Summary of Safety and Effectiveness Data

I. General Information

Device Generic Name:	Device for Detection of HER-2/*neu* Gene Amplification in Human Breast Tissue
Device Trade Name:	PathVysion™ HER-2 DNA Probe Kit
Applicant's Name and Address:	Vysis, Inc. 3100 Woodcreek Drive Downers Grove, IL 60515
Premarket Approval Application (PMA) Number:	P980024/S001
Date of Notice of Approval to the Applicant:	December 31, 2001

II. Indications for Use

The PathVysion™ HER-2 DNA Probe Kit (PathVysion Kit) is designed to detect amplification of the HER-2/*neu* gene via fluorescence *in situ* hybridization (FISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens. Results from the PathVysion Kit are intended for use as an adjunct to existing clinical and pathologic information currently used as prognostic factors in stage II, node-positive breast cancer patients. The PathVysion Kit is further indicated as an aid to predict disease-free and overall survival in patients with stage II, node positive breast cancer treated with adjuvant cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) chemotherapy.

The PathVysion Kit is indicated as an aid in the assessment of patients for whom HERCEPTIN® (Trastuzumab) treatment is being considered (refer to HERCEPTIN package insert).

III. Device Description

The PathVysion Kit is used for the identification and quantification of HER-2/*neu* gene amplification by fluorescent *in situ* hybridization on formalin-fixed, paraffin-embedded tissue sections fixed on slides. The kit contains four principal component reagents: Locus Specific Identifier® (LSI) HER-2/*neu* and Chromosome Enumeration Probe® (CEP) 17 probe mixture, DAPI (4,6 diamidino-2-phenylindole) counterstain, Nonidet P-40 (NP-40), and 20X sodium chloride/sodium citrate (SSC).

The LSI HER-2/*neu* DNA probe is a 190 Kb SpectrumOrange directly labeled fluorescent DNA probe specific for the HER-2/*neu* gene locus and hybridizes to region 17q11.2-q12 on human chromosome 17. The CEP 17 DNA probe is a 5.4 Kb SpectrumGreen directly labeled fluorescent DNA probe specific for the alpha satellite DNA sequence (D17Z1 locus) at the centromeric region of chromosome 17 (17q11.2-q11.1). The CEP 17 probe is used as a control for determining the copy number for chromosome 17. In each cell, the copy numbers of HER-2/*neu* and CEP 17 are enumerated. The presence of amplified HER-2/*neu* is determined by the ratio of the average copy number of HER-2/neu to CEP 17.

IV. Contraindications

None known

V. Warnings and Precautions

Refer to the product labeling for a list of warnings and precautions

VI. Alternative Practices and Procedures

Other commercially available FISH devices for gene amplification determination in breast tissue of lymph node negative patients with localized invasive tumor. Alternative procedures for detection of gene product overexpression in human breast tissue include immunohistochemical (IHC), or polymerase chain reaction (PCR) techniques.

VII. Marketing History

The PathVysion Kit received a premarket approval decision from the FDA on December 11, 1998. The product has been on the market since January 1999 and is available in the following countries:

Argentina	Colombia	India	Slovenia
Bolivia	Denmark	Israel	South Africa
Paraguay	Egypt	Italy	Spain
Uruguay	Finland	Jordan	Portugal
Australia	Estonia	Korea	State of Bahrain
New Zealand	Latvia	Malaysia	Sweden
Austria	Lithuania	Mexico	Switzerland
Czech Republic	France	Norway	Taiwan
Slovakia	Greece	Philippines	Thailand
Hungary	Germany	Poland	Turkey
Brazil	Holland	Singapore	United Kingdom
Canada	Belgium	Indonesia	
China, Hong Kong	Luxembourg	Vietnam	

This product has not been withdrawn from any of these markets for any reason.

VIII. Potential Adverse Effects of the Device on Public Health

A potential risk associated with misuse of the assay, or a false positive test result is to assign patients to receive a more aggressive adjuvant therapy regimen than needed, possibly exposing the patient to serious side effects and, in rare cases, death. Alternatively, a false negative test result may exclude a patient who might benefit from more aggressive therapy from a treatment regimen, potentially resulting in a poor outcome.

IX. Summary of Studies

A. Non-Clinical Studies

1. Analytical

a) Hybridization Efficiency

Hybridization efficiency was established using ProbeChek™ quality control slides prepared from paraffin-embedded breast cancer cell lines. The average percentage of cells with no hybridization signal was 0.0 to 2.0%. Under these optimal conditions, the hybridization efficiency was 98%, with <2% cells having no signal for either probe.

b) Analytical Sensitivity

The analytical sensitivity of the PathVysion Kit probes was determined using the data from the reproducibility study described in 2 below. For the normal specimen (1.0 to 1.2 HER-2/*neu* to CEP 17 ratio), the estimated mean ratio was 1.05 (S.D. = 0.03). For the amplified specimen with a 1.6 to 2.0 HER-2/*neu* to CEP 17 ratio, the estimated mean ratio was 1.81 (S.D. = 0.08). The upper 95% Confidence Interval (CI) was 1.11 for the normal specimen and the lower 95% CI was 1.65 for the amplified specimen. The limit of detection for the PathVysion Kit in interphase cells was estimated to be a ratio of 1.5.

c) Analytical Specificity

i. Locus specificity

To determine locus specificity, metaphase spreads from normal lymphocytes were analyzed according to standard Vysis QC protocols. A total of 254 metaphase spreads were examined sequentially by G-banding to identify chromosome 17 and the HER-2/*neu* gene locus by FISH. No cross-hybridization to other chromosome loci was observed in the 254 cells examined; hybridization was limited to the intended target regions of the two probes.

ii. Stringency studies

Stringency studies included determination of the optimum denaturation time and temperature; hybridization time and

temperature; post-hybridization wash time and temperature; and post-hybridization wash buffer composition. These studies were performed on formalin-fixed, paraffin-embedded tissue specimens using the standard Vysis protocols.

For the denaturation step, three temperatures (65°C, 73°C, and 80°C) were tested for 2 minutes, 5 minutes and 8 minutes each. The results showed no statistical difference in the overall rating among all denaturation temperatures and durations.

Stringency of the hybridization step was tested in two parts; first, hybridizations were conducted at 5 different temperatures (27°C, 32°C, 37°C, 42°C, and 47°C) for 18 hours, then for 5 different durations (10 hr, 14 hr, 18 hr, 22 hr, and 26 hr) at the recommended temperature (37°C). Both hybridization temperature and time significantly affected hybridization quality. Hybridization at 37°C for 18 hours showed the highest overall quality ratings. Since the differences in hybridization quality were not statistically significant between 14 and 18 hours, an incubation time of 14-18 hours is recommended.

The post-hybridization wash step was tested in a similar manner by first performing the assay at 5 different temperatures (69°C, 71°C, 73°C, 76°C, and 80°C), followed by different durations, ranging from 2 to 8 minutes at 73°C. Wash temperature was a significant factor, with 73°C giving the best results. All wash times between 2 and 5 minutes produced acceptable results, but if the wash time was increased to 8 minutes, the overall quality in some samples was significantly decreased. Based on these results, the recommended post-hybridization wash conditions are $72 \pm 1^{\circ}$C for 2 minutes.

The wash buffer composition was also analyzed to determine the effect on signal intensity and probe specificity. Increasing the salt concentration from 0.4X Sodium Chloride and Sodium Citrate (SSC) to 2X SSC increased the signal intensity, but did not appear to compromise the probe specificity. Thus, a wash buffer composition of 2X SSC/0.3% NP-40 is recommended.

d) Methods Comparison

The Vysis FISH assay was compared to Southern, Northern and Western blot analyses in 143 archival breast cancer tissue specimens, as well as IHC analysis on frozen specimens using the Vysis DNA probes for HER-2/neu and CEP 17 [1]. FISH was found to have a positive agreement of 96.5% relative to the IHC method on frozen specimens, while Southern blot analysis had an agreement of 92.4% relative to IHC. The agreement of Vysis FISH and IHC on negative specimens was 100%. There was one failure with the FISH assay due to loss of tissue from the slide. For FISH analysis with the direct-labeled probe, the first-attempt success rate was 99% as compared to 83% for

Southern blot, 82% for Northern blot, 92% for Western blot, and 80% for FISH analysis with an indirect-labeled probe.

e) Stability

Expiration dating for this device has been established at 12 months. The protocol 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).

2. Reproducibility and Repeatability Studies

a) Breast Tissue Sections

The repeatability of the FISH assay for HER-2/*neu* was determined on consecutive sections of normal and amplified breast tissue, as well as on different thickness of the same tissue. On 10 consecutive tissue sections from one normal breast tissue, the average ratio of HER-2/*neu* to CEP 17 copy number was 1.19 (S.D. = 0.05); the results are shown in Table 1.

Table 1

Average Number of Signals per Cell and Ratio of HER-2/*neu*:CEP 17 Copy Number in Consecutive Sections (with normal HER-2/*neu*)

	Section Number									
	1	2	3	4	5	6	7	8	9	10
HER-2	3.8	3.2	3.6	3.5	3.6	3.5	3.4	3.5	3.3	3.1
CEP 17	3.1	3.1	3.0	2.7	3.0	3.0	2.8	2.1	3.0	2.7
Ratio	1.2	1.2	1.2	1.3	1.2	1.2	1.2	1.6	1.1	1.1

On 10 consecutive tissue sections from one specimen with amplified HER-2/*neu*, the average ratio of HER-2/*neu* to CEP 17 copy number was 3.61 (S.D. = 0.50); the results are shown in Table 2.

Table 2

Average Number of Signals per Cell and Ratio of HER-2/*neu*:CEP 17 Copy Number in Consecutive Sections (with amplified HER-2/*neu*)

	Section Number									
	1	2	3	4	5	6	7	8	9	10
HER-2	4.7	4.9	5.9	4.5	3.6	4.6	4.6	4.8	4.5	4.2
CEP 17	1.2	1.3	1.3	1.3	1.3	1.3	1.4	1.3	1.4	1.3
Ratio	3.9	3.7	4.7	3.6	2.8	3.7	3.3	3.8	3.3	3.3

Similarly, on 8 consecutive normal tissue sections of different thickness (2 to 8 microns), the average ratio of HER-2/*neu* to CEP 17 copy number was 1.15 (S.D. = 0.16); the results are shown in Table 3. These results

demonstrated an acceptable degree of reproducibility of the HER-2/*neu* FISH assay in tissue sections with thicknesses between 4 and 8 microns.

Table 3

Average Number of Signals per Cell and Ratio of HER-2/*neu*:CEP 17 Copy Number in Consecutive Sections of Different Thickness

	Thickness of Section (microns)							
	2	2	4	4	6	6	8	8
HER-2	2.3	2.4	2.4	2.7	2.7	2.8	2.6	3.3
CEP 17	1.7	1.8	2.3	2.5	2.7	2.7	2.5	3.2
Ratio	1.4	1.4	1.1	1.1	1.0	1.1	1.1	1.0

b) Control Slides

To determine the reproducibility of the HER-2/*neu* and CEP 17 assay, the ratios of HER-2/*neu* to CEP 17 were assessed for inter-site, inter-lot, inter-day and inter-observer reproducibility on control slides with differing levels of HER-2/*neu* gene amplification. Four specimens with normal (1.0-1.2) and amplified (1.6-2.0, 3-5, and 7-11) ratios of HER-2/*neu* to CEP 17 were evaluated according to the instructions for signal enumeration in the package insert. The overall hybridization success rate was 98.3% (118/120). Hybridization of the two replacement slides was also successful.

Using ANOVA, statistically significant variations were observed between observers, which reflects the subjectivity in signal interpretation and enumeration. No statistically significant variations were observed in any of the other study parameters. The mean, standard deviation, and percent CV of the observed ratios of HER-2/*neu* to CEP 17 are shown in Tables 4-7.

Table 4

Site-to-Site Reproducibility

Ratio of HER-2/*neu* to CEP 17	Statistics	Site #1	Site #2	Site #3
1.0-1.2	Mean S.D C.V.(%) N	1.08 0.03 2.66 8	1.01 0.04 3.58 8	1.07 0.07 6.77 8
1.6-2.0	Mean S.D. C.V.(%) N	1.81 0.05 2.88 8	1.71 0.05 2.78 8	1.78 0.19 10.50 8
3.0-5.0	Mean S.D. C.V.(%) N	4.39 0.22 4.99 8	3.65 0.18 4.93 8	4.49 0.79 17.64 8
7.0-11	Mean S.D. C.V.(%) N	7.21 0.15 2.07 8	8.26 0.83 10.10 8	8.23 0.87 10.55 8

S.D. (Standard Deviation), C.V.(%) (Coefficient of Variation).

Table 5
Lot-to-Lot Reproducibility

Ratio of HER-2/*neu* to CEP 17	Statistics	Lot #1	Lot #2	Lot #3	Lot #4
1.0-1.2	Mean S.D C.V.(%) N	1.05 0.07 6.48 6	1.07 0.06 6.06 6	1.02 0.03 3.21 6	1.04 0.05 4.87 6
1.6-2.0	Mean S.D. C.V.(%) N	1.78 0.10 5.65 6	1.77 0.13 7.49 6	1.77 0.15 8.54 6	1.75 0.09 5.07 6
3.0-5.0	Mean S.D. C.V.(%) N	4.08 0.44 10.78 6	3.92 0.34 8.74 6	4.57 0.96 20.92 6	4.14 0.40 9.56 6
7.0-11	Mean S.D. C.V.(%) N	7.67 0.69 8.97 6	7.72 0.72 9.36 6	7.89 0.88 11.16 6	8.33 1.06 12.68 6

S.D. (Standard Deviation), C.V.(%) (Coefficient of Variation).

Table 6

Day-to-Day Reproducibility

Ratio of HER-2/*neu* to CEP 17	Statistics	Assay Day #1	Assay Day #2	Assay Day #3	Assay Day #4
1.0-1.2	Mean	1.06	1.07	1.02	1.04
	S.D	0.06	0.07	0.05	0.04
	C.V.(%)	5.65	6.61	4.58	4.03
	N	6	6	6	6
1.6-2.0	Mean	1.76	1.77	1.77	1.77
	S.D.	0.17	0.14	0.08	0.10
	C.V.(%)	9.62	7.99	4.31	5.65
	N	6	6	6	6
3.0-5.0	Mean	4.24	4.48	4.10	3.89
	S.D.	0.48	0.97	0.36	0.38
	C.V.(%)	11.25	21.56	8.89	9.71
	N	6	6	6	6
7.0-11	Mean	7.91	8.01	7.72	7.97
	S.D.	1.11	0.90	0.57	0.89
	C.V.(%)	13.99	11.22	7.39	11.20
	N	6	6	6	6

S.D. (Standard Deviation), C.V.(%) (Coefficient of Variation).

Table 7
Observer-to-Observer Reproducibility

Ratio of HER-2/*neu* to CEP 17	Statistics	Observer #1	Observer #2
1.0-1.2	Mean	1.06	1.04
	S.D	0.07	0.03
	C.V.(%)	7.00	2.85
	N	12	12
1.6-2.0	Mean	1.71	1.82
	S.D.	0.10	0.11
	C.V.(%)	6.01	6.20
	N	12	12
3.0-5.0	Mean	4.05	4.31
	S.D.	0.44	0.73
	C.V.(%)	10.80	16.84
	N	12	12
7.0-11	Mean	7.52	8.28
	S.D.	0.49	0.95
	C.V.(%)	6.55	11.44
	N	12	12

S.D. (Standard Deviation), C.V.(%) (Coefficient of Variation).

3. Comparison of 20 Nuclei vs. 60 Nuclei

For signal enumeration, the applicant proposed changing the number of nuclei counted from 60 to 20. The study used to substantiate this change was the same described below for assay portability. This study was a five-center, blinded, randomized, comparative study using formalin-fixed, paraffin-embedded human breast cancer specimens with varying levels of HER-2/neu gene amplification. For each specimen, signal enumeration was performed on sets of 20 and 60 nuclei. Table 8 shows the comparison of the mean difference of LSI Her-2/neu to CEP 17 ratios [2].

Table 8

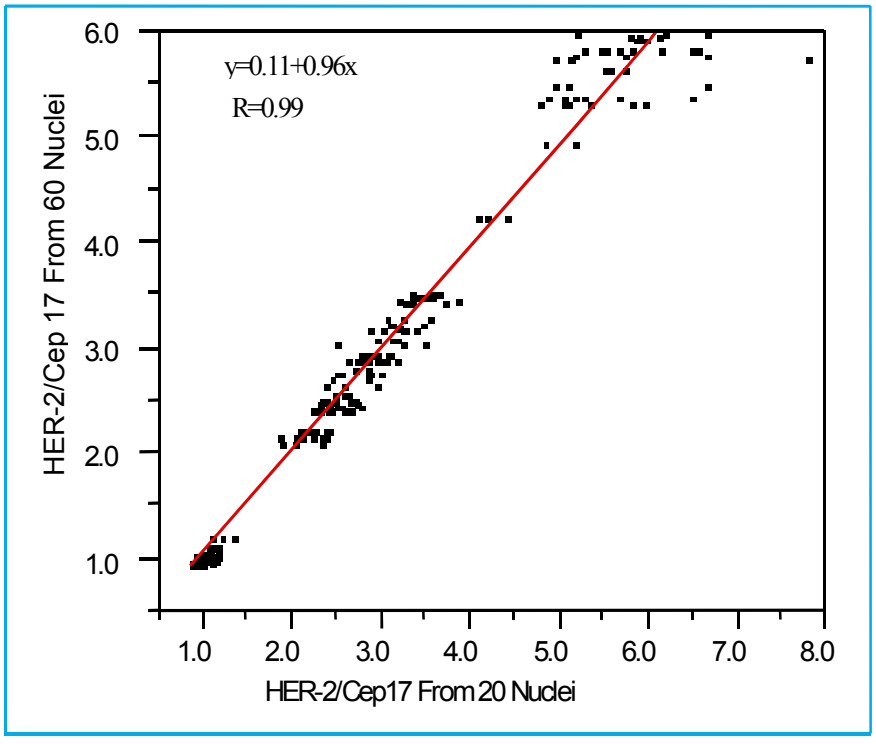
Comparison of Mean Difference of Ratios of LSI HER-2/neu to CEP 17

Expected Ratio	Mean		P-value
	20 nuclei (n=45)	60 nuclei (n=15)	
1.0-1.2	1.04	1.04	0.77
2.1-2.8	2.47	2.46	0.82
2.5-3.5	3.07	3.06	0.89
5.0-7.0	5.67	5.63	0.29

Regression analysis indicated a slope of 0.96 and a correlation coefficient of 0.99, indicating acceptable correlation between 20 and 60 nuclei results (Figure 1).

Figure 1

Correlation of Ratio of HER-2/*neu* to CEP 17 for Enumerating 60 vs. 20 Nuclei



4. Assay Portability Study

A five-center, blinded, randomized, comparative study using formalin-fixed, paraffin-embedded human breast cancer specimens with varying levels of HER-2/neu gene amplification was conducted to assess assay portability [2]. The specimens included one normal (no amplification), two with low-level, and one with moderate level HER-2/*neu* gene amplification, as determined by FISH. For each specimen, three sets of 20 nuclei were counted in different target areas on the slide. Sixty nuclei enumeration was also performed on the same slides. The results were compared between study sites.

a) Intra-assay Reproducibility

The intra-assay variations for all four ratios of LSI HER-2/*neu* to CEP 17 were estimated and presented in Table 9 [2].

Table 9

Ratios of LSI HER-2/*neu* to CEP 17

Ratio of HER-2/*neu* to CEP 17	**Mean**	**Standard Deviation**	**C.V. (%)**	**N**
20 nuclei				
1.0-1.2	1.04	0.10	9.60	45
2.1-2.8	2.47	0.32	12.96	45
2.5-3.5	3.07	0.31	10.10	45
5.0-7.0	5.67	0.63	11.11	45
60 nuclei				
1.0-1.2	1.04	0.07	6.73	15
2.1-2.8	2.46	0.27	10.98	15
2.5-3.5	3.06	0.28	9.15	15
5.0-7.0	5.63	0.30	5.33	15

b) Day-to-Day Reproducibility

Table 10 shows the mean observed ratios of LSI HER-2/*neu* to CEP 17 for the three assay days, based on enumerating 20 nuclei per specimen. Results for 60 nuclei are shown in parentheses [2]. There were no statistically significant variations in ratio values across the three study days ($p>0.05$). The results of this study demonstrated that day-to-day reproducibility was acceptable for either method of nuclei counting.

Table 10

Summary Statistics of LSI HER-2/neu to CEP 17 by Assay Day

Expected Ratio	Statistics	Assay Day #1	Assay Day #2	Assay Day #3	P-value
1.0-1.2	**Mean**	**1.01 (1.01)**	**1.06 (1.05)**	**1.05 (1.04)**	0.6826
	S.D.	0.10 (0.08)	0.12 (0.10)	0.08 (0.05)	(0.6395)
	C.V.(%)	9.90 (7.92)	11.32 (9.52)	7.62 (4.81)	
	N	15 (5)	15 (5)	15 (5)	
2.1-2.8	**Mean**	**2.54 (2.53)**	**2.43 (2.42)**	**2.43 (2.42)**	0.5535
	S.D.	0.19 (0.11)	0.32 (0.28)	0.22 (0.39)	(0.7623)
	C.V.(%)	7.48 (4.34)	13.17 (11.57)	17.70 (16.12)	
	N	15 (5)	15 (5)	15 (5)	
2.5-3.5	**Mean**	**3.18 (3.17)**	**2.98 (2.98)**	**3.03 (3.03)**	0.2083
	S.D.	0.30 (0.27)	0.31 (0.30)	0.32 (0.30)	(0.5815)
	C.V.(%)	9.43 (8.52)	10.40 (10.07)	10.56 (9.90)	
	N	15 (5)	15 (5)	15 (5)	
5.0-7.0	**Mean**	**5.69 (5.66)**	**5.63 (5.60)**	**5.69 (5.02)**	0.9620
	S.D.	0.53 (0.29)	0.49 (0.25)	0.86 (0.42)	(0.9652)
	C.V.(%)	9.31 (5.12)	8.70 (4.46)	15.11 (7.47)	
	N	15 (5)	15 (5)	15 (5)	

c) Site-to-Site Reproducibility

There was some statistically significant variation in the mean observed ratios of LSI HER-2/*neu* to CEP 17 across the five study sites for the normal and 2.5-3.5 specimen ($p=<0.05$) based on enumerating 20 nuclei per specimen as shown in Table 11 [2]. These differences did not affect clinical assessment since 99% of the specimens were correctly classified as positive or negative for HER-2/*neu* gene amplification.

Table 11

Summary Statistics of LSI HER-2/*neu* to CEP 17 by Assay Day

Expected Ratio	Statistics	Site #1	Site #2	Site #3	Site #4	Site #5	P-value
1.0-1.2	**Mean**	**1.00 (1.00)**	**1.16 (1.15)**	**1.01 (1.01)**	**1.04 (1.04)**	**0.97 (0.98)**	0.0001
	S.D.	0.09 (0.03)	0.09 (0.06)	0.07 (0.06)	0.09 (0.02)	0.04 (0.02)	(0.0032)
	C.V.(%)	9.00 (3.00)	7.76 (5.22)	6.93 (5.94)	8.65 (1.92)	4.12 (2.04)	
	N	9 (3)	9 (3)	9 (3)	9 (3)	9 (3)	
2.1-2.8	**Mean**	**2.40 (2.39)**	**2.46 (2.45)**	**2.57 (2.55)**	**2.26 (2.26)**	**2.65 (2.65)**	0.0965
	S.D.	0.19 (0.15)	0.26 (0.24)	0.52 (0.46)	0.22 (0.18)	0.24 (0.20)	(0.4919)
	C.V.(%)	7.92 (6.28)	10.60 (9.80)	20.20 (18.04)	9.73 (7.95)	9.06 (7.55)	
	N	9 (3)	9 (3)	9 (3)	9 (3)	9 (3)	
2.5-3.5	**Mean**	**3.01 (3.00)**	**3.09 (3.09)**	**3.41 (3.41)**	**2.74 (2.73)**	**3.08 (3.08)**	<0.0001
	S.D.	0.21 (0.16)	0.35 (0.38)	0.20 (0.12)	0.23 (0.08)	0.16 (0.12)	(0.0269)
	C.V.(%)	6.98 (5.33)	11.3 (12.30)	5.87 (3.52)	8.39 (2.93)	5.19 (3.90)	
	N	9 (3)	9 (3)	9 (3)	9 (3)	9 (3)	
5.0-7.0	**Mean**	**5.48 (5.42)**	**5.22 (5.19)**	**5.94 (5.89)**	**5.82 (5.73)**	**5.91 (5.91)**	0.0568
	S.D.	0.66 (0.07)	0.43 (0.21)	0.56 (0.07)	0.89 (0.08)	0.18 (0.05)	(<0.0001)
	C.V.(%)	12.0 (1.29)	8.24 (4.05)	9.43 (1.19)	15.30 (1.40)	3.05 (0.85)	
	N	9 (3)	9 (3)	9 (3)	9 (3)	9 (3)	

Results for 60 nuclei are shown in parentheses

The assay variations for all five sites are summarized in Table 12. The standard deviation (S.D.) and the coefficient of variation (C.V.) were relatively small and stable across all ratios of LSI HER-2/*neu* to CEP 17. The hybridization success rate for this study was 100%.

Table 12

Summary of Site-to-Site Reproducibility

HER-2/*neu* To CEP 17 Ratio	Mean	Standard Deviation	C.V. (%)	N
1.0-1.2	1.04	0.10	9.60	45
2.1-2.8	2.47	0.32	12.96	45
2.5-3.5	3.07	0.31	10.10	45
5.0-7.0	5.67	0.63	11.11	45

B. Clinical Studies

1) Dose of Cyclophosphamide, Adriamycin and 5-fluorouracil (CAF) (CALGB 8869 Study)

The objectives of this study were to determine whether the amplification of HER-2/*neu*, as assessed by FISH with DNA probe, provided statistically significant and independent prognostic information pertaining to disease-free survival and overall survival in stage II node-positive patients receiving adjuvant therapy.

Subject Selection and Exclusion Criteria

Only patients who met all of the following inclusion criteria were included:

1. Patients with node positive stage II breast cancer receiving adjuvant therapy in CALGB protocol 8869.

2. Sufficient archival paraffin-embedded tissue available for FISH assay.

3. Complete information available on relapse, survival, as well as other relevant clinical data.

Patients not meeting the inclusion criteria as specified above were excluded from the study.

Vysis Protocol 302 investigated whether HER-2/*neu* gene amplification could be used to identify those patients more likely to benefit from high doses of chemotherapy. FISH assay with the PathVysion Kit was performed on a sample of 572 patients, randomly selected from those patients included in the CALGB 8869 study. Among these 572 patients, 45 were excluded due to FISH assay failures, and 3 were duplicate assays. The remaining 524 cases were used in the analysis of clinical utility (92% of the specimens were evaluable by FISH assay).

Analysis of Clinical Investigation

The results of analysis with Cox proportional hazard model for disease-free survival using FISH measurement of HER-2/*neu* gene amplification showed a statistically significant (p=0.033) interaction between HER-2/*neu* gene amplification and the cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) dose regimen. Similarly, the results of Cox proportional hazard model for overall survival also showed a statistically significant (p=0.028) interaction between HER-2/*neu* gene amplification and the CAF dose regimen (see Table 13).

Table 13

Likelihood-Ratio Tests for Disease-free Survival

	Disease-Free Survival			Overall Survival		
Source	DF	ChiSq	P value	DF	ChiSq	P value
CAF	2	5.56	0.06	2	4.57	0.10
Square root: #positive nodes	1	72.87	0.0000	1	56.32	0.0000
Tumor>2 cm	1	13.77	0.0002	1	12.93	0.0003
PREMENOPAUSAL	1	1.96	0.16	1	0.10	0.76
HER-2 ratio	1	10.05	0.0015	1	10.52	0.0012
HER-2 ratio interaction of CAF dose	2	6.84	0.033	2	7.15	0.028

As expected from the significance tests for the HER-2/*neu* by CAF inter-action from the proportional hazards models, there was a significant dose-response effect of adjuvant chemotherapy with CAF in patients with HER-2/*neu* gene amplification, but not in patients with no or minimal HER-2/*neu* amplification. Disease-free survival probabilities (Figure 2a & b) were comparable among the three dose groups of patients with HER-2/*neu* -negative tumors.

At 7 years post-randomization, the estimated disease-free survival probabilities were 55%, 63%, and 61% for low (L), moderate (M), and high (H) dose, respectively. The dose effect is greater for HER-2/*neu* positive tumors, with disease-free survival at 7 years of 36%, 44%, and 66% for L, M, and H, respectively (Table 14).

Table 14

Disease-free Probabilities

Dose	**HER-2/*neu* Negative**	**HER-2/*neu* positive**
Low	55%	***36%***
Moderate	63%	***44%***
High	61%	***66%***

The corresponding figures for 7-year overall survival (Figure 2c & d) have a similar relationship: 64%, 75%, and 70% for HER-2/*neu* negative and 48%, 50%, and 76%, again for L, M, and H, respectively.

Table 15

Overall Survival Probabilities

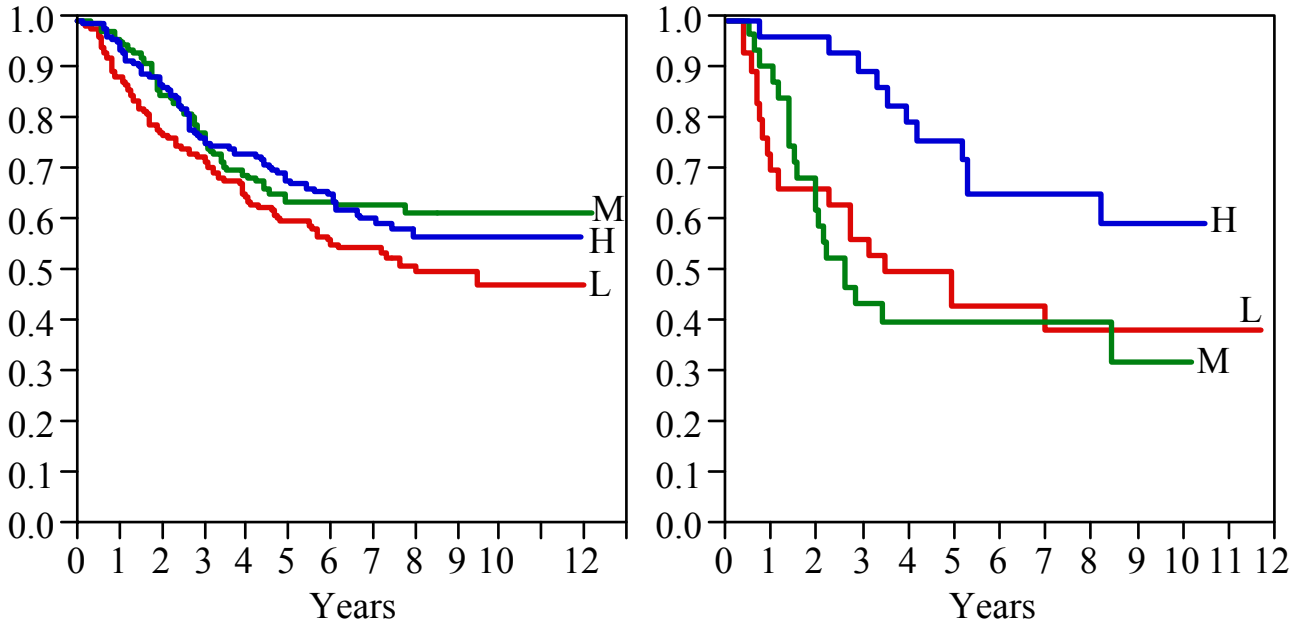
Dose	**HER-2/*neu* Negative**	**HER-2/*neu* positive**
Low	64%	***48%***
Moderate	75%	***50%***
High	70%	***76%***

This association was found in both disease-free and overall survival, and was consistent with those observed with HER-2/neu expression by the immunohistochemistry method.

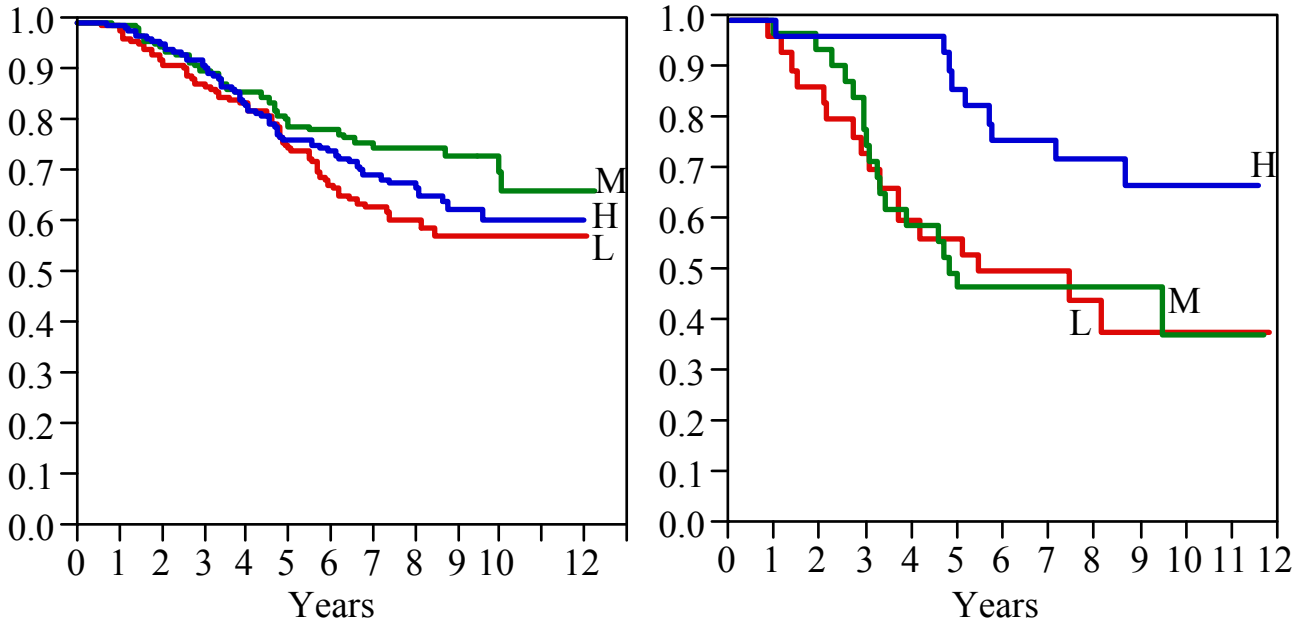
Figure 2

Disease-free (a, b) and overall (c, d) survival for patients with HER-2/*neu* negative (a, c) and positive (b, d) tumors for the three CAF dose groups, H, M, and L*

(a) Disease-free survival for HER2/*neu* negative (b) Disease-free survival for HER2/*neu* positive



(c) Overall survival for HER2/*neu* negative (d) Overall survival for HER2/*neu* positive



* HER-2/*neu* positivity means HER/CEP ≥ 2. Sample sizes in (a, c) are 149, 136, and 148 (for H, M, and L) and in (b, d) are 30, 31, and 30. The significance levels for the HER2/*neu* by CAF interaction from the proportional hazards models (Table 12) are 0.033 for disease-free survival—(a) vs. (b)—and 0.028 for overall survival—(c) vs. (d).

2) Concordance with the Clinical Trial Assay (CTA)

The primary mechanism of HER-2 protein overexpression in human breast cancer appears to be via gene amplification [3,4]. Fluorescence *in situ* hybridization (FISH) detection of HER-2/*neu* gene amplification provides an additional diagnostic method to define HER-2 overexpression.

The PathVysion Kit was compared to the Clinical Trial Assay (CTA), which was used to enroll patients into the Genentech-sponsored pivotal HERCEPTIN trials (H0648g, H0649g, H0650g) [4]. To establish concordance between FISH and the CTA, a subset of 623 specimens (317 positive and 306 negative, as determined by the CTA), were randomly selected in an intended 1:1 ratio from the specimens screened for enrollment in the HERCEPTIN trials. FISH assays were performed on all specimens, with informative results achieved on 529 specimens. The results from the analysis of the 529 informative cases are presented in Table 16.

Table 16

CTA versus FISH

	CTA Score				
FISH	0	1+	2+	3+	Total
Negative	207	28	67	21	323
Positive	7(3.2%)	2(6.7%)	21(23.9%)	176(89.3%)	206
Total	214	30	88	197	529

The results showed a 2x2 concordance of 82% (95% CI 79% - 85%), where concordance was defined as the proportion of samples rated 0 or 1+ by CTA and not amplified by FISH plus the proportion of samples rated 2+ or 3+ by CTA and amplified by FISH. These data are consistent with a high concordance between protein overexpression [as determined by immunohistochemistry (CTA)] and gene amplification [as determined by FISH (Vysis PathVysion)].

X. Conclusions Drawn from the Studies

It is believed that the previous studies demonstrate the following:

- Acceptable performance is obtained with the PathVysion HER-2 DNA Probe assay on formalin-fixed, paraffin-embedded human breast cancer tissue sections with varying levels of gene amplification.

- The PathVysion HER-2 DNA Probe assay demonstrated inter-day, inter-lot and inter-site reproducibility of <10% CV for normal and weakly amplified specimens and <20% CV for moderately and highly amplified specimens. The variability between observers reflects subjectivity in signal interpretation and enumeration and can be minimized by adequate training and proficiency assessment prior to test implementation. The overall hybridization success rate is 98.3%.

- The limit of detection as defined by the HER-2/*neu* to CEP 17 ratio for the PathVysion HER-2 DNA Probe assay is estimated to be 1.5. The estimated mean ratio for a normal specimen with a HER-2/*neu* to CEP 17 ratio of 1.0 to 1.2 was 1.05±0.03 and for an amplified specimen with a HER-2/*neu* to CEP 17 ratio of 1.6 to 2.0 in this analysis was 1.81±0.08. The upper 95% Confidence Interval (CI) is 1.11 for the normal specimen and the lower 95% CI is 1.65 for the amplified specimen in this analysis.

- Comparable amplification results can be obtained by enumerating 20 nuclei instead of 60 nuclei as originally recommended. Additional nuclei should be counted for results at or near the cutoff point (1.8 to 2.2) or if there is significant variability in signal number from nucleus to nucleus.

- The PathVysion HER-2 DNA Probe Kit can be stored up to 12 months at -20ºC when protected from light and humidity. The 20X SSC salts and NP-40 can be stored at room temperature. Same storage conditions apply for both opened and unopened reagents.

- Based on clinical laboratory studies, the PathVysion HER-2 DNA Probe assay when used in accordance with the provided directions and in conjunction with clinical information, is safe and effective in the determination of the HER-2/neu amplification status in patients with stage II, node-positive breast cancer. Concordance with immunohistochemistry (IHC) was found to be 82% (95% CI: 97-85%) for samples that were rated 0 or 1+ by the Clinical Trial Assay (CTA) and not amplified by FISH and those that were rated 2+ or 3+ by CTA and amplified by FISH.

Safety

As a diagnostic test, the PathVysion HER-2/neu DNA Probe assay involves testing on formalin-fixed, paraffin embedded human breast cancer tissue sections. These tissue sections are routinely removed for breast cancer diagnosis. The test, therefore, presents no additional safety hazard to the patient being tested.

Benefit/Risk

The submitted clinical studies have shown that the PathVysion HER-2 DNA Probe Kit, when compared to the reference methods IHC and CTA, has similar ability to detect HER-2/neu amplification in specimens from patients with stage II, node positive breast cancer. The rate of false positivity and false negativity are within acceptable limits compared to the reference methods. Thus, this device should benefit the physician in assessing patients for HERCEPTIN (Trastuzumab) treatment and patients treated with adjuvant CAF chemotherapy.

Based on the results of the preclinical and clinical studies, the PathVysion HER-2 DNA Probe Kit, when used according to the provided directions and in conjunction with clinical information, should be safe and effective and pose minimal risk to patient due to false test results.

XI. Panel Recommendation

Pursuant to Section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Immunology Devices Advisory Panel meeting because the information in the PMA substantially duplicated information previously reviewed by this Panel.

XII. CDRH Decision

CDRH issued an approval order for the applicant's PathVysion Kit on December 31, 2001.

XIII. Approval Specifications

Directions for Use: See labeling

Hazards to Health from Use of the Device: See Contraindications, Warnings, Precautions and Adverse Events in the attached labeling.

Postapproval Requirements and Restrictions: See approval order.

XIV. References

1. Press MF, Zhou JY, Ma Y, *et al.* Evaluation of HER-2/*neu* gene amplification by fluorescence *in situ* hybridization in invasive breast carcinoma. In: FISH: Clinical Applications in Cancer and Genetics; February 8-11, 1994; Lake Tahoe, CA.
2. Persons DL, Bui MM, Lowery MC, *et al*. Fluorescence *in situ* hybridization (FISH) for detection of HER-2/*neu* amplification in breast cancer: a multicenter portability study. *Ann Clin Lab Science*. 2000;30(1):41-48.
3. Pauletti G, Dandekar S, Rong H; *et al.* Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. J Clin Oncol. 2000;18(21):3651-3664.
4. Slamon DJ, Leyland-Jones B, Shak S, *et al*. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med.* 2001;344:783-792.