



NATIONAL SURGICAL ADJUVANT
BREAST AND BOWEL PROJECT

Norman Wolmark, MD
Chairman

OPERATIONS CENTER
Medical Affairs
412/330-4600 412/330-4660 Fax
412/330-4661 Fax
Administrative and Fiscal Affairs
412/330-4600 412/330-4662 Fax
Clinical Coordinating Section
1-800/477-7227 412/330-4660 Fax

To: NSABP Investigators and Program Coordinators

From: Elizabeth Tan-Chiu, MD; NSABP B-31 Protocol Officer
NSABP Operations Center

Date: March 14, 2001

We have completed the quality assessment of HER2 assays on the first 104 tumor specimens from patients registered in NSABP Protocol B-31. Included in this memo is the summary of the findings of this assessment which we urge you to share with your referring pathologist(s) and co-investigators since it has important patient safety implications.

To be considered eligible for the study, a positive HER2 assay has to be one of the following: 3+ staining by HercepTest; or $\geq 33\%$ positively stained cells if assayed with other antibodies; or positive gene amplification by fluorescent in situ hybridization (FISH). Eighty of the 104 patients met the eligibility criteria based on HercepTest; the remaining 24 were eligible based on another immunohistochemical (IHC) assay; and none of the 104 patients met the eligibility requirement based on FISH.

For all 104 tumor specimens, the original HER2 assessment was validated both by centralized HercepTest and by FISH (Vysis PathVysion Test). Table 1, on page 2, shows two apparent trends:

1. If a 3+ HercepTest used to establish patient eligibility was done in a reference laboratory, it almost always was confirmed as 3+ on central HercepTest assay, and was almost always amplified by FISH: only 1/28 (4%) of such cases failed to be confirmed as positive by both central HercepTest and FISH. On the other hand, HercepTest assays done at non-reference laboratories often could not be confirmed centrally by either HercepTest or FISH: 10/52 (19%) of such assays were judged to be negative by both central assays.
2. If IHC assays other than the HercepTest were used at non-reference laboratories to establish patient eligibility, the results often could not be confirmed centrally: 8/23 (35%) of such assays were judged centrally to be negative by both HercepTest and FISH. Immunohistochemical assays other than the HercepTest were rarely done at reference laboratories.

Table 1. Quality Assessment Results of HER2 Analyses (first 104 tumor specimens)

Test Used for Eligibility	Type of Lab Used	Central HercepTest	Central FISH	Negative by both Central Assays
		0-2+	Not Amplified	
HercepTest 3+ (n=80)	Non-reference lab 52	19% (10/52)	23% (12/52)	19% (10/52)
	Reference lab 28	4% (1/28)	4% (1/28)	4% (1/28)
Other Anti-bodies \geq 33% stain positive (n=24)	Non-reference lab 23	48% (11/23)	39% (9/23)	35% (8/23)
	Reference lab 1	0% (0/1)	0% (0/1)	0% (0/1)

Based on these results, the NSABP recommends the following changes for patient entry to Protocol B-31. A formal protocol amendment will be prepared as quickly as possible, at which time the recommended changes will become mandatory.

- You may establish the eligibility of a patient for participation in Protocol B-31 by use of FISH, without any requirement for further validation.
- **Any HER2 assay done at a non-reference laboratory by any method other than FISH will require validation at one of the reference laboratories listed below, before a patient may be entered in Protocol B-31.** The method of validation to be used by the reference laboratory may be either FISH or HercepTest, although our preference is for the use of FISH.

We recommend FISH based on preliminary data that suggest HER2 gene amplification by FISH may correlate more closely with clinical response to Herceptin than 3+ staining by IHC in the advanced-disease setting. (Mass et al. ASCO 2000 abstr 291). We also recommend FISH because it may be more likely to be reimbursed by a third party payor than a repeat IHC done in a reference lab.

Any reference laboratory from the list below may be utilized for HER2 validation:

1. Impath
2. Quest (Nichols Laboratory)
3. LabCorp
4. Mayo Clinical Laboratory
5. Memorial Sloan Kettering Cancer Center Laboratory
6. St. Barnabas Medical Center (New Jersey)
7. Dianon Systems
8. Rush Presbyterian/ St. Luke's Medical Center
9. LDS Hospital, Salt Lake City
10. US Labs-Irvine, CA
11. Kaiser, San Francisco

March 14, 2001 – NSABP B-31 Mass Fax

The NSABP has determined these laboratories have processed an average of 100 HER2 samples per month for the past 6 months. In order to propose a laboratory for consideration as a reference lab, please contact NSABP Clinical Coordinating Section at 1-800-477-7227 for instruction. Sufficient time must be allowed for this process.

In order to defray the additional cost of having tissue samples sent to a reference laboratory for validation, NSABP will reimburse your institution \$100 per registered patient upon receipt of HER2 assay results from both the reference and the non-reference laboratories. This reimbursement is underwritten by a grant from Genentech Inc., and will be applicable from April 1, 2001 to March 31, 2002. This reimbursement is in addition to the current *industrial* PSA reimbursement for tissue block submission (\$200), baseline blood sample submission (\$50), tissue block submission on relapse (\$100) and blood sample submission on relapse (\$50).

The forthcoming protocol amendment will include a change in eligibility criteria allowing entry up to 84 days from the last surgery for breast cancer treatment to date of randomization. This should allow investigators enough time to obtain validation of IHC performed by a non-reference lab prior to patient entry. The current entry criteria of 63 days from biopsy to date of randomization will remain in effect until the amendment is approved by your institution's IRB.

Information sent in this memo will also be available on the NSABP Web site.

Questions concerning any information in this memo should be directed to the NSABP Clinical Coordinating Section (1-800-477-7227).