



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

P940004

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Ms. Barbara H. Keech
Director
Regulatory Affairs and Quality Assurance
Oncor, Inc.
209 Perry Parkway
Gaithersburg, Maryland 20877

DEC 30 1997

Re: P940004
Oncor® INFORM™ HER-2/*neu* Gene Detection System
Filed: February 14, 1994
Amended: February 28, 1994; February 21, March 13,
August 16, August 25, September 5, September 11, November 1,
December 26, 1995; April 1, April 15, July 25, August 8, August 23,
1996; February 7, March 10, June 10, October 7, December 11, and 19,
1997.

Dear Ms. Keech:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Oncor® Inform™ HER-2/*neu* Gene Detection System. The Oncor® INFORM™ HER-2/*neu* Gene Detection System is a fluorescence *in situ* hybridization (FISH) DNA probe assay that determines the qualitative presence of HER-2/*neu* gene amplification on formalin-fixed, paraffin-embedded human breast tissue as an aid to stratify breast cancer patients according to risk for recurrence or disease-related death. It is indicated for use as an adjunct to existing clinical and pathologic information currently used as prognostic indicators in the risk stratification of breast cancer in patients who have had a primary, invasive, localized breast carcinoma and who are lymph node-negative. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution, and use of this device are restricted to prescription use in accordance with 21 CFR 801.109 within the meaning of section 520(e) of the Federal Food, Drug, and Cosmetic Act (the act) under the authority of section 515(d)(1)(B)(ii) of the act. FDA has also determined that to ensure the safe and effective use of the device that the device is further

section 515(d)(1)(B)(ii), (1) insofar as the labeling specify the requirements that apply to the training of practitioners who may use the device as approved in this order and (2) insofar as the sale, distribution, and use must not violate sections 502(q) and (r) of the act.

In addition to the postapproval requirements in the enclosure, the postapproval reports must include the following information: The results of the proficiency testing provided as part of the sponsor's training program will be provided in Annual Reports to the FDA.

Expiration dating for this device has been established and approved at eighteen (18) months. This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(8).

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

Page 3 - Ms. Barbara H. Keech

If you have any questions concerning this approval order, please contact Peter Maxim, Ph.D. at (301) 594-1293.

Sincerely yours,

Kimber C. Richter

Kimber C. Richter, M.D.
Deputy Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

3

CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

4

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the **addition** of, but **not the replacement** of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. **This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.**

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

- (1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).
- (2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:
 - (a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

- (b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

- (1) A mixup of the device or its labeling with another article.
- (2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and
 - (a) has not been addressed by the device's labeling or
 - (b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.
- (3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984, and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to FDA whenever they receive or otherwise became aware of information that reasonably suggests that one of its marketed devices

- (1) may have caused or contributed to a death or serious injury or
- (2) has malfunctioned and that the device or any other device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for this PMA, you shall submit the appropriate reports required by the MDR Regulation and identified with the PMA reference number to the following office:

Division of Surveillance Systems (HFZ-531)
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, Room 240
Rockville, Maryland 20850
Telephone (301) 594-2735

Events included in periodic reports to the PMA that have also been reported under the MDR Regulation must be so identified in the periodic report to the PMA to prevent duplicative entry into FDA information systems.

Copies of the MDR Regulation and an FDA publication entitled, "An Overview of the Medical Device Reporting Regulation," are available by written request to the address below or by telephoning 1-800-638-2041.

Division of Small Manufacturers Assistance (HFZ-220)
Center for Devices and Radiological Health
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. General Information

Generic Name: Fluorescence *in situ* hybridization (FISH) DNA probe assay for the qualitative detection of HER-2/*neu* genomic sequences in human breast tissue.

Trade Name: Oncor® INFORM™ HER-2/*neu* Gene Detection System.

Applicant's Name and Address: Oncor, Inc.
209 Perry Parkway
Gaithersburg, MD 20877

Premarket Approval Application (PMA) Number: P940004

Date of Panel Recommendation: On November 30, 1995 the FDA 's Immunology Devices Advisory Panel recommended that the PMA not be approved.

Date of Notice of Approval to the Applicant: December 30, 1997

II. Indications For Use

The Oncor® INFORM™ HER-2/*neu* Gene Detection System is a fluorescence *in situ* hybridization (FISH) DNA probe assay that determines the qualitative presence of HER-2/*neu* gene amplification on formalin-fixed, paraffin-embedded human breast tissue as an aid to stratify breast cancer patients according to risk for recurrence or disease-related death. It is indicated for use as an adjunct to existing clinical and pathologic information currently used as prognostic indicators in the risk stratification of breast cancer in patients who have had a primary, invasive, localized breast carcinoma and who are lymph node-negative.

Background

Studies have shown positive correlation between HER-2/*neu* gene amplification and other common indicators of poor prognosis (1,2). However, even strong breast cancer prognostic factors, such as number of positive lymph nodes, tumor size and histograde, do not predict patient outcome unfalteringly (3,4). Current evidence indicates that HER-2/*neu* protein overexpression and gene amplification are indicative of poor patient prognosis at all stages of breast cancer development (2,3,16). Because HER-2/*neu* amplification appears early in breast cancer progression (5,6) and, when present is homogeneously distributed throughout the cancer (5,7) it can serve as a prognostic marker for this disease (when used as an adjunct with other accepted prognostic indicators).

Using FISH targeted to the HER-2/*neu* gene, has successfully demonstrated gene amplification in breast cancer cell lines and primary tumors, and has shown that FISH results are concordant with other measures of gene amplification (8). FISH technology combines the advantages of direct gene amplification assessment with direct localization in morphologically identified tumor cells. FISH is applicable to tumors of all sizes because studies can be performed on sections from the original specimen blocks used for diagnosis. In many samples, direct comparison can be made with FISH assay on normal cells from the same preparation. Further, if amplification were localized rather than diffusely distributed within a tumor, it would be detectable by FISH but could be diluted below detectable limits in extracted tumor DNA required for other procedures.

Breast cancer remains a major cause of illness and death among women in the United States, with over 180,000 new cases and 44,000 deaths per year (9). Possibly the most important predictor of clinical course in breast cancer is the presence or absence of lymph node metastases. Many prognostic indicators aid in evaluation of invasive cancers in addition to the presence or absence of lymph node metastasis, including tumor size, histologic type, tumor grade (differentiation reflected in extent of gland formation), nuclear grade (extent of nuclear alteration and frequency of mitosis), DNA content (ploidy), and hormone receptor status. Those indicators considered to be the strongest breast cancer prognostic factors, such as number of positive lymph nodes, tumor size, and histograde, do not predict patient outcome unfalteringly (10,11). A reasonable and desirable approach would be the use of prognostic factors to risk-stratify invasive breast cancer patients into low-risk and high-risk groups in terms of the probability of recurrence (12).

HER-2/*neu* gene amplification status is useful as an adjunct in the evaluation of the prognosis of node negative breast cancer patients and is an independent marker of high risk in node-negative patients. Amplification of HER-2/*neu* is indicative of poor patient prognosis at all stages of breast cancer development and correlates with relatively shorter disease-free and overall survival.

III. Device Description

A. HER-2/*neu* System

The HER-2/*neu* System is an *in vitro* diagnostic device consisting of a biotin-labeled DNA probe which detects the HER-2/*neu* gene, a detection reagent, Fluorescein-labeled Avidin and counterstain reagent, DAPI/Antifade. The DNA probe yields a green fluorescent signal at the site of the HER-2/*neu* gene. The DAPI/Antifade is an intercalating fluorescent counterstain which resulting in a blue fluorescent background of nuclear DNA. The test yields these results on thin sections of formalin-fixed, paraffin-embedded human breast tissue mounted on slides, which are then subjected to the above reagents following a specific set of instructions.

B. Control Kit for the HER-2/*neu* System.

Controls must be used with every assay run for monitoring the performance of the HER-2/*neu* test. The Oncor® INFORM™ HER-2/*neu* Control Kit ("Control Kit") is an accessory to the HER-2/*neu* test and is available separately. The Control Kit consists of intact cultured cell lines, with different levels of HER-2/*neu* amplification, that have been formalin-fixed, paraffin-embedded, sectioned, and applied to silanized slides to mimic tissue specimens. The levels include a Level 1 control, having a mean signal per nucleus value of less than or equal to three; a Level 2 control, having a mean signal per nucleus value greater than three to less than ten; and a Level 3 control, having a mean signal per nucleus value of equal to or greater than ten. In addition, it may be preferable to use qualified and validated tissue specimens as controls. For those that prefer to use tissue specimens as controls, it is recommended that control tissue specimens periodically be obtained and validated in the user's laboratory.

Contraindications:

There are no known contraindications for the HER-2/*neu* System.

Warnings and Precautions:

The assay is intended to be performed and interpreted by users certified by the Oncor proficiency program. Additional Warnings and Precautions for use of the device are stated in the product labeling.

IV. Alternative Practices And Procedures

Generally accepted alternative prognostic indicators that are currently in use include presence or absence of lymph node metastases, histopathological

classification comprising histologic and nuclear grade, tumor size, estrogen and progesterone receptor status, and DNA ploidy (13,14) Nodal status is considered essential for prognostication, and while nuclear and histologic grades are informative, they have been cited for poor reproducibility (15). The importance of tumor size is reflected in the recommendation of the National Institutes of Health Breast Cancer Consensus Conference in 1990 that tumors less than one centimeter in diameter should rarely be treated with systemic adjuvant therapy (16). Finally, estrogen receptor status is a marker of differentiation that appears most useful when combined with other prognostic information.

These practices and procedures, along with the clinical evaluation of the patient, are currently used by clinicians to assess the risk for recurrence of the cancer and determination of post-surgical treatment.

V. Marketing History

Oncor has received permission to market the HER-2/*neu* System in Australia, Austria, Canada, Denmark, Germany, Greece, Ireland, Luxemburg, The Netherlands, Portugal, South Africa, Sweden, Switzerland, and the United Kingdom. The HER-2/*neu* Kit has never been withdrawn from the market in any country for any reason related to the safety and effectiveness of the device.

VI. Potential Adverse Effects of the Device on Health

Failure of the HER-2/*neu* System could result in one of two situations: 1) a false positive where the test results showed amplification, yet the cancer did not recur; and 2) a false negative where the test results did not show amplification and there was recurrence of disease.

VII. Summary of Studies

A. NONCLINICAL STUDIES

The objective of the nonclinical laboratory studies performed with the HER-2/*neu* System was to determine the probe performance by testing analytical specificity and sensitivity, and test performance reproducibility and stability.

Cross-hybridization

A study was performed in which three sets of normal and amplified control tissues were assayed with the HER-2/*neu* System. One set was tested as directed, and the other two sets were tested using denaturation, hybridization and post-wash temperatures that were 1°C and 2°C lower than the directed temperatures. No significant differences in the mean

number of HER-2/*neu* signals were detected between the sets tested at the normal and less stringent conditions.

Analytical Sensitivity

The analytical sensitivity of the HER-2/*neu* System was determined from analysis of a non-amplified control cell line which is known to contain only two copies of the HER-2/*neu* gene. Cell pellets from this cell line were fixed, and slides prepared, using standard cytogenetic procedures. The slides were processed, hybridized and scored with a modified protocol for cytogenetic samples. Whole cells were used in this analysis to avoid the truncation artifact that occurs during scoring sectioned, paraffin-embedded cell pellets.

In this study, the scores were 1.6 to 1.8 HER-2/*neu* signals (99 per cent Confidence Intervals (C.I.) of 27 samples) per nucleus (40 nuclei scored) when assayed with the HER-2/*neu* System. The close concordance of these results with the known integral copy number indicates that a single copy of the HER-2/*neu* gene can be detected. Therefore, the analytical sensitivity of the test was one copy of the HER-2/*neu* gene per nucleus.

Analytical Specificity

The analytical specificity of the HER-2/*neu* System was determined in studies that assessed the effect of lower or higher stringency in denaturation, hybridization and post-washing on the number of HER-2/*neu* signals detected in breast cancer specimens.

The potential effect of raising the stringency of denaturation, hybridization, and post-washing was weak or no signals causing a decrease in the apparent number of signals per cell. The potential effect of lowering the stringency was greater base mismatching between the DNA probe and target sequences, and encouragement of potential cross-hybridization, resulting in an apparent increase of signals.

The effect of lower or higher stringency during the denaturation step on the number of HER-2/*neu* signals detected was assessed in one HER-2/*neu* amplified breast cancer and one HER-2/*neu*, non-amplified breast cancer specimen. The effect of lower or higher stringency during the hybridization and post-washing steps on the number of HER-2/*neu* signals detected was assessed using one HER-2/*neu*, highly amplified breast cancer and one low amplified breast cancer specimen.

Denaturation Stringency

Three sets of the two specimens (one amplified and one non-amplified) were prepared and assayed as directed in the HER-2/*neu* System Procedure and Interpretation Guide with the exception that each set was processed under different denaturation temperature conditions. One set of the two specimens was denatured at the recommended temperatures, another set was denatured at -10°C from the recommended temperatures, and the third set was assayed at +10°C from the recommended temperatures. The sample sets were evaluated in a blinded fashion (as to the stringency) and scored using the scoring criteria provided under Interpretation without truncation of scores at 20. Forty nuclei in each specimen were scored, and the mean number of signals per nucleus and the 99 per cent C. I. were determined. For the denaturation stringency experiments, the results are summarized in Table 1.

Table 1

	At Recommended Temperature Mean: (99% CI)	10°C Below Recommended Temperature Mean: (99%CI)	10°C Above Recommended Temperature Mean: (99% CI)
SAMPLE 1	2.4 (1.9 - 3.0)	2.0 (1.7 - 2.4)	2.7 (1.9 - 3.6)
SAMPLE 2	23.1 (20.3 - 25.8)	24.4 (21.4 - 27.5)	22.8 (20.3 - 25.3)

Sample 1 is a Non-Amplified Breast Cancer Specimen

Sample 2 is an Amplified Breast Cancer Specimen

The mean number of signals did not increase in either the amplified or non-amplified specimens under either more or less stringent denaturation conditions, which indicated that there was no effect on the outcome of the assay as a result of altering the denaturation temperature by $\pm 10^\circ\text{C}$

Hybridization and Post-Wash Stringency

Three sets of two specimens (one amplified and one low amplified) were prepared and assayed as directed in the HER-2/*neu* System Procedure and Interpretation Guide, with the exception that each set was processed under different hybridization and post-wash temperature conditions. One set of the two specimens was hybridized and post washed at the recommended temperatures, another set was hybridized and

13

post-washed at 10°C above the recommended temperatures and the third set was hybridized and post washed at 10°C below the recommended temperatures. The sample sets were elevated in a blinded fashion (as to the stringency) and scored using the scoring criteria provided under Interpretation without truncation of scores at 20. Forty nuclei in each specimen were scored, and the mean number of signals per nucleus and the 99% C.I. were determined.

For the hybridization and post-wash stringency experiments, the results are summarized in Table 2.

Table 2

	At Recommended Temperatures Mean (99% CI)	10°C Below Recommended Temperatures Mean (99% CI)	10°C Above Recommended Temperatures Mean (99% CI)
SAMPLE 1	6.1 (5.4 - 6.8)	7.0 (5.8 - 8.2)	7.1 (6.1 - 8.1)
SAMPLE 2	18.6 (16.6 - 20.5)	17.8 (15.6 - 20.0)	20.2 (17.5 - 22.9)

Sample 1 is a Low Amplified Breast Cancer Specimen

Sample 2 is an Amplified Breast Cancer Specimen

The mean number of signals did not increase in either the amplified or the low amplified specimens under either more or less stringent hybridization and post-wash conditions, which indicated that there was no effect on the outcome of the assay as a result of altering the hybridization and post-wash temperatures by $\pm 10^\circ\text{C}$

Reproducibility Studies

Reproducibility of the HER-2/*neu* System was analyzed using data collected in two distinct studies designed to assess reproducibility. The first study evaluated reproducibility with three different lots of the kit and among three different sites; two technicians at each site performing the assay on three different sample sets. Another study evaluated the intra- and inter-laboratory variability following the implementation of the Oncor Training Program at five sites.

Multifactorial Reproducibility Study

Reproducibility was assessed using a factorial study design protocol, analyzed as 3x3x3x2 (sample-by-lot-by-site-by-technician) between-subject factorial, was tested by assessing the number of HER-2/*neu* signals in each of three different sample sets consisting of one amplified, one low amplified, and one non-amplified breast cancer specimen.

Clinical sites participating in this Study, in addition to Oncor, were the University of Southern California, University of Iowa, and University of Wisconsin.

Lot-To-Lot Reproducibility

Each sample set (amplified, low amplified and non-amplified HER-2/*neu* breast cancer sections) was assayed with three different lots of the HER-2/*neu* System. All specimens were prepared and assayed as directed in the HER-2/*neu* System Procedure and Interpretation Guide. Forty nuclei in each specimen were scored, and the mean number of signals per nucleus and the standard deviation (S.D.) for each specimen for the three kit lots were determined.

The lot-to-lot reproducibility study yielded a Total Mean of 1.9 for the Non-Amplified Sample with a C.V. of 22.2% when tested with the three lots.

For the Low Amplified Sample the Total Mean was 4.3 with a C.V. of 24.1%.

The Amplified Sample yielded a Total Mean of 18.9 with a C.V. of 34.0% across the three lots.

Multi-site Reproducibility/ Transportability

Reproducibility performance of the device was assessed at five study sites using twelve breast cancer tissue specimens. The purpose of the study was to assess inter- and intra-site reproducibility of a series of breast cancer specimens with varying levels of HER-2/*neu* values. For this study only one lot of HER-2/*neu* reagent was used and processing and analysis was done by a single person at each study site. The twelve specimens were supplied to the study sites "blinded" in replicates of three. The twelve unique breast cancer specimens had the following levels of HER-2/*neu* amplification.

Level 1 (< 3 signals/nucleus): 3 Specimens

15

Level 2 (>3 and <10 signals/nucleus): 6 Specimens
 Level 3 (>= 10 signals/nucleus): 3 Specimens

The five clinical sites participating in this study were University of Chicago, Beth Israel Deaconess Medical Center, Brigham and Women's Hospital, University of Nebraska Medical Center and Georgetown University.

Table 3 shows the cumulative results from the five clinical sites.

Table 3 Multi-Site Reproducibility

Total Sites	Level 1 Specimens	Level 2 Specimens	Level 3 Specimens
Total Specimens	3	6	3
Mean	1.9 - 3.2	2.9 - 4.4	13-6 - 21-6
N	45	90	45
STD DEV	0.1 - 14.5	1.5 - 4.7	2.2 - 6.6
C.V. (%)	17.5 - 129.0	47.7 - 120.4	15.56 - 35.3

Validation of Positive and Negative Controls

The controls consist of three cell lines which have been plasma/thrombin clotted, fixed in formalin, paraffin-embedded, sectioned, and mounted onto microscope slides. All three cell lines originated from invasive breast cancers. The cell lines were chosen to provide three levels of HER-2/*neu* signals. The Oncor Control Specimens are divided into three categories.

A Level 1 cell line control has a mean signal per nucleus value of less than or equal to 3. This range of assay scores (0 to <= 3) is defined as non-amplified for the HER-2/*neu* gene. Based on 393 observations (40 nuclei scored per observation) of 4 µm sections of the Level 1 control cell line a mean of 2.39 ± 0.25 SD HER-2/*neu* signals per nucleus was determined.

A Level 2 cell line control has a mean signal per nucleus value of greater than 3 to less than 10. This range of assay scores (>3 to <10) is defined as low amplified for the HER-2/*neu* gene. Based on 102 observations (40 nuclei scored per observation) of 4 µm sections of the recommended Level 2 control cell line, a mean of 3.47 ± 0.71 SD HER-2/*neu* signals per nucleus was determined.

A Level 3 cell line control has a mean signal per nucleus value equal to or greater than 10. This control is amplified for the HER-2/*neu* gene. Based on 338 observations (40 nuclei scored per observation, truncating scores

160

greater than 20 to 20) of 4 μm sections of the recommended Level 3 control cell line, an acceptance range of 15.8 to 20.0 HER-2/*neu* signals per nucleus was determined.

Stability

Stability of the HER-2/*neu* System was assessed using both accelerated temperature stress testing methods and real time stability testing methods. Three different lots were evaluated for each method. The proposed expiration dating of 18 months was supported by both testing methods. In the accelerated stability study, three breast cancer specimens (2 amplified and 1 non-amplified) were used to demonstrate that DAPI counterstain is the most temperature sensitive reagent in the kits. Following this preliminary study, two control cell lines were assayed to determine the stability of three lots of DAPI counterstain.

In the "Real Time" stability study, three different lots were tested over a period of 18 months on components that had been stored at the labeled temperatures. All kits passed the testing criteria. The stability testing program of the HER-2/*neu* System was initiated in May of 1996 using systems that were manufactured in July and August of 1995. Testing was conducted at approximately 9, 12, 15, and 18 months. The testing conducted was identical to that of the final system Quality Control testing used to determine compliance with established kit acceptance criteria. Each test procedure was based on the procedures described in the HER-2/*neu* Procedure and Package Interpretation Guide insert. The tests represented customer use once the product is commercially available.

Additionally, a shipping study was conducted to evaluate the stability of the finished components under simulated shipping and stressed temperature conditions. The study demonstrated that the kit was stable under stressed temperature conditions.

For the HER-2/*neu* Cell Line Controls, stability testing conducted from September 1995 to October 1997 shows that the Control Slides are stable for up to 24 months under the labeled conditions in one lot and up to 19 months in two lots of control cell lines, thus supporting the shelf-life of 18 months.

B. CLINICAL STUDIES

A retrospective study of 242 node-negative breast cancer patient specimens was conducted at three (3) sites in the United States. Additional specimen material was re-acquired for patients studied at two sites and then assayed at a fourth site

under a retest study protocol. Data from a total of 145 patient specimens was then combined with the data generated at the third original site (75 patient specimens). This combined data set was used to determine the association of the HER-2/*neu* System result to recurrence of breast cancer and death due to breast cancer. See Figure 1.

Figure 1

<p>Original Clinical Sites 242 node-negative breast cancer specimens University of Iowa University of Southern California University of Wisconsin</p>
<p>Re-acquired Specimens for Retest from the University of Iowa and the University of Southern California 145 node-negative breast cancer specimens Retested at Brigham and Women's Hospital</p>
<p>Retest Data (145 specimens) and University of Wisconsin Data (75 specimens) 220 node-negative breast cancer specimens Used for Final Analysis</p>

NOTE: The clinical performance characteristics of the HER-2/*neu* System are described with amplification defined as >4 signals per nucleus and non-amplification defined as ≤ 4 signals per nucleus. A borderline result at or near the cutoff of 4.0 (3.5 to 4.5) is expected in 3.6 per cent of the patient population. Borderline results should be interpreted with caution and increased emphasis should be given to the other clinical and prognostic information available to the practitioner.

Inclusion/Exclusion Criteria for Safety and Effectiveness Study

The HER-2/*neu* System was used to retrospectively identify the recurrence and death risk for node-negative breast cancer patients meeting the following criteria:

- 1) Diagnosis of invasive breast cancer;
- 2) Available formalin-fixed, paraffin-embedded tissue for HER-2/*neu* analysis;
- 3) Primary treatment was surgery only; and
- 4) Clinical follow up for at least 2 years for early recurrence, 3 years for recurrence and death.

19

Relationship of the HER-2/*neu* System to Outcome Endpoints

The safety and effectiveness of the HER-2/*neu* System was evaluated in a population of 220 node-negative, invasive breast cancer patients. The relationship of the HER-2/*neu* System assay result to disease-free survival is presented in Table 4 and overall survival is shown in Table 5.

Table 4 Probability of disease-free survival of breast cancer patients with non-amplified and amplified lesions

Not Amplified					Amplified			
Time from Surgery (in years)	N**	Cumulative No. Events	Cumulative No. Cases Censored	Probability of Remaining Disease Free	N**	Cumulative No. Events	Cumulative No. Cases Censored	Probability of Remaining Disease Free
0.5	179	0	0	100.0%	31	2	0	93.9%
1.0	176	3	0	98.3%	27	6	0	81.8%
1.5	173	6	0	96.7%	25	8	0	75.8%
2.0	169	10	0	94.4%	25	8	0	75.8%
2.5	168	11	0	93.9%	24	9	0	72.7%
3.0	167	12	0	93.3%	23	10	0	69.7%
5.0	121	24	34	85.9%	19	11	3	66.7%
10.0	23	35	121	70.5%	4	12	17	61.9%

** Number of Cases = number of cases remaining in analyses at the time interval specified. The N values decrease with time due to patients experiencing an event (death or recurrence) or being lost to follow-up.

Table 5 Probability of overall survival of breast cancer patients with non-amplified and amplified lesions.

Not Amplified					Amplified			
Time from Surgery (in years)	N**	Cumulative No. Events	Cumulative No. Cases Censored	Probability of Remaining Disease Free	N**	Cumulative No. Events	Cumulative No. Cases Censored	Probability of Remaining Disease Free
0.5	178	0	0	100.0%	32	0	0	100.0%
1.0	178	0	0	100.0%	32	0	0	100.0%
1.5	178	0	0	100.0%	31	1	0	96.9%
2.0	178	0	0	100.0%	30	2	0	93.8%
2.5	177	1	0	99.4%	30	2	0	93.8%
3.0	177	1	0	99.4%	28	4	0	87.5%
5.0	135	5	38	97.0%	19	10	3	68.8%
10.0	32	15	131	84.7%	4	11	17	55.0%

** Number of Cases = number of cases remaining in analyses at the time interval specified. The N values decrease with time due to patients experiencing an event (death or recurrence) or being lost to follow-up.

Receiver-Operating Characteristic (ROC)

The accuracy of the HER-2/*neu* System was evaluated by calculation of the area under receiver-operating characteristic (ROC) curves and the Mann-Whitney test (18). The area under the ROC curves for each outcome is as follows: early recurrence (within 2 years) = 0.69; recurrence (within 3 years) = 0.67; and disease-related death (within 3 years) = 0.90. Results of the Mann-Whitney test for each outcome indicated that areas under the curves are significantly greater than 0.5 (for each $p \leq 0.01$), indicating that the test is accurate in detecting amplification in patients that experience recurrence or disease-related death.

Utility of HER-2/*neu* System with Respect to Other Prognostic Factors

During the clinical study, other factors associated with prognosis in breast cancer were collected from patient histories. These factors included patient age at diagnosis, tumor size, and estrogen receptor status. In a separate study, tumor grade was systematically determined for these patients using archival material.

Of the 220 node negative invasive breast cancer cases, the following was determined:

- 1) Tumor Grade Status for 173 patients (Scarff-Bloom-Richardson, categories: Grade 1, 2, or 3)
- 2) Tumor Size for 191 patients (categories at 1 cm: ≤ 1 cm; > 1 cm; and categories at 2 cm: < 2 cm; ≥ 2 cm)
- 3) Estrogen Receptor Status for 178 patients (categories: positive, ≤ 10 fmoles/mg cytosol protein; negative, > 10 fmoles/mg cytosol protein)
- 4) Age at diagnosis for 220 patients (categories: < 50 ; 50 to 59; 60 to 69; 70+)

HER-2/*neu* amplification was analyzed along with and controlling for other prognostic factors (tumor grade, tumor size, estrogen receptor status and age at diagnosis) in both univariate and multivariate analysis. The significance of the prognostic factor to the clinical outcomes of early recurrence, recurrence, and disease-related death was examined.

For Early Recurrence (recurrence of disease within 24 months of surgery):

In the univariate analysis, HER-2/*neu* amplification status was the most powerful predictor of early recurrence and the only predictor to be statistically significant except tumor size at 2 cm where the "> 2 cm" category was a statistically significant predictor of early recurrence. The patient age groups of "60 to 69", and "70+" were statistically significant in predicting non-early recurrence relative to patients in the "< 50" age group.

In the multivariate analysis, HER-2/*neu* amplification status was shown to be independent of other prognostic factors and the most powerful predictor of early recurrence; its power was not diminished when controlling (adjusting) for the other prognostic factors. HER-2/*neu* amplification status was the only factor to be statistically significant in the multivariate analyses. The patient age groups "60 to 69" (at tumor size 1 cm and 2 cm), and "70+" (at tumor size 1 cm) were marginally significant predictors of non-early recurrence (no recurrence within 24 months) relative to patients in the "< 50" age group.

For Recurrence (at anytime):

In the univariate analysis, HER-2/*neu* amplification status was the most powerful predictor of recurrence and the only predictor to be statically significant, although the patient age group of "60 to 69" was statistically significant in predicting non-recurrence relative to patients in the "< 50" age group.

In the multivariate analysis, HER-2/*neu* amplification status was shown to be independent of the other prognostic markers and the most powerful predictor of recurrence; the power of HER-2/*neu* amplification was not diminished when controlling (adjusting) for the other prognostic factors. HER-2/*neu* amplification status was the only factor to be statistically significant in the multivariate analysis with the exception of patient age group "60-69" which was a statistically significant predictor of non-recurrence relative to patients in the "<50" age group.

For Disease-related death (at anytime):

In the univariate analysis, HER-2/*neu* amplification status was the most powerful predictor of disease-related death and the only predictor to be statistically significant except the patient age group "60 to 69" which was a marginally significant predictor of survival relative to the "< 50" age group.

In the multivariate analysis, HER-2/*neu* amplification status was shown to be independent of the other prognostic markers and the most powerful

predictor of disease-related death. HER-2/*neu* amplification status was the only factor to be statistically significant in the multivariate analysis.

Expected Results

Summary of Normal and Abnormal Values

The expected HER-2/*neu* System assay result in normal breast tissue (non-cancerous) was estimated in a population of 20 breast tissues samples from reduction mammoplasties. The overall observed mean was 2.2 signals per nucleus with a 95 per cent C. I. of 1.5 to 2.9 signals per nucleus.

The target population for analysis using the Oncor® INFORM™ HER-2/*neu* Gene Detection System was patients with primary node-negative, invasive breast carcinoma. The expected prevalence of early recurrence within 2 years is 4 to 6%. The expected prevalence of recurrence within 3 years is 2 to 10%. The expected prevalence of disease-related death (within 3 years) is 10 to 15% (Clinical Oncology, 1993, page 207).

Oncor's Clinical study evaluated HER-2/*neu* gene amplification status in 220 women with node negative invasive breast cancer whose only course of treatment was surgery, unless diagnosed with disease recurrence. For this study population HER-2/*neu* amplification was shown to have predictive power independent of the other prognostic markers evaluated (patient age at diagnosis, tumor size, tumor grade, and estrogen receptor. HER-2/*neu* was shown to be the strongest predictor for early recurrence (within 24 months), recurrence, and disease-related death.

The negative predictive value, probability of no disease being present in women with HER-2/*neu* non amplified tumors, was found to be high three years after diagnosis (93.3% based on a prevalence of 10.4%). The probability of being alive three years after diagnosis was 99.4%, based on a prevalence of 2.4%.

Laboratory Baseline Data

In conjunction with routine clinical use of the HER-2/*neu* System, each laboratory should establish baseline data in accordance with good clinical laboratory practice.

Validation of Counting 20 Cell/Tumor Nuclei in 2 Different Sections of the Slide

HER-2/*neu* amplification status in breast cancer tissue has been shown to be relatively homogeneous throughout the tumor. A study was conducted in which 100 individual non overlapping nuclei were counted for each specimen as representative for the entire tumor. The data were analyzed to determine if there was a significant difference between HER-2/*neu* signals counted in each cell for the first 20 nuclei and last 20 nuclei. The data were also analyzed for significant differences from the average count for the total 100 nuclei and the first and last 20 nuclei. The statistical analysis showed there was no significant difference therefore all subsequent studies and interpretation guide require signal enumeration in 20 nuclei located in two distinct areas of invasion. The mean of the signals counted in 40 nuclei determined the assay result.

Selection of 4 as the Cutoff Value

HER-2/*neu* amplification cutoff values between 1.5 and 21 signals per nucleus were evaluated in an assessment of the clinical utility of the HER-2/*neu* System assay. Cutoff analysis was performed for amplification status relative to both recurrence within 3 years and DRD. The relationship of amplification status to clinical outcomes was determined for all cases. Overall, a cutoff of 4 is the most appropriate way of classifying HER-2/*neu* results as either amplified or non-amplified.

There are 2 reasons for using a cutoff of 4 as an absolute cutoff. First, and most important, taking into account all data analyses, a cutoff of 4 signals per nucleus was indicated to achieve optimal clinical performance of the test. Second, a cutoff value of 4 was supported by a rational, scientific basis and is frequently used in the published literature.

Overall, a cutoff of 4 is the most appropriate way of classifying HER-2/*neu* results as either gene amplified or non-amplified.

Probability Tables and Survival Curves for Tumor Size and HER-2/*neu* Amplification Interactions

The interactions between tumor size and HER-2/*neu* amplification status are shown in the following probability tables and survival curves. Table 6 shows the probability of disease-free survival of tumor size and HER-2/*neu* amplification status for small (≤ 1 cm) amplified and non-amplified tumors and for large (> 1 cm) amplified and non-amplified tumors. The survival curve in Figure 2 shows the graphical representation of the probability of disease-free survival with tumor size alone. Figure 3 (without error bars) shows the probability of disease-free

survival of the interaction of tumor size (≤ 1 cm vs. > 1 cm) and HER-2/*neu* amplification (amp- vs. Amp+). Error bars show the standard error around the values in Figures 4 and 5.

The same analyses are shown for overall survival in Table 7 and Figures 6, 7, 8, and 9.

75

Table 6
Probability of Disease-free Survival
Tumor size large (> 1 cm) / small (\leq 1 cm)
and Oncor® INFORM™ HER-2/*neu* Amplification Status

Probability of disease-free survival of breast cancer patients with large / small and non-amplified / amplified tumors.

Time from Surgery (in Years)	Probability of Survival*			
	Small (\leq 1 cm); Non-Amplified (\leq 4)		Small (\leq 1 cm); Amplified ($>$ 4)	
	(95% CI)†	N**	(95% CI)†	N**
0.5	100% (100% to 100%)	39	80.0% (44.9% to 100.0%)	4
1.0	100% (100% to 100%)	39	80.0% (44.9% to 100.0%)	4
1.5	100% (100% to 100%)	39	80.0% (44.9% to 100.0%)	4
2.0	97.4% (92.5% to 100.0%)	38	80.0% (44.9% to 100.0%)	4
2.5	94.9% (88.0% to 100.0%)	37	80.0% (44.9% to 100.0%)	4
3.0	92.3% (83.9% to 100.0%)	36	80.0% (44.9% to 100.0%)	4
5.0	92.3% (83.9% to 100.0%)	27	60.0% (17.1% to 100.0%)	2
10.0	86.5% (73.0% to 100.0%)	4	60.0% (17.1% to 100.0%)	1

Time from Surgery (in Years)	Probability of Survival*			
	Large (>1 cm), Non-Amplified (\leq 4)		Large (>1 cm); Amplified ($>$ 4)	
	(95% CI)†	N**	(95% CI)†	N**
0.5	100% (100% to 100.0%)	118	96.0% (88.4% to 100.0%)	24
1.0	97.5% (94.7% to 100.2%)	115	80.0% (64.3% to 95.7%)	20
1.5	95.8% (92.0% to 99.5%)	113	76.0% (59.3% to 92.7%)	19
2.0	93.2% (88.7% to 97.7%)	110	76.0% (59.3% to 92.7%)	19
2.5	93.2% (88.7% to 97.7%)	110	72.0% (54.4% to 89.6%)	18
3.0	93.2% (88.7% to 97.7%)	110	68.0% (49.8% to 86.2%)	17
5.0	82.2% (75.1% to 89.2%)	77	68.0% (49.8% to 86.2%)	15
10.0	69.5% (57.9% to 81.0%)	16	61.8% (41.6% to 82.0%)	3

* Point estimate generated from the Kaplan Meier Statistic (Kaplan, E.L., and Meier, P., 1958).

† 95% Confidence Interval (C.I.) generated from the Greenwood estimate of standard error (Greenwood, M., 1926)

**Number of Cases = number of cases at risk remaining in analyses at the time interval specified. Tumor size was available for 187 specimens out of the 212 specimens in the "recurrence-free at any time" database. The Table above is calculated from these 187 specimens. The N values decrease with time due to patients experiencing an event (death or recurrence) or being lost to follow-up.

FIGURE 2

**Tumor Size
Cumulative Probability -- 1 cm
Disease-free Survival**

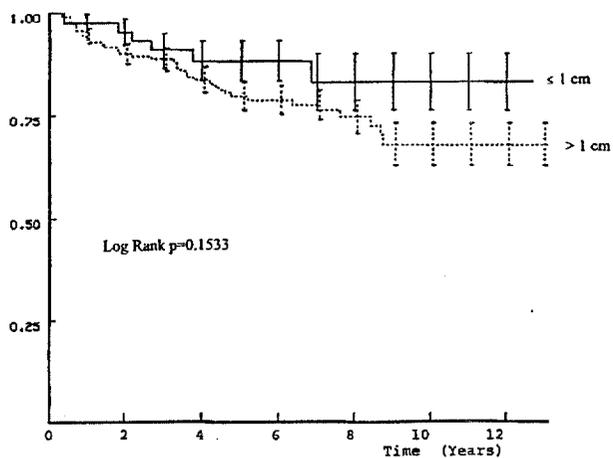


FIGURE 3

**Interaction (without error bars) of Oncor® INFORM™ HER-2/*neu* Amplification
and Tumor Size
Cumulative Probability of Disease-free Survival
HER-2/*neu* Amplification Status (amp+/amp-) and Tumor Size (≤ 1 cm/ > 1 cm)**

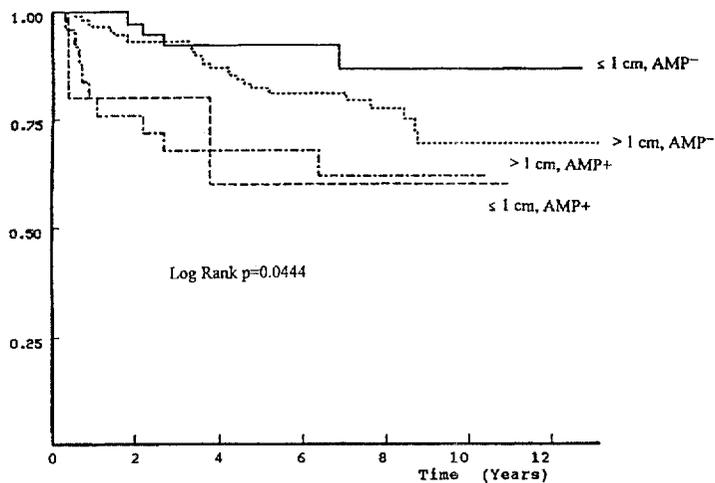


FIGURE 4

Interaction (with error bars) of Oncor® INFORM™ HER-2/*neu* Amplification and Tumor Size
Cumulative Probability of Disease-free Survival
HER-2/*neu* Amplification Status (amp+ / amp-) vs. Tumor Size (≤ 1 cm)

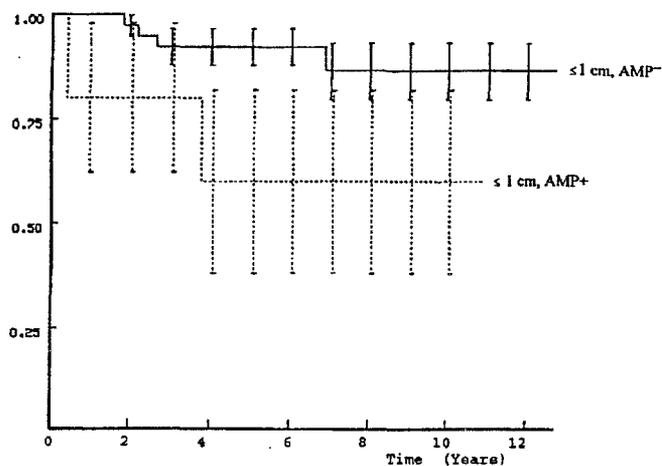


FIGURE 5

Interaction (with error bars) of Oncor® INFORM™ HER-2/*neu* Amplification and Tumor Size
Cumulative Probability of Disease-free Survival
HER-2/*neu* Amplification Status (amp+ / amp-) vs. Tumor Size (> 1 cm)

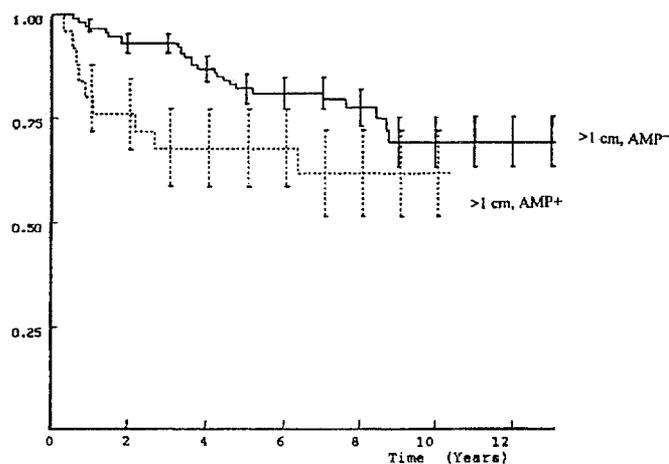


Table 7
Probability of Overall Survival
Tumor Size large (> 1 cm) / small (≤ 1 cm)
and Oncor® INFORM™ HER-2/neu Amplification Status

Probability of overall survival of breast cancer patients with large/small and non-amplified/amplified tumors.

Time from Surgery (in Years)	Probability of Survival*			
	Small (≤1 cm); Non-Amplified (≤ 4)		Small (≤1 cm); Amplified (>4)	
	(95% CI)†	N**	(95% CI)†	N**
0.5	100% (100.0% to 100.0%)	39	100% (100.0% to 100.0%)	5
1.0	100% (100.0% to 100.0%)	39	100% (100.0% to 100.0%)	5
1.5	100% (100.0% to 100.0%)	39	100% (100.0% to 100.0%)	5
2.0	100% (100.0% to 100.0%)	39	80.0% (44.9% to 100.0%)	4
2.5	100% (100.0% to 100.0%)	39	80.0% (44.9% to 100.0%)	4
3.0	100% (100.0% to 100.0%)	39	80.0% (44.9% to 100.0%)	4
5.0	100% (100.0% to 100.0%)	29	60.0% (17.1% to 100.0%)	2
10.0	100% (100.0% to 100.0%)	6	60.0% (17.1% to 100.0%)	1

Time from Surgery (in Years)	Probability of Survival*			
	Large (>1 cm), Non-Amplified (≤ 4)		Large (>1 cm); Amplified (>4)	
	(95% CI)†	N**	(95% CI)†	N**
0.5	100% (100% to 100.0%)	117	100% (100% to 100.0%)	25
1.0	100% (100% to 100.0%)	117	100% (100% to 100.0%)	25
1.5	100% (100% to 100.0%)	117	96.0% (88.4% to 100.0%)	24
2.0	100% (100% to 100.0%)	117	96.0% (88.4% to 100.0%)	24
2.5	99.2% (97.4% to 100.0%)	116	96.0% (88.4% to 100.0%)	24
3.0	99.2% (97.4% to 100.0%)	116	88.0% (75.3% to 100.0%)	22
5.0	96.4% (92.9% to 99.9%)	89	68.0% (49.8% to 86.2%)	15
10.0	83.4% (74.2% to 92.6%)	21	51.0% (19.1% to 82.9%)	3

* Point estimate generated from the Kaplan Meier Statistic (Kaplan, E.L., and Meier, P., 1958).

† 95% Confidence Interval (C.I.) generated from the Greenwood estimate of standard error (Greenwood, M., 1926)

**Number of Cases = number of cases at risk remaining in analyses at the time interval specified. Tumor size was available for 186 specimens out of the 210 specimens in the "disease-related death" database. The table above is calculated from these 186 specimens. The N values decrease with time due to patients experiencing an event (death or recurrence) or being lost to follow-up.

FIGURE 6

**Tumor Size
Cumulative Probability -- 1 cm
Overall Survival**

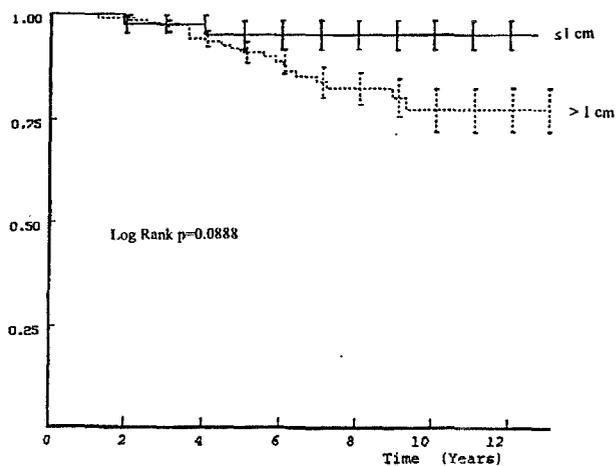


FIGURE 7

**Interaction (without error bars) of Oncor® INFORM™ HER-2/*neu* Amplification
and Tumor Size
Cumulative Probability of Overall Survival
HER-2/*neu* Amplification Status (amp+/amp-) and Tumor Size (≤1 cm/>1 cm)**

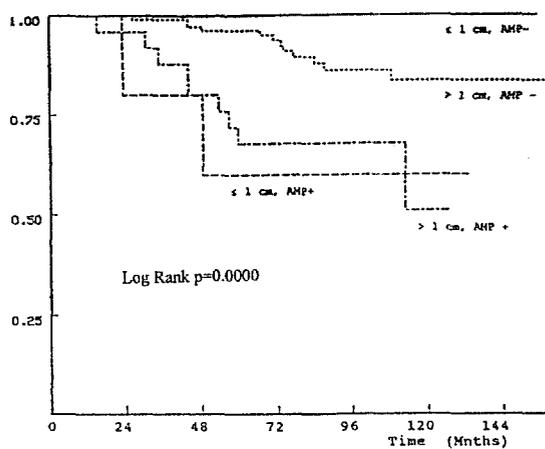


FIGURE 8

Interaction (with error bars) of Oncor® INFORM™ HER-2/*neu* Amplification and Tumor Size
Cumulative Probability of Overall Survival
HER-2/*neu* Amplification Status (amp+/amp-) vs. Tumor Size (≤ 1 cm)

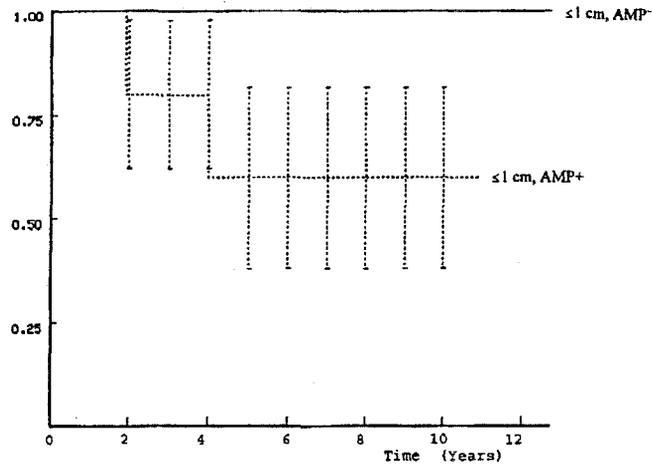
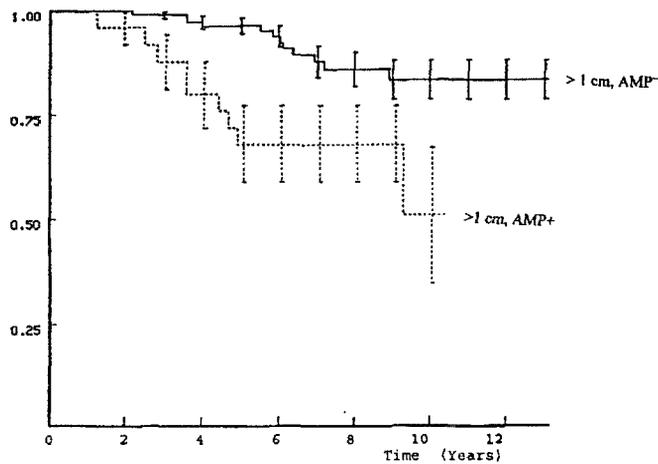


FIGURE 9

Interaction (with error bars) of Oncor® INFORM™ HER-2/*neu* Amplification and Tumor Size
Cumulative Probability of Overall Survival
HER-2/*neu* Amplification Status (amp+/amp-) vs. Tumor Size (> 1 cm)



31

The same analyses for interactions were also calculated with large and small tumors defined as ≥ 2 cm and < 2 cm, respectively. Table 8 shows the probability of disease-free survival of tumor size and Oncor® INFORM™ HER-2/*neu* amplification status for small (< 2 cm) amplified and non-amplified tumors and for large (≥ 2 cm) amplified and non-amplified tumors. The survival curve in Figure 10 shows the graphical representation of the probability of disease-free survival of tumor size alone (< 2 cm vs. ≥ 2 cm). Figure 11 shows the probability (without error bars) of disease-free survival of the interaction of tumor size (< 2 cm vs. ≥ 2 cm) and HER-2/*neu* amplification (amp- vs. amp+). Error bars show the standard error around the values in Figures 12 and 13.

The same analyses are shown for overall survival (disease-related death) in Table 9 and Figures 14, 15, 16, and 17.

Table 8
Probability of Disease-free Survival
Tumor Size large (≥ 2 cm) / small (< 2 cm)
and Oncor® INFORM™ HER-2/neu Amplification Status

Probability of disease-free survival of breast cancer patients with large / small and non-amplified / amplified tumors.

Time from Surgery (in Years)	Probability of Survival*			
	Small (< 2 cm); Non-Amplified (≤ 4)		Small (< 2 cm); Amplified (>4)	
	(95% CI)†	N**	(95% CI)†	N**
0.5	100% (100.0% to 100.0%)	85	90.0% (71.4% to 100.0%)	9
1.0	100% (100.0% to 100.0%)	85	80.0% (55.3% to 100.0%)	8
1.5	100% (100.0% to 100.0%)	85	80.0% (55.3% to 100.0%)	8
2.0	97.7% (94.5% to 100.0%)	83	80.0% (55.3% to 100.0%)	8
2.5	96.5% (92.6% to 100.0%)	82	80.0% (55.3% to 100.0%)	8
3.0	95.3% (90.8% to 99.8%)	81	70.0% (41.6% to 98.4%)	7
5.0	89.0% (82.1% to 95.9%)	55	60.0% (29.6% to 90.4%)	4
10.0	80.6% (69.4% to 91.8%)	7	60.0% (29.6% to 90.4%)	1

Time from Surgery (in Years)	Probability of Survival*			
	Large (≥ 2 cm), Non-Amplified (≤ 4)		Large (≥ 2 cm); Amplified (>4)	
	(95% CI)†	N**	(95% CI)†	N**
0.5	100% (100% to 100.0%)	72	95.0% (85.4% to 100.0%)	19
1.0	95.8% (91.1% to 100.0%)	69	80.00% (62.6% to 97.4%)	16
1.5	93.1% (87.2% to 98.9%)	67	75.0% (56.0% to 94.0%)	15
2.0	90.3% (83.4% to 97.1%)	65	75.0% (56.0% to 94.0%)	15
2.5	90.3% (83.4% to 97.1%)	65	70.0% (50.0% to 90.0%)	14
3.0	90.3% (83.4% to 97.1%)	65	70.0% (50.0% to 90.0%)	14
5.0	79.7% (70.1% to 89.3%)	49	70.0% (50.0% to 90.0%)	13
10.0	67.7% (54.0% to 81.4%)	13	63.0% (40.7% to 85.3%)	3

* Point estimate generated from the Kaplan Meier Statistic (Kaplan, E.L., and Meier, P., 1958).

† 95% Confidence Interval (C.I.) generated from the Greenwood estimate of standard error (Greenwood, M., 1926)

**Number of Cases = number of cases at risk remaining in analyses at the time interval specified. Tumor size was available for 187 specimens out of the 212 specimens in the "recurrence-free at any time" database. The table above is calculated from these 187 specimens. The N values decrease with time due to patients experiencing an event (death or recurrence) or being lost to follow-up.

FIGURE 10

**Tumor Size
Cumulative Probability -- 2 cm
Disease-free Survival**

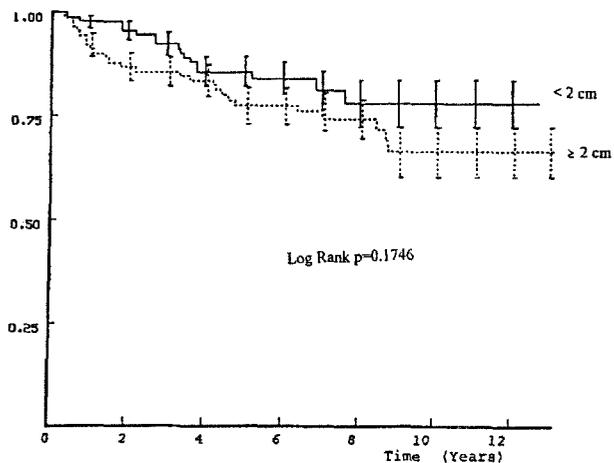


FIGURE 11

**Interaction (without error bars) of Oncor® INFORM™ HER-2/*neu* Amplification
and Tumor Size
Cumulative Probability of Disease-free Survival
HER-2/*neu* Amplification Status (amp+/amp-) and Tumor Size (< 2 cm/≥ 2 cm)**

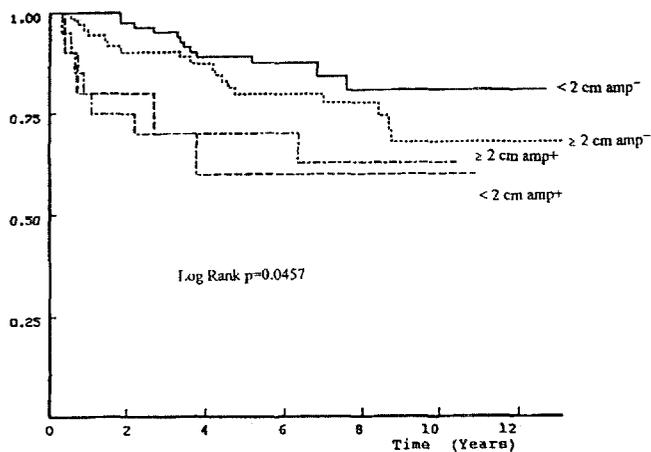


FIGURE 12

Interaction (with error bars) of Oncor® INFORM™ HER-2/*neu* Amplification and Tumor Size
Cumulative Probability of Disease-free Survival
HER-2/*neu* Amplification Status (amp+/amp-) vs. Tumor Size (< 2 cm)

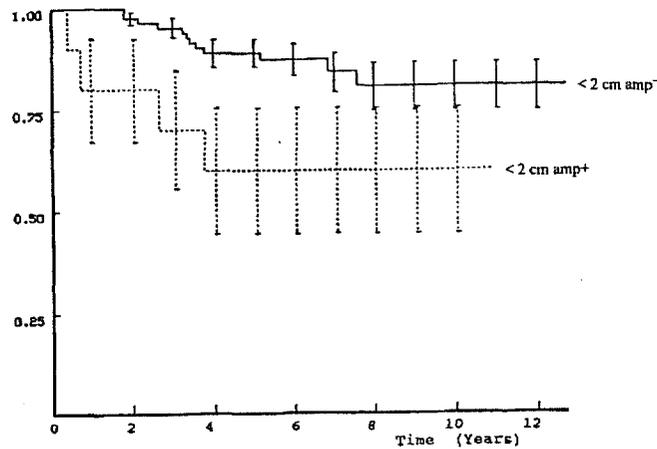


FIGURE 13

Interaction (with error bars) of Oncor® INFORM™ HER-2/*neu* Amplification and Tumor Size
Cumulative Probability of Disease-free Survival
HER-2/*neu* Amplification Status (amp+/amp-) vs. Tumor Size (≥ 2 cm)

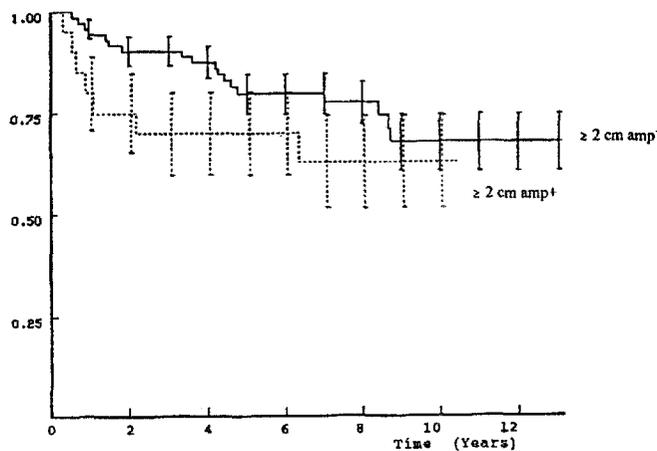


Table 9
Probability of Overall Survival
Tumor Size large (≥ 2 cm) / small (< 2 cm)
and Oncor® INFORM™ HER-2/*neu* Amplification Status

Probability of overall survival of breast cancer patients with large / small and non-amplified / amplified tumors.

Time from Surgery (in Years)	Probability of Survival*			
	Small (< 2 cm); Non-Amplified (≤ 4)		Small (< 2 cm); Amplified (> 4)	
	(95% CI)†	N**	(95% CI)†	N**
0.5	100% (100.0% to 100.0%)	85	100% (100.0% to 100.0%)	10
1.0	100% (100.0% to 100.0%)	85	100% (100.0% to 100.0%)	10
1.5	100% (100.0% to 100.0%)	85	100% (100.0% to 100.0%)	10
2.0	100% (100.0% to 100.0%)	85	90.0% (71.4% to 100.0%)	9
2.5	100% (100.0% to 100.0%)	85	90.0% (71.4% to 100.0%)	9
3.0	100% (100.0% to 100.0%)	85	90.0% (71.4% to 100.0%)	9
5.0	98.7% (96.2% to 100.0%)	61	60.0% (29.6% to 90.4%)	4
10.0	91.2% (82.6% to 99.9%)	10	60.0% (29.6% to 90.4%)	1

Time from Surgery (in Years)	Probability of Survival*			
	Large (≥ 2 cm), Non-Amplified (≤ 4)		Large (≥ 2 cm); Amplified (> 4)	
	(95% CI)†	N**	(95% CI)†	N**
0.5	100% (100.0% to 100.0%)	71	100% (100.0% to 100.0%)	20
1.0	100% (100.0% to 100.0%)	71	100% (100.0% to 100.0%)	20
1.5	100% (100.0% to 100.0%)	71	95.0% (85.4% to 100.0%)	19
2.0	100% (100.0% to 100.0%)	71	95.0% (85.4% to 100.0%)	19
2.5	98.6% (95.8% to 100.0%)	70	95.0% (85.4% to 100.0%)	19
3.0	98.6% (95.8% to 100.0%)	70	85.0% (69.3% to 100.0%)	17
5.0	95.6% (90.7% to 100.0%)	57	70.0% (50.0% to 90.0%)	13
10.0	84.2% (73.6% to 94.8%)	17	52.5% (19.2% to 85.8%)	3

* Point estimate generated from the Kaplan Meier Statistic (Kaplan, E.L., and Meier, P., 1958).

† 95% Confidence Interval (C.I.) generated from the Greenwood estimate of standard error (Greenwood, M., 1926)

**Number of Cases = number of cases at risk remaining in analyses at the time interval specified. Tumor size was available for 186 specimens out of the 210 in the "disease-related death" database. The table above is calculated from these 186 specimens. The N values decrease with time due to patients experiencing an event (death or recurrence) or being lost to follow-up.

FIGURE 14
Tumor Size
Cumulative Probability -- 2 cm
Overall Survival

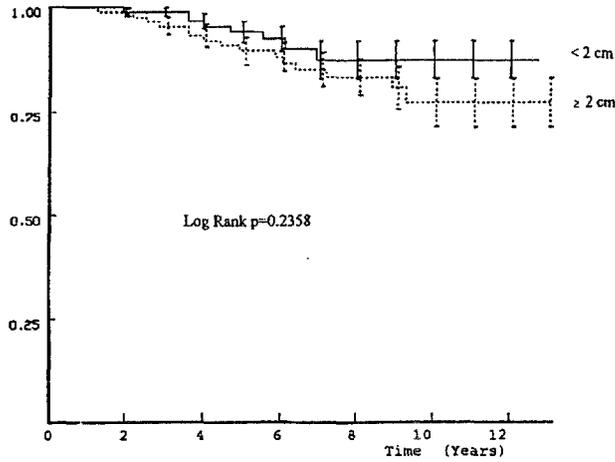
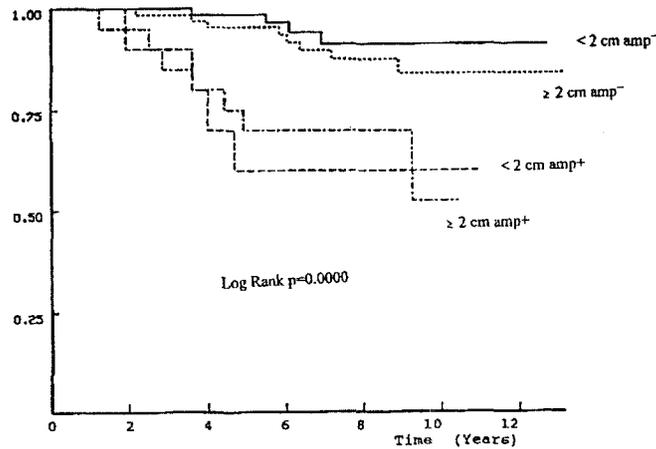


FIGURE 15

Interaction (without error bars) of Oncor® INFORM™ HER-2/*neu*
Amplification and Tumor Size
Cumulative Probability of Overall Survival
HER-2/*neu* Amplification Status (amp+/amp-) and Tumor Size (< 2 cm/≤ 2 cm)



27

FIGURE 16

Interaction (with error bars) of Oncor® INFORM™ HER-2/*neu* Amplification and Tumor Size
Cumulative Probability of Overall Survival
HER-2/*neu* Amplification Status (amp+/amp-) vs. Tumor Size (<2 cm)

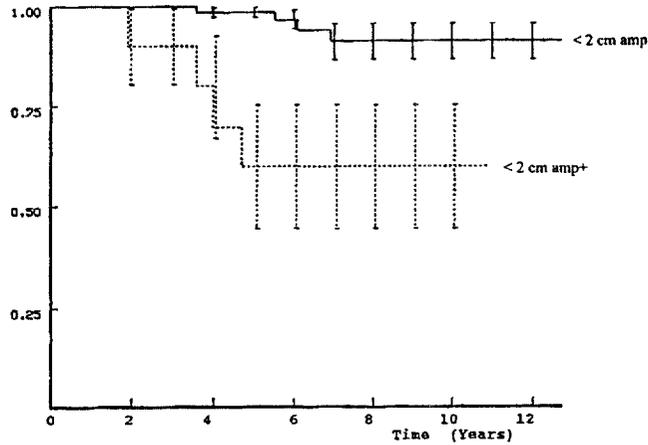
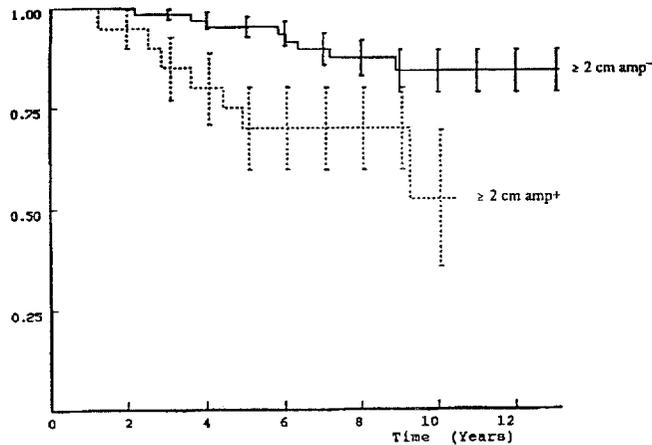


FIGURE 17

Interaction (with error bars) of Oncor® INFORM™ HER-2/*neu* Amplification and Tumor Size
Cumulative Probability of Overall Survival
HER-2/*neu* Amplification Status (amp+/amp-) vs. Tumor Size (≥ 2 cm)



Clinical Sensitivity and Specificity

When the HER-2/*neu* test results and clinical outcomes were compared, the HER-2/*neu* System showed the following clinical sensitivities, clinical specificities and positive predictive values (Table 14).

Table 14 Clinical Sensitivities; Specificities, and Positive Predictive Values

	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)	Positive Predictive Value (95% Confidence Interval)
Early Recurrence (within 2 years)	44.4% (21.5% - 69.2%)	86.6% (81.2% - 91.0%)	22.9% (10.4% - 40.1%)
Recurrence (within 3 years)	45.5% (24.4% - 67.8%)	87.9% (82.4% - 92.2%)	30.3% (15.6% - 48.7%)
Death (within 3 years)	80.0% (28.4% - 99.5%)	86.3% (80.9% - 90.7%)	12.5% (3.5% - 29.0%)

The comparison of the HER-2/*neu* test results and clinical outcome are summarized in the following tables.

Table 15 Early Recurrence

HER-2/ <i>neu</i> Gene Detection System	Outcome: Early Recurrence (Within 2 years)		
	Recurrence	No Recurrence	Total
Amplification Positive	8	27	35
Amplification Negative	10	175	185
Total	18	202	220

			95% C.I.
Prevalence	18/220	8.2%	(4.98% - 12.6%)
Sensitivity	8/18	44%	(21.5% - 69.2%)
Specificity	175/202	86.6%	(81.2% - 91.0%)
Positive Predictive Value		22.9%	(10.4% - 40.1%)
Negative Predictive Value		94.6%	(90.3% - 97.4%)

Table 16 Recurrence

	Outcome: Recurrence (Within 3 years)		
HER-2/ <i>neu</i> Gene Detection System	Recurrence	No Recurrence	Total
Amplification Positive	10	23	33
Amplification Negative	12	167	179
Total	22	190	212

			95% C.I.
Prevalence	22/212	10.4%	(6.6% - 15.3%)
Sensitivity	10/22	45.5%	(24.4% - 67.8%)
Specificity	167/190	87.9%	(82.4% - 92.2%)
Positive Predictive Value		30.3%	(15.6% - 48.7%)
Negative Predictive Value		93.3%	(88.6% - 96.5%)

Table 17 Disease Related Death

	Outcome: Disease Related Death (Within 3 years)		
HER-2/ <i>neu</i> Gene Detection System	Death	No Death	Total
Amplification Positive	4	28	32
Amplification Negative	1	177	178
Total	5	205	210

			95% C.I.
Prevalence	5/210	2.4%	(0.7% - 5.5%)
Sensitivity	4/5	80.0%	(28.4% - 99.5%)
Specificity	177/205	86.3%	(80.9% - 90.7%)
Positive Predictive Value		12.5%	(3.5% - 29.0%)
Negative Predictive Value		99.4%	(96.9% - 100.0%)

40

VIII. CONCLUSIONS DRAWN FROM THE STUDIES

The preclinical and clinical studies provide reasonable assurance that the Oncor® INFORM™ HER-2/*neu* Gene Detection System is safe and effective when used in accordance with the directions for use.

IX. PANEL RECOMMENDATION

The Immunology Devices Panel recommended against approval of this PMA on November 30, 1995. The following concerns were raised by the Panel during the discussion of this PMA:

1. Most panel members felt there was a need for a larger study and suggested that histological grade should be included in the analysis to see if HER-2/*neu* is an independent predictor and adds significant information.
2. Panel members also wanted to see data to assess local vs. Distant recurrence, nuclear grade, ploidy, and S-phase in relation to amplification. It was suggested that younger women should be more represented.
3. Concern was expressed about the time frame of the study - 1983 to 1987. The current standard of patient management is not reflected in the patient population sampled during this time frame. With current practices smaller tumors are being detected. However, no alternative was suggested by the Panel.
4. There is a need to assure the level of training and experience is adequate to obtain reliable results. It was suggested that this technology is not ready for the community hospital setting.

The Panel concluded that the safety and effectiveness of the device had not been established and that further analysis and labeling changes were needed. Therefore the Panel recommended that the PMA be found not approvable for the indications stated in the PMA.

X. CDRH ACTION ON THE APPLICATION

Following the November 30, 1995 panel meeting the applicant has submitted eleven amendments. DCLD has worked closely with the PMA applicant since the panel meeting to review the information required to answer the panel's concerns. CDRH considered the concerns raised by the Panel and did not concur with the need for a larger study. Instead it was felt that a reanalysis of the data would provide the information needed. In addition, the applicant was asked to assess the assay's

performance when combined with tumor size and to provide this information in the package insert.

The applicant has provided further information on the cell line controls required for use with the assay.

The applicant tested several different cutoffs, as suggested by the panel. However, it was concluded after a review of the data, that the original cutoff was the most optimal for the intended use of the assay. Several revisions were made to the product labeling to provide a better representation of the strengths and weaknesses of the device.

At the request of the FDA, the applicant has provided a protocol for a post market study to assess the performance of the assay in the hands of actual users, as well as the effectiveness of the training program provided by the applicant. The data will be submitted to the FDA for evaluation with the annual report.

CDRH determined that, based on the data submitted in the PMA and the additional study, the device has been shown to be safe and effective for the indications as specified in the labeling and issued an approval letter on December 30, 1997.

The manufacturing facility was inspected on June 11, 1997 and found to be in compliance with the Good Manufacturing Practices (GMP) regulations.

XI. APPROVAL SPECIFICATIONS

Directions for use: See labeling.

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order.

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Oncor[®] INFORM[™] HER-2/*neu* Gene Detection System

(Refer to the Oncor[®] INFORM[™] HER-2/*neu* Gene Detection System (S8000-KIT) Procedure and Interpretation Guide for further explanation and information)

INTENDED USE/INDICATIONS FOR USE

The Oncor[®] INFORM[™] HER-2/*neu* Gene Detection System is a fluorescence *in situ* hybridization (FISH) DNA probe assay that determines the qualitative presence of HER-2/*neu* gene amplification on formalin-fixed, paraffin-embedded human breast tissue as an aid to stratify breast cancer patients according to risk for recurrence or disease-related death. It is indicated for use as an adjunct to existing clinical and pathologic information currently used as prognostic indicators in the risk stratification of breast cancer in patients who have had a primary, invasive, localized breast carcinoma and who are lymph node-negative.

SUMMARY AND EXPLANATION OF THE TEST

Current evidence indicates that HER-2/*neu* protein overexpression and gene amplification are indicative of poor patient prognosis at all stages of breast cancer development (Seshadri, *et al.*, 1993, Wright, *et al.*, 1989 and Niehans *et al.*, 1993). HER-2/*neu* amplification appears early in breast cancer progression (Iglehart, *et al.*, 1990 and van de Vijver, *et al.*, 1988) and, when present is homogeneously distributed throughout the cancer (Iglehart, *et al.*, 1990 and Press, *et al.*, 1994). It is a logical choice as a prognostic marker when used as an adjunct with other accepted prognostic indicators.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Oncor[®] INFORM[™] HER-2/*neu* Gene Detection System is a kit consisting of DNA probe and detection reagents that yields a green fluorescent signal at the site of the HER-2/*neu* gene, on a blue fluorescent background of stained nuclear DNA.

The kit is intended to be used with sections (4 μ m) of formalin-fixed, paraffin-embedded breast cancer tissue mounted on microscope slides. The tissue sections are pretreated chemically and enzymatically to remove proteins that block DNA access. The DNA in the sections is converted from double- to single-strand by solution denaturation at 75°C. A hybridization solution is applied to the tissue section, which is then incubated under conditions favorable for annealing of probe DNA and genomic DNA sequences. Unannealed probe is washed off. The hybridized probe is detected using a fluorescently-tagged ligand (fluorescein-labeled avidin) which binds to the label on the DNA probe, thereby immobilizing the fluorescein at the site of the HER-2/*neu* gene. The remainder of the DNA is then stained with an intercalating fluorescent counterstain (DAPI in Antifade).

Excitement of fluorescein and DAPI results in the emission of green and blue light, respectively. The observer selects for these two colors by using a microscope filter set designed for simultaneous viewing of DAPI and fluorescein, and scores nuclei in the tissue section for the number of green signals on a blue background.

WARNINGS

The assay is intended to be performed and interpreted by users certified by Oncor's proficiency program.

The Oncor® INFORM™ HER-2/*neu* Gene Detection System is to be used as an adjunct to the data obtained by evaluation of other accepted prognostic indicators. It is not a screening test for breast cancer, nor is it intended for use as a test method for the diagnosis of breast cancer. It should not be used as the sole basis for making decisions regarding patient risk stratification.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use only.
2. The Oncor® INFORM™ HER-2/*neu* Gene Detection System is not intended for any diagnostic or prognostic use on non-breast cancers or fresh tissue.
3. Formamide is a potential teratogen and an eye, skin and respiratory irritant. Exercise extreme caution when using this reagent. Gloves, eye or face protection and lab-coat should be worn when handling. For handling and disposal, follow your institution's biosafety and hazardous waste disposal procedures and observe all applicable federal, state and local laws.
4. Detection Reagent (Fluorescein-labeled Avidin), Blocking Reagents, and Anti-Avidin Antibody contain sodium azide. Sodium azide may be fatal if swallowed and can cause skin and eye irritation. Gloves, eye or face protection and lab-coat should be worn when handling. For handling and disposal, follow your institution's biosafety and hazardous waste disposal procedures. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing of these reagents, flush with a copious amount of water and observe all applicable federal, state and local laws.