on HER2/neu amplification by FISH may predict improved clinical benefit from the addition of H [Herceptin®] to C [chemotherapy] compared to selection by IHC (2+/3+). This includes a substantial survival benefit. This data supports FISH testing for selecting patients for Herceptin therapy."

### Research Leader Commentary

In our previous studies, FISH was the most accurate method for assessing HER2 status, and commercially-available FDA-approved IHC assays were significantly less accurate. We collaborated to re-evaluate the tissue from women who had been enrolled in the trastuzumab pivotal trials.

We found somewhere between one out of four and one out of five women entered into those trials, based upon IHC, actually did not have HER2 gene amplification. In other words, there were false-positive results by IHC. When we analyzed the data, FISH was found to be a stronger predictor of response to trastuzumab.

*Michael F Press MD, PhD*

Vogel CL et al. **Efficacy and Safety of Trastuzumab as a Single Agent in First-Line Treatment of HER2-Overexpressing Metastatic Breast Cancer.** *Journal of Clinical Oncology* 2002;20:719-26.

- Objective**
- To assess the activity and safety of two different trastuzumab doses as first-line therapy in women who did not want to receive chemotherapy for metastatic breast cancer
- Eligibility**
- Metastatic breast cancer, previously untreated with chemotherapy
  - HER2 overexpression (2+ or 3+) measured by IHC with two murine monoclonal antibodies (4D5 and CB11)
- Schema**
- ARM 1: Trastuzumab 4 mg/kg → 2 mg/kg weekly  
ARM 2: Trastuzumab 8 mg/kg → 4 mg/kg weekly

Response Rates for Single-Agent Trastuzumab In Women with HER2-Overexpressing Metastatic Breast Cancer: A Subset Analysis		
	Objective Response n (%)	Clinical Benefit* n (%)
Overall Response (n=111)	29 (26%)	42 (38%)
Complete Response	7 (6%)	
Partial Response	22 (20%)	
Trastuzumab Dose		
2 mg/kg per week (n=58)	14 (24%)	20 (34%)
4 mg/kg per week (n=53)	15 (28%)	22 (42%)
IHC		
3+ (n=84)	29 (35%)	40 (48%)
2+ (n=27)	0	2 (7%)
FISH		
Positive (n=79)	27 (34%)	38 (48%)
Negative (n=29)	2 (7%)	3 (10%)

\* Complete response + partial response + minor response + ≥ 6 months stable disease

**Authors’ Conclusions**

“The results of this trial indicate that trastuzumab is active as a single agent and produces durable objective responses in women with HER2-overexpressing breast cancer who have not previously received chemotherapy for their metastatic disease.... Although an accurate assessment of the median duration of response was not possible because of censoring, 57% of the responding patients were known to be free of disease progression at 12 months or more of follow-up, underscoring the durability of the responses.”

“The higher dose of trastuzumab showed no apparent benefit over the standard dose based on the efficacy end points in this relatively small trial... . The data from this and the trastuzumab pivotal trials suggest that FISH is a superior method for selecting patients likely to benefit from trastuzumab therapy.”

*Research Leader Commentary*

It became readily apparent to me early on that there was a subset of women with metastatic HER2-positive disease who really did not want to receive chemotherapy up front, so I lobbied for having a first-line, single-agent trastuzumab trial. Many other investigators — including Melody Cobleigh and Debu Tripathy — were also very instrumental in moving this concept forward. So, this was really the third major initial trial to look at what trastuzumab could do in metastatic breast cancer. All of these were basically proof-of-principle trials.



Our trial was a Phase II study, and we accrued 114 patients. The patients were quite gratified because they were treated with a relatively nontoxic form of therapy, at least from the standpoint of subjective toxicities.

The overall, published response rate for all the patients with IHC 2+/3+, HER2-positive disease was 26 percent. We've subsequently learned that there is a very high false-positive rate for the patients with IHC 2+ disease. Consequently, further analyses were done using only the patients with IHC 3+ disease, and ultimately, the patients with FISH-positive disease.

Another interesting outcome measurement is prolonged stable disease, because it seemed that patients were responding to trastuzumab more like they would to hormonal therapy than to chemotherapy. We were seeing prolonged periods of disease stabilization, even though we weren't able to objectively record definitive responses, as classically defined. So, we also evaluated the group of patients with prolonged stable disease for greater than six months. In the group of patients with FISH-positive disease, if you add the patients with prolonged stable disease to those with objective responses, about half the patients responded to first-line, single-agent trastuzumab.

I use single-agent trastuzumab in a similar manner as hormonal therapy. There are subsets of women with HER2-positive disease who don't have horribly aggressive metastatic breast cancer. In those relatively asymptomatic patients who do not have visceral crisis or rapidly progressive disease and are not incapacitated by symptoms, I have no problem at all starting them on first-line, single-agent trastuzumab. However, the patients must be fully informed that they may be giving away something in terms of response rate, based on an analysis of crosstrial comparisons with the combination regimens.

*Charles Vogel, MD, FACP*

Until a few years ago, the only other option for a woman with HER2-positive indolent disease that was nonetheless progressing would have been chemotherapy. The toxicities of many chemotherapy agents would make me less enthusiastic about this approach. The availability of single-agent trastuzumab changes the playing field. In this type of patient, I would feel most justified in using single-agent trastuzumab.

Randomized trial data clearly shows a time-to-progression and survival advantage for chemotherapy plus trastuzumab compared to chemotherapy alone, and no data demonstrates that trastuzumab alone is equivalent to trastuzumab plus chemotherapy. There is indirect data, however, suggesting that trastuzumab can be initiated, and if there is disease progression, chemotherapy can subsequently be started while continuing the trastuzumab, without any real loss of apparent benefit.

From Chuck Vogel's data there is good evidence that in patients with HER2-positive (FISH-

positive or IHC 3+) metastatic disease, single-agent trastuzumab before chemotherapy is comparable to conventional chemotherapy. That data provides me with the basis for using single-agent trastuzumab.

*Clifford Hudis, MD*

Trastuzumab monotherapy is an attractive therapeutic approach. It is analogous to the use of sequential single-agent endocrine therapy for indolent metastatic disease. HER2-positive tumors are not necessarily always aggressive. Chuck Vogel demonstrated a very acceptable response rate and clinical benefit with single-agent trastuzumab. Howard Burris and his colleagues gave trastuzumab up front and used chemotherapy in those whose disease failed to respond or progressed. This is a reasonable strategy and should be considered in appropriately chosen patients.

*Nicholas Robert, MD*



Esteva FJ et al. **Phase II Study of Weekly Docetaxel and Trastuzumab for Patients with HER2-Overexpressing Metastatic Breast Cancer.** *Journal of Clinical Oncology* 2002;20:1800-8.

- Objectives**
- To assess the safety and response rate of trastuzumab plus docetaxel in women with HER2-overexpressing metastatic breast cancer
  - To evaluate the role of circulating HER2 extracellular domain (ECD) concentration as a predictor of response to trastuzumab therapy

- Eligibility**
- Metastatic breast cancer previously treated with no more than three chemotherapy regimens
  - HER2-overexpression assessed by IHC with the monoclonal antibody, e2-4001, or by FISH with the PathVysion™ FISH assay

**Schema** (Trastuzumab + docetaxel) q week x 3 every 4 weeks

*Treatment was continued until disease progression or the appearance of prohibitively toxic effects.*

## Results

- The estimated median time to progression was nine months.
- There was a longer time to progression in patients receiving weekly trastuzumab plus docetaxel as first-line therapy than in patients receiving it as second-line therapy.

Response Rates for Weekly Trastuzumab Plus Docetaxel in Patients with Metastatic Breast Cancer According to HER2 Status		
	Partial Response	p value
IHC (n=24)		
0-2+	3/5 (60%)	0.99
3+	12/19 (63%)	
FISH (n=28)		
Negative	2/4 (50%)	0.60
Positive	16/24 (67%)	
Baseline Serum HER2 ECD (n=30)		
Negative	3/9 (33%)	0.04
Positive	16/21 (76%)	

### Authors' Conclusions

"Weekly administration of docetaxel and trastuzumab is safe and effective for patients with metastatic breast cancer whose tumors overexpress HER-2. Further research is warranted to determine the value of serum HER-2 ECD testing in selecting and monitoring patients undergoing trastuzumab-based therapy."

Robert N et al. **Phase III Comparative Study of Trastuzumab and Paclitaxel with and without Carboplatin in Patients with HER-2/neu-Positive Advanced Breast Cancer.** *Breast Cancer Research and Treatment* 2002;76 (Suppl 1);Abstract 35. [San Antonio Breast Cancer Symposium Highlights Streaming Webcast. General Session II. ([www.smdn.net/smdn/sabcs/](http://www.smdn.net/smdn/sabcs/))]

### Objective

- To compare the response rates, times to progression, survivals and toxicities of trastuzumab/paclitaxel/carboplatin (TPC) with trastuzumab/paclitaxel (TP) in patients with HER2-positive advanced breast cancer

- Eligibility**
- Metastatic breast cancer, previously untreated with chemotherapy
  - HER2-overexpression (2+ or 3+) assessed by IHC with the HercepTest™
  - Tumors that were 2+ also had to have gene amplification measured by FISH

- Schema**
- ARM 1: Trastuzumab q week + paclitaxel q 21 days  
 ARM 2: Trastuzumab q week + [paclitaxel + carboplatin] q 21 days

*Chemotherapy was continued for at least six cycles, and trastuzumab was continued until disease progression.*

**Results**

**Objective Response Rate for Trastuzumab/Paclitaxel/Carboplatin (TPC) and Trastuzumab/Paclitaxel (TP) in Patients with HER2-Positive Metastatic Breast Cancer**

	TPC (n=92)	TP (n=94)	p Value
Overall response rate	48/92 (52%)	34/94 (36%)	0.04
Subset analysis			
IHC 3+	35/61 (57%)	23/63 (37%)	0.03
FISH+	26/44 (59%)	13/31 (42%)	0.17

**Time to Progression (TTP) for Trastuzumab/Paclitaxel/Carboplatin (TPC) and Trastuzumab/Paclitaxel (TP) in Patients with HER2-Positive Metastatic Breast Cancer**

	TPC	TP	p value
Overall median TTP	11.2 months	6.9 months	0.007
Subset analysis			
IHC 3+	13.5 months	7.2 months	0.006
FISH+	13.5 months	7.2 months	0.205

**Survival**

- Trend for improved survival with TPC at 36 months (53 versus 47 percent,  $p = 0.2$ )

## Significant Adverse Events Associated with Trastuzumab/Paclitaxel/Carboplatin (TPC) and Trastuzumab/Paclitaxel

	TPC (n=96)	TP (n=95)
Neutropenia		
Grade 3	19%	15%
Grade 4	36%*	12%*
Thrombocytopenia		
Grade 3	9%*	1%*
Grade 4	0	0

\* $p \leq 0.01$  between groups

### Authors' Conclusions

"The addition of carboplatin to TP (trastuzumab/paclitaxel) significantly increases the overall response and time-to-progression."

*Nicholas Robert, MD  
2002 San Antonio Breast Cancer Symposium*

### Research Leader Commentary

This Phase III study of trastuzumab/paclitaxel with or without carboplatin in advanced breast cancer was spawned by the results of the pivotal trial by Slamon and colleagues, in which the combination of paclitaxel/trastuzumab improved the response rate to the 40 percent range and the time to progression to 6.9 months compared to paclitaxel alone.

We couldn't add doxorubicin to the paclitaxel/trastuzumab combination because in the pivotal trial, 28 percent of patients in the group given an anthracycline, cyclophosphamide and trastuzumab had cardiotoxicity. We knew of preclinical synergy between the taxanes and carboplatin, as well as three first-line therapy trials showing response rates between 52 percent and 62 percent with the combination of paclitaxel and carboplatin. Therefore, adding carboplatin seemed an obvious next step in evaluating the paclitaxel/trastuzumab combination.

We recruited 196 patients with Stage IV, HER2-positive breast cancer, of whom 191 were eligible and 186 were evaluable for response. As in the trial by Slamon and colleagues, we enrolled patients with IHC 2+ and 3+ disease, but as the data became available, we found that only 30 percent of the patients with IHC 2+ had FISH-positive disease. Therefore, we changed our eligibility requirements so that patients with IHC 2+ disease also had to have FISH-positive disease. Patients had to have measurable disease and a normal left ventricular ejection fraction. They were ineligible if they received adjuvant taxanes or more than 360 mg/m<sup>2</sup> of doxorubicin.

Patients were randomized to receive trastuzumab/paclitaxel, the successful arm of the pivotal trial, or the combination plus carboplatin. Paclitaxel was administered at 175

mg/m<sup>2</sup> over three hours every 21 days, trastuzumab was administered at a standard loading dose of 4 mg/kg followed by weekly 2 mg/kg, and carboplatin was administered at an AUC of six every 21 days. As in the pivotal trial, physicians had to give six cycles of chemotherapy, but could discontinue chemotherapy and continue the trastuzumab after that.

The addition of carboplatin improved both the response rate and time to progression. The primary endpoint was the response rate, which improved from 36 percent with the two-drug regimen to 52 percent with the addition of carboplatin, with a P value of 0.04.

Time to progression was a secondary endpoint in the trial. The time to progression in the trastuzumab/paclitaxel control arm was similar to what was seen in the pivotal trial by Slamon and colleagues. The addition of carboplatin increased the time to progression from 6.9 months to 11.2 months.

We looked at survival, although it was early to do so as over 120 patients are still alive. The preliminary analysis shows a trend for improvement with the three-drug regimen. In the patients with IHC 3+ disease, we saw an improvement in survival, with a P value of 0.06, approaching 0.05, and the population with FISH-positive disease showed a similar trend. It will be important to see if the survival advantage persists.

The trastuzumab/paclitaxel/carboplatin regimen was well-tolerated. The only significant difference in toxicity was increased myelosuppression, which we expected to see from the addition of carboplatin. However, there were no significant differences in terms of serious complications, such as infectious complications, significant neutropenia or fever. Other toxicities, such as neuropathy, allergic responses, nausea and arthralgias, were comparable in both arms.

It is important to note that we did not use prophylactic growth factors or attempt a dose-dense trial. We utilized dose reduction or dose delay when needed. In responding patients, only about 25 percent continued treatment beyond six cycles, so there are a number of important caveats when administering this regimen in order to get the benefits and avoid unacceptable toxicities.

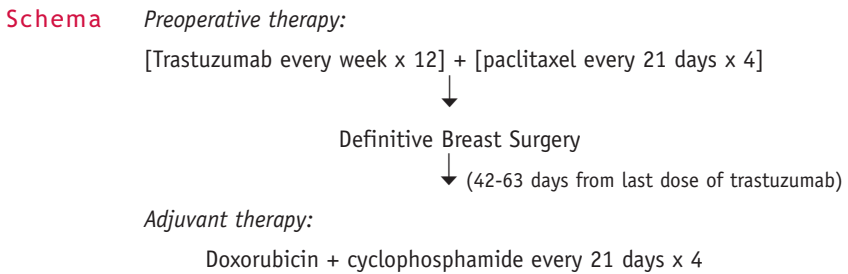
One of the questions our trial evoked was: Could we achieve the same results by treating patients with paclitaxel/trastuzumab and switching to carboplatin and trastuzumab when they progress? Historically, carboplatin is not a very effective agent when given outside the first-line setting, with response rates in the range of 10 percent; but it's possible that in combination with trastuzumab it's a different drug. This may be a strategy to consider in future clinical trials.

*Nicholas Robert, MD*

Burstein HJ et al. **Preoperative Therapy with Trastuzumab and Paclitaxel Followed by Sequential Adjuvant Doxorubicin/Cyclophosphamide for HER2-Overexpressing Stage II or III Breast Cancer: A Pilot Study.** *Journal of Clinical Oncology* 2003;21:46-53.

- Objectives**
- To assess the clinical effects, complete pathologic response rate and safety/feasibility of trastuzumab plus paclitaxel before breast surgery
  - To characterize pathologic changes in response to trastuzumab-based therapy

- Eligibility**
- Clinical Stage II or III invasive breast cancer, including inflammatory breast cancer
  - HER2 2+ or 3+ measured by the HercepTest™



## Results

Influence of Preoperative Trastuzumab Plus Paclitaxel on HER2 Status		
	Baseline HER2 Status	
	HER2 2+ (n=8)	HER2 3+ (n=32)
HER2 status after therapy		
3+	1 (13%)	17 (53%)
2+	0	2 (6%)
1+ or 0	3 (37%)	4 (13%)
Not accessible	3 (37%)	3 (9%)
pCR	1 (13%)	6 (19%)

pCR=pathologic complete response

### Response Rates for Preoperative Trastuzumab Plus Paclitaxel

	HER2 2+ (n=8)	HER2 3+ (n=32)	p value
Pathologic complete response	13%	19%	1.0
Clinical overall response	38%	84%	0.01
Clinical partial response	25%	50%	-
Clinical complete response	13%	34%	-

### Changes In Left Ventricular Ejection Fraction

	Preoperative Trastuzumab Plus Paclitaxel (n=40)	Adjuvant Doxorubicin Plus Cyclophosphamide (n=34)
Grade 1 (decline of $\geq 10\%$ but $< 20\%$ )	4 (10%)	5 (14.7%)
Grade 2 (decline of $\geq 20\%$ or below laboratory normal)	1 (2.5%)	4 (11.8%)
Grade 3 (symptomatic heart failure)	0	0
Grade 4 (symptomatic heart failure)	0	0

### Authors' Conclusions

"The administration of trastuzumab for patients with early-stage breast cancer remains investigational. Our trial of preoperative therapy demonstrates the feasibility of using trastuzumab treatment as part of a multimodality treatment program for stage II and III breast cancer."

### Research Leader Commentary

This study is novel for several reasons. It is the first trial evaluating neoadjuvant trastuzumab, and there is a lot of interest in defining the response rate. Also, we performed cardiac analyses during the trastuzumab/paclitaxel therapy and again during the postsurgical adjuvant AC chemotherapy. Our results are very similar to George Sledge's — a significant number of women had a 10-20 percent decline in their ejection fraction. Fortunately, none of the patients developed any symptoms of congestive heart failure, and the changes in ejection fraction appeared to reverse with time.

The decline in ejection fraction occurred either during or at the end of adjuvant AC and did not change much during the trastuzumab/paclitaxel therapy. Most of us believe these kinds of changes in ejection fraction are consistent with what occurs with AC alone, but since this is not a randomized trial, we do not know if the addition of trastuzumab influences the ejection fraction.

*Harold J Burstein, MD, PhD*



We published the results from a preoperative trastuzumab trial in 40 patients with Stage II or III breast cancer that had HER2 overexpression (IHC 2+ or 3+). A small number of patients didn't have surgery and some had a pathologic complete response, so obviously in those situations we couldn't reassess the tumor.

Of the patients with residual tumor, a small number of them had a change in their IHC status. The numbers get extraordinarily small, but it looks like the change in IHC status might be more common in patients with 2+ tumors initially, rather than 3+ tumors. The change in HER2 status typically went from 2+ or 3+ to 0 or 1+.

I don't think we can say exactly what is happening. Perhaps it is just variability in testing, or it may be an effect of trastuzumab. Since we don't have the FISH status on these patients yet, one possibility may be that those patients with a change in HER2 status may really have had FISH-negative tumors. We have a patient in our current study who has HER2-negative and HER2-positive tumor cells adjacent to each other, as assessed both by IHC and FISH.

*Eric P Winer, MD*

#### **Related Publications:** *HER2 Status and Response to Trastuzumab*

Burstein HJ et al. **Clinical activity of trastuzumab and vinorelbine in women with HER2-overexpressing metastatic breast cancer.** *J Clin Oncol* 2001;19(10):2722-30.

Christodoulou C et al. **Prolonged administration of weekly paclitaxel and trastuzumab in patients with advanced breast cancer.** *Anticancer Res* 2003;23(1B):737-44.

Cobleigh MA et al. **Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease.** *J Clin Oncol* 1999;17(9):2639-48.

Jahanzeb M et al. **Phase II trial of weekly vinorelbine and trastuzumab as first-line therapy in patients with HER2(+) metastatic breast cancer.** *Oncologist* 2002;7(5):410-7.

Miller KD et al. **Gemcitabine, paclitaxel, and trastuzumab in metastatic breast cancer.** *Oncology (Huntingt)* 2001;15(2 Suppl 3):38-40.

Montemurro F et al. **Safety and activity of docetaxel and trastuzumab in HER2 overexpressing metastatic breast cancer: A pilot phase II study.** *Am J Clin Oncol* 2003;26(1):95-7.

O'Shaughnessy J. **Gemcitabine and trastuzumab in metastatic breast cancer.** *Semin Oncol* 2003;30(2 Suppl 3):22-6.

Seidman AD et al. **Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification.** *J Clin Oncol* 2001;19(10):2587-95.

Yardley DA et al. **Final results of the Minnie Pearl Cancer Research Network first-line trial of weekly paclitaxel/carboplatin/trastuzumab in metastatic breast cancer.** *Breast Cancer Res Treat* 2002;Abstract 439.

# College of American Pathologists

Cell Markers and Cytogenetics Committees, College of American Pathologists. **Clinical Laboratory Assays for HER-2/neu Amplification and Overexpression Quality Assurance, Standardization, and Proficiency Testing.** *Archives of Pathology and Laboratory Medicine* 2002;126:803-8.

- Objective**
- To present the results of the College of American Pathologists' (CAP) proficiency testing, which assesses the variability in the interpretation of clinical laboratory results for HER2 gene amplification (FISH) and protein expression (IHC).

- Methods**
- The College of American Pathologists (CAP) provides two proficiency surveys for HER2 testing in breast cancer:
    - Cases for IHC surveys are distributed twice annually.
    - Two cases for FISH survey are distributed once annually.
    - If possible, the same pathologic material is used for both the IHC and FISH survey. They are known as shared specimens.
    - A series of six photomicrographs that were scored by a referee were also distributed.

## Results

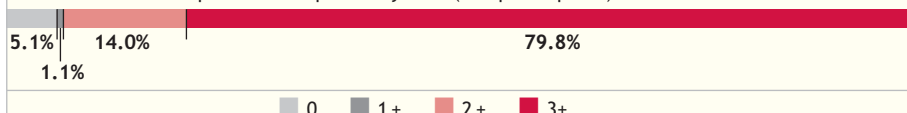
### Laboratory Concordance for FISH Proficiency Surveys

	2000 Survey	2001 Survey
Number of participating laboratories	35	63
Concordance	100%	100%*

\*Not all 63 participants submitted results

### IHC Results for the Shared Survey Specimens

IHC results for specimens amplified by FISH (371 participants)



IHC results for specimens not amplified by FISH (381 participants)



## Accuracy of Participants' Scores on Photomicrographs

Photomicrograph	Referees' Score	Number of Participants	Participants with Correct Score
HER2 - 01 - specimen	1+	457	77.0%
HER2 - 02 - specimen	2+	456	82.5%
HER2 - 03 - specimen	3+	456	97.5%
HER2 - 04 - specimen	1+	455	77.0%
HER2 - 05 - specimen	2+	454	42.7%
HER2 - 06 - specimen	2+	455	62.0%

### Authors' Conclusions

"The level of concordance observed for the FISH surveys may reflect the nonambiguous and quantitative nature of FISH as compared with immunohistochemistry. ...The immunohistochemistry survey results for the shared cases had much greater variability, particularly for the FISH-negative case... ."

"...a series of 6 photomicrographs were also assembled as a 1-page color print and distributed.... For even the case scored as 3+ by referees, 8 pathologists in participating laboratories interpreted the results as negative (a score of 0). This exercise also highlighted substantial disagreement for cases scored 1+ and 2+ by referees."

### Research Leader Commentary

The College of American Pathologists (CAP) interlaboratory testing program implemented by Cell Markers and Cytogenetics Committees sends laboratories unknown controls. The laboratory sends back the results, and CAP tells them whether they are doing a good or bad job. The more we can participate in voluntary programs to improve our performance, the better off we'll be. This study found that using either IHC or FISH and the feedback from the CAP quality assurance program, laboratories can improve their test performance and standardization. Clearly, quality control and proficiency standards testing are very, very important. A laboratory needs to constantly re-evaluate whether they're doing a good job.

*Ann D Thor, MD*

It has been consistently shown that FISH is superior to IHC. FISH is not a subjective test. If one can count dots, there should not be false positives. If false-positive FISH results were a real phenomenon, the College of American Pathologists' (CAP) study should have detected it. CAP took cases it characterized and sent them to pathologists in community practice, not university pathologists. Ray Tubbs has done this study, and they are seeing great concordance.

*Dennis Slamon, MD, PhD*

- Objective**
- Derive areas of practical agreement, define the current state-of-the-art and point out opportunities for improvement in HER2 testing

- Methods**
- On May 4 and 5, 2002, the College of American Pathologists assembled a group of expert speakers to integrate evolving basic, clinical and scientific data about HER2 testing with aspects of laboratory management.
  - The program faculty included: Noel Weidner, MD; Daniel Hayes, MD; Robert Mass, MD; Jon Askaa, DVM, PhD; Kenneth Bloom, MD; Steven Gutman, MD; Jack Bierig; Raymond Tubbs, DO; and Jeffery Ross, MD.
  - The conference had more than 100 attendees.

## Conclusions

### *Clinical value of HER2 as a prognostic and predictive factor*

Patients with tumors that have HER2 protein overexpression or gene amplification:

- Have a worse prognosis in terms of disease-free and overall survival
- Obtain more benefit from trastuzumab
- Obtain equal or more benefit from anthracyclines
- Obtain equivocal benefit from taxanes
- Obtain less benefit from nonanthracycline chemotherapy and hormones

### *Standardization of HER2 testing*

- Use of 10 percent neutral buffered formalin as a fixative
- Optimal fixation time is 6 to 12 hours
- Use of control cell lines, fixed exactly as the test sample, to calibrate the assay with each episode of testing
- Selection of well-fixed areas of tumor and benign breast tissue, without artifacts or decalcified materials
- If antigen lability is suspected, then a negative IHC result can be verified by evaluating ubiquitous antigen preservation
- Better training, improved interpretation guidelines, or quantitation by image analysis may reduce interobserver variation in IHC interpretation
- Verification that invasive tumor is being assessed

### *Consensus algorithm for HER2 testing*

- Laboratories should confirm their concordance rates of FISH to IHC for IHC scores of 3+ and 0, or they should perform both tests on all breast cancer specimens.
- IHC can be used as a screening tool if there is at least a 90 percent concordance for IHC 3+/FISH-amplified and IHC 0/FISH-nonamplified.
- If IHC is used as a screening tool, FISH should confirm all 1+ and 2+ cases.
- If the concordance rate for IHC 1+/FISH is more than 95 percent, there may not be a need to confirm cases with FISH.
- Testing may be considered for all patients newly diagnosed with breast cancer, not just those with metastatic disease.

### *Liability issues for the pathologist*

Basing a therapeutic decision on non-FDA-approved HER2 tests raises a number of liability issues, such as the potential for malpractice exposure should a patient experience injury from the treatment.

### *Quality control*

Measures of quality control for HER2 testing should include the percentage of positive cases obtained and whether the percentage varies by pathologist.

## **Authors' Conclusions**

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“Pathologists should view the current Her-2/neu testing challenge as an emerging opportunity to play a larger and more pivotal role in clinical medicine by providing the laboratory determination responsible for the selection of patients for future targeted therapies. This larger role in pathology practice requires the ongoing education of colleagues regarding the significance of new testing as it becomes available and leadership in algorithm development to provide consistent and accurate testing. This rigorous approach to Her-2/neu testing should become the standard by which we view our role as future laboratory assays that determine therapeutic decisions become available.”

## **Related Publications:** *College of American Pathologists*

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Fitzgibbons PL et al. **Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999.** *Arch Pathol Lab Med* 2000;124(7):966-78.

Hammond ME et al. **College of American Pathologists Conference XXXV: solid tumor prognostic factors-which, how and so what? Summary document and recommendations for implementation. Cancer Committee and Conference Participants.** *Arch Pathol Lab Med* 2000 Jul;124(7):958-65.

Mascarello JT et al. **Cytogenetics Resource Committee of the College of American Pathologists and American College of Medical Genetics. Proficiency testing for laboratories performing fluorescence in situ hybridization with chromosome-specific DNA probes.** *Arch Pathol Lab Med* 2002;126(12):1458-62.

## Post-test and Evaluation

- The NCCTG, NSABP and BCIRG cooperative research groups found a significant number of tumors that were reported as HER2-positive by local laboratories to be HER2-negative when retested at a central laboratory.
  - True
  - False
- Because of the discordance reported between central and local laboratory HER2 testing, NCCTG-N9831 was amended to require confirmation of the tumor's HER2 status at the Mayo Clinic laboratory.
  - True
  - False
- Because of the discordance reported between central and local laboratory HER2 testing, NSABP-B-31 was amended to require that HER2 testing by IHC only be allowed from NSABP-approved laboratories. Since the volume of HER2 testing was found to influence the number of false-positive HER2 results, NSABP-approved laboratories had to demonstrate high concordance between IHC and FISH or perform:
  - >50 HercepTest™ cases/month
  - >100 HercepTest™ cases/month
  - <50 HercepTest™ cases/month
  - <100 HercepTest™ cases/month
- When NSABP-B-31 was amended to require that HER2 testing by IHC only be allowed from NSABP-approved laboratories, the reliability of HER2 testing by IHC improved.
  - True
  - False
- According to Dr Thor, testing that looks at the ability of trastuzumab to produce HER2 activation and downstream signaling may be a better predictor of response to trastuzumab than HER2 gene amplification or protein overexpression.
  - True
  - False
- The paper by Zarbo and Hammond, published in the *Archives of Pathology and Laboratory Medicine*, identified a consensus algorithm for HER2 testing. Which of the following statements is false according to this paper?
  - All laboratories should perform both FISH and IHC on all breast cancer specimens.
  - IHC can be used as a screening tool if there is at least a 90 percent concordance for IHC 3+/FISH-amplified and IHC 0/FISH-nonamplified.
  - If IHC is used as a screening tool, FISH should confirm all 1+ and 2+ cases.
  - If the concordance rate for IHC 1+/FISH is more than 95 percent, there may not be a need to confirm cases with FISH.
- Which of the following clinical variables tend to be correlated with HER2 status?
  - Lymph node status
  - Time to relapse
  - Estrogen-receptor status
  - All of the above
  - None of the above
- In the abstract that reanalyzed the tumor specimens from the trastuzumab pivotal trial, by Mass et al in the *Proceedings American Society of Clinical Oncology*, FISH was found to be a stronger predictor of responsiveness to trastuzumab than IHC.
  - True
  - False
- Clinical trial results have been reported for the following trastuzumab-containing regimens:
  - Trastuzumab monotherapy
  - Trastuzumab/paclitaxel
  - Trastuzumab/docetaxel
  - Trastuzumab/paclitaxel/carboplatin
  - All of the above
- The paper published by Gancberg et al in the *Annals of Oncology* supports the belief that patients should routinely have metastatic sites retested for HER2 status.
  - True
  - False

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### Answer Key

1a, 2a, 3b, 4a, 5a, 6a, 7d, 8a, 9e, 10b

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# Evaluation Form: HER2 Testing for Breast Cancer Management

NL Communications Inc respects and appreciates your opinions. To assist us in evaluating the effectiveness of this activity and to make recommendations for future educational offerings, please complete this evaluation form. A certificate of completion is issued upon receipt of a completed evaluation form.

*Please answer the following questions by circling the appropriate rating:*

5 = Outstanding    4 = Good    3 = Satisfactory    2 = Fair    1 = Poor

## LEARNING OBJECTIVES

Upon completion of this activity, participants should be able to:

- Critically evaluate the clinical implications of the emerging data about the discordance between local and central laboratory HER2 testing . . . . . 5 4 3 2 1
- Describe and implement an algorithm for HER2 testing . . . . . 5 4 3 2 1
- Explain the components of a quality assurance program for HER2 testing 5 4 3 2 1
- Define clinical variables that should be considered when evaluating the accuracy of HER2 test results . . . . . 5 4 3 2 1
- Assess the need to retest a patient's HER2 status . . . . . 5 4 3 2 1

## EFFECTIVENESS OF THE INDIVIDUAL FACULTY MEMBERS

Faculty	Knowledge of Subject Matter	Effectiveness as an Educator
Mark Pegram, MD	5 4 3 2 1	5 4 3 2 1
Edith A Perez, MD	5 4 3 2 1	5 4 3 2 1
Michael F Press, MD, PhD	5 4 3 2 1	5 4 3 2 1
Ann D Thor, MD	5 4 3 2 1	5 4 3 2 1

## OVERALL EFFECTIVENESS OF THE ACTIVITY

- Objectives were related to overall purpose/goal(s) of activity . . . . . 5 4 3 2 1
- Related to my practice needs . . . . . 5 4 3 2 1
- Will influence how I practice . . . . . 5 4 3 2 1
- Will help me improve patient care . . . . . 5 4 3 2 1
- Stimulated my intellectual curiosity . . . . . 5 4 3 2 1
- Overall quality of material . . . . . 5 4 3 2 1
- Overall, the activity met my expectations . . . . . 5 4 3 2 1
- Avoided commercial bias or influence . . . . . 5 4 3 2 1

# Evaluation Form: HER2 Testing for Breast Cancer Management

Please Print Clearly

Name: \_\_\_\_\_

ME#: \_\_\_\_\_ Last 4 digits of SS# (required): \_\_\_\_\_

Street Address: \_\_\_\_\_ Box/Suite: \_\_\_\_\_

City: \_\_\_\_\_ State: \_\_\_\_\_ Zip Code: \_\_\_\_\_

Phone Number: \_\_\_\_\_ Fax Number: \_\_\_\_\_ Email: \_\_\_\_\_

NL Communications Inc designates this educational activity for a maximum of 2.5 category 1 credits towards the AMA Physician's Recognition Award. Please claim only the actual time spent to complete this activity. I certify my actual time spent to complete this educational activity to be \_\_\_ hour(s).

Signature: \_\_\_\_\_

Will the information presented cause you to make any changes in your practice?

\_\_Yes \_\_No

If Yes, please describe any change(s) you plan to make in your practice as a result of this activity.

\_\_\_\_\_  
\_\_\_\_\_

What other topics would you like to see addressed in future educational programs?

\_\_\_\_\_  
\_\_\_\_\_

What other faculty would you like to hear interviewed in future educational programs?

\_\_\_\_\_  
\_\_\_\_\_

Degree:

MD  DO  PharmD  RN  NP  PA  BS  Other \_\_\_\_\_

Specialty:

Medical Oncology  Pathology  Other

To obtain a certificate of completion and receive credit for this activity, please complete the post-test, fill out the evaluation form and mail or fax both to: NL Communications Inc, 400 SE Second Avenue, Suite 401, Miami, FL 33131-2117, FAX 305-377-9998.