Controversies in HER2 Testing

The accurate assessment of HER2 status is paramount for the management of patients with metastatic breast cancer and the enrollment of patients into adjuvant trastuzumab trials. Two trials evaluating adjuvant trastuzumab — NSABP-B-31 and NCCTG-N9831 — have reported poor concordance between community and central laboratories' assessments of HER2 status. NSABP subsequently demonstrated that a quality assurance program in which NSABP-approved community laboratories were used could improve the reliability of HER2 testing in the community. Recent studies have also evaluated concordance between different HER2 assays, concordance of HER2 status in the primary lesion, lymph nodes and distant metastases, and the impact of neoadjuvant trastuzumab on HER2 status.

CONCORDANCE BETWEEN COMMUNITY AND CENTRAL LABORATORIES' RESULTS FOR HER2-POSITIVE TUMORS 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

QUALITY ASSURANCE PROGRAM FOR NSABP-B-31: 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%

SOURCES: Paik S et al. *J Natl Cancer Inst* 2002;94(11):852-4.

Paik S. Presentation. San Antonio Breast Cancer Symposium, 2002;Abstract 9.

CONCORDANCE RATES BETWEEN CHROMOGEN *IN SITU* HYBRIDIZATION AND FISH IN CORE CUT BIOPSIES OF PRIMARY T2 BREAST CANCER

Samples	N	Concordance rate
IHC score 2+ Differentiation between HER2 positivity or negativity	56	98.2%
IHC score 3+ Differentiation between HER2 positivity	6	100%
All samples (IHC 0/1+, 2+, 3+) Differentiation between HER2 positivity	71	96.6%
All samples (IHC 0/1+, 2+, 3+) Differentiation between HER2 negativity	71	97.9%

SOURCE: Raab GH et al. *Proc ASCO* 2004;Abstract 569.

CONCORDANCE OF HER2 STATUS IN SAMPLES FROM PRIMARY BREAST CANCER AND DISTANT METASTASES IN THE SAME PATIENT

IHC score	Primary breast cancer (n=31)	Distant metastases (n=31)
0 or 1+	80.6%	54.8%
2+	9.7%	25.8%
3+	9.7%	19.4%

CONCORDANCE OF HER2 STATUS IN SAMPLES FROM PRIMARY BREAST CANCER AND REGIONAL LYMPH NODE METASTASES IN THE SAME PATIENT

IHC score	Primary breast cancer (n=10)	Regional lymph node metastases (n=10)
0	80%	80%
1+	10%	10%
3+	10%	10%

SOURCE: Regitnig P et al. *J Pathol* 2004;203(4):8-26.

CONCORDANCE BETWEEN LOCAL AND CENTRAL LABORATORIES' RESULTS FOR THE INITIAL HER2-POSITIVE TUMOR SPECIMENS FROM N9831

Local HER2 testing	Central results		
	Total	FISH-amplified	IHC-positive (3+)
IHC 3+	110	73 (66%)	81 (74%)
FISH-positive	9	6 (67%)	7 (78%)
Total	119	79 (66%)	88 (74%)

CONCORDANCE BETWEEN LOCAL, CENTRAL AND REFERENCE LABORATORIES' RESULTS FOR SUBSEQUENT HER2-POSITIVE TUMOR SPECIMENS FROM N9831

Local HER2 testing	Central HER2 testing	
	FISH-positive	HercepTest® (3+)
FISH-positive	204/240 (85%)	—
HercepTest® (3+)	—	376/473 (79.5%)

Central HER2 testing	Reference HER2 testing	
	FISH-negative	HercepTest® (0, 1+, 2+)
FISH-negative	122/128 (95.3%)	—
HercepTest® (0, 1+, 2+)	—	130/135 (96.3%)

SOURCES: Perez EA et al. Presentation. *ASCO* 2004;Abstract 567.

Roche PC et al. *J Natl Cancer Inst* 2002;94(11):855-7.

FREQUENCY OF HER2 GENE AMPLIFICATION ACCORDING TO HER2 PROTEIN EXPRESSION IN A COHORT OF 6,556 SPECIMENS FROM IMPATH LABORATORIES

IHC score	Percent of cases amplified
0	4.1
1+	7.4
2+	23.3
3+	91.7

SOURCE: Owens MA et al. *Clin Breast Cancer* 2004;5(1):63-9.

HER2 STATUS FOLLOWING PREOPERATIVE TRASTUZUMAB AND PACLITAXEL

HER2 status following preoperative therapy	Baseline HER2 status			
	3+ (n=32)		2+ (n=8)	
	No. of patients	Percent	No. of patients	Percent
3+	17	53	1	13
2+	2	6	0	0
1+ or 0	4	13	3	37
Not assessable	3	9	3	37
Pathologic complete response	6	19	1	13

SOURCE: Burstein HJ et al. *J Clin Oncol* 2003;21(1):46-53.

SELECT PUBLICATIONS

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.** *J Clin Oncol* 2003;21(1):46-53.

Owens MA et al. **HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues.** *Clin Breast Cancer* 2004;5(1):63-9.

Paik S et al. **Real world performance of HER2 testing — National Surgical Adjuvant Breast and Bowel Project Experience.** *J Natl Cancer Inst* 2002;94(11): 852-4.

Paik S et al. **Successful quality assurance program for HER2 testing in the NSABP trial for Herceptin®.** *Breast Cancer Res Treat* 2002;76(Suppl 1):31;Abstract 9.

Perez EA et al. **HER2 testing by local, central, and reference laboratories in the NCCTG N9831 Intergroup adjuvant trial.** *Proc ASCO* 2004;Abstract 567.

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.** Poster. San Antonio Breast Cancer Symposium, 2002;Abstract 238.

Raab GH et al. **Chromogen in situ hybridisation (CISH) for HER2 assessment is highly concordant with FISH in core cut biopsies of primary T2 breast cancers.** *Proc ASCO* 2004;Abstract 569.

Regitnig P et al. **Change of HER-2/neu status in a subset of distant metastases from breast carcinomas.** *J Pathol* 2004;203(4):8-26.

Roche PC et al. **Concordance between local and central laboratory HER2 testing in the Breast Intergroup Trial N9831.** *J Natl Cancer Inst* 2002;94(11):855-7.

QUALITY CONTROL FOR HER2 TESTING

When the NSABP designed the B-31 adjuvant trastuzumab trial, we were reluctant to require central testing for HER2. I always believed that it was not possible for a pathologist to misclassify patients with IHC 3+ overexpression, and the entry criteria for the study required patients' tumors to be IHC 3+. However, we built a safeguard into the protocol, such that we would perform central testing of the initial 100 patients entered into the study.

HER2 status was measured by both IHC and FISH, so HER2-negative tumors were truly negative. We were shocked because the false-positive rate was 18 percent. The Intergroup trial demonstrated essentially the same finding, and these results were a big "wake-up call" for the community.

Based on the false-positive rate, we revised the protocol so that patients had to be tested by an approved laboratory that performs over 100 tests per month or performs fewer tests but demonstrates a concordance rate between IHC and FISH of over 95 percent. The end result was a dramatic improvement in the quality of test results; the false-positive rate dropped from 18 percent to three percent.

— Soonmyung Paik, MD

We were surprised when we found poor concordance between community and central laboratory HER2 testing, in terms of both HER2 protein expression and gene amplification. The data from the first 119 cases were so important that we actually changed the eligibility criteria for this trial (NCCTG-N9831).

Physicians can still conduct local HER2 testing, but we test the tumor specimens again by the HercepTest® and the PathVysion® FISH assay. If neither demonstrates HER2 positivity, we send the specimen to another central laboratory and if that laboratory also finds that the tumor is HER2-negative by both assays, then we notify the physician that the patient should not participate in the trial.

— Edith A Perez, MD

HER2 TESTING ALGORITHM

We initially perform IHC for HER2 testing and then FISH if the IHC result is 2+. We view zero and 1+ results as HER2-negative and 3+ results as HER2-positive. However, we know from concordance data that approximately 10 percent of IHC zero and 1+ cases will be FISH-positive and approximately 10 percent of IHC 3+ cases will be FISH-negative, so that has to be taken into consideration.

We have learned that labs must perform a high volume of FISH testing to be proficient, and community labs have low concordance rates. At the 2004 ASCO meeting, an interesting technique for evaluating the HER2 status was presented, called chromogen *in situ* hybridization (CISH). The concordance rates between this technique and FISH were high, and I believe this new assay will change our current patterns of testing.

— Adam M Brufsky, MD, PhD

INTRAPATIENT STABILITY OF HER2 STATUS

"For most patients with residual tumor after 12 weeks of neoadjuvant treatment, HER2 expression as measured by immunohistochemistry was unchanged. However, a subset of patients whose initial tumors were 3+ was found, on testing after induction therapy, to have lost immunohistochemical expression of HER2.

"The clinical significance of this finding is not known. It may represent downregulation of HER2 expression following anti-HER2 antibody exposure, as reported in preclinical tumor models. It may also represent intrinsic heterogeneity of HER2 expression and tumor response, or an artifact of tumor sampling or testing. It is not clear whether this finding implies resistance or sensitivity to trastuzumab."

— Burstein HJ et al. J Clin Oncol *2003;21(1):46-53.*