Polysomy 17 in Breast Cancer: Clinicopathologic Significance and Impact on HER-2 Testing

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Submitted July 9, 2007; accepted May 14, 2008; published online ahead of print at www.jco.org on September 15, 2008.

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/08/2630-4869/\$20.00

DOI: 10.1200/JCO.2007.13.4296

ABSTRACT

Purpose

Polysomy 17 is frequently found in breast cancer and may complicate the interpretation of HER-2 testing results. We investigated the impact of polysomy 17 on HER-2 testing and studied its clinicopathologic significance in relation to *HER2* gene amplification.

Patients and Methods

In 226 patients with primary invasive breast carcinoma, *HER2* gene and chromosome 17 copy numbers were determined by dual-color fluorescent in situ hybridization (FISH). The interpretation of FISH results was based on either absolute *HER2* gene copy number or the ratio *HER2*/chromosome 17. Results were correlated with HER-2 protein expression on immunohistochemistry (IHC), *HER2* mRNA expression by reverse transcriptase polymerase chain reaction (RT-PCR), and with various clinicopathologic parameters.

Results

All cases with an equivocal HER-2 result by FISH, either by absolute *HER2* copy number (44 of 226 patients; 19.5%) or by the ratio *HER2*/chromosome 17 (three of 226 patients; 1.3%), displayed polysomy 17. On its own, polysomy 17 was not associated with HER-2 overexpression on IHC or increased *HER2* mRNA levels by RT-PCR. Moreover, and in contrast with *HER2* gene amplification, polysomy 17 was not associated with high tumor grade, hormone receptor negativity, or reduced disease-free survival.

Conclusion

Polysomy 17 affects HER-2 testing in breast cancer and is a major cause of equivocal results by FISH. We show that tumors displaying polysomy 17 in the absence of *HER2* gene amplification resemble more HER-2-negative than HER-2-positive tumors. These findings highlight the need for clinical trials to investigate whether polysomy 17 tumors benefit from HER-2-targeted therapy.

J Clin Oncol 26:4869-4874. © 2008 by American Society of Clinical Oncology

INTRODUCTION

The search for prognostic markers and therapeutic targets in human breast cancer has revealed a major role for the HER-2 oncoprotein. Overexpression of HER-2 has been reported in 15% to 25% of invasive breast carcinomas.^{1,2} In most cases, this can be attributed to amplification of the *HER2* oncogene located on the long arm of chromosome 17 (17q12).³ Both HER-2 overexpression and *HER2* gene amplification have been correlated with poor clinical outcome.⁴⁻⁶ Apart from its prognostic value, the HER-2 status has major therapeutic implications. Not only does HER-2 overexpression predict response to certain chemotherapeutic agents, such as anthracyclines or paclitaxel, it is also considered to be a strong predictive marker for clinical benefit from HER-2-targeted therapy (trastuzumab) in the meta-

static setting and, more recently, also in the adjuvant setting.⁷⁻¹² Although tumors not expressing HER-2 have virtually no chance of responding to trastuzumab, moderate or even high levels of expression are not always associated with a therapeutic success. Moreover, treating patients with breast cancer with trastuzumab is expensive and not without risk, because serious cardiac toxicity has been observed in approximately 1% to 4% of patients.¹³ Therefore, correct identification of patients who will benefit from trastuzumab therapy is of utmost importance.

A wide variety of techniques can be applied to determine the HER-2 status in breast cancer tissue. Of these, immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) are most frequently used. Although both methods have shown high concordance in some studies, reproducibility remains poor in others.^{14,15} Recently, an expert team

assembled by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) has developed guidelines for HER-2 testing in breast cancer.¹⁶ Accordingly, HER-2 testing results should be reported as either positive, negative, or equivocal. The latter group represents a gray area of breast tumors scoring 2+ on IHC or having a modest increase in *HER2* gene copy number by FISH. Interestingly, equivocal HER-2 testing results have been related to chromosome 17 polysomy.¹⁷⁻¹⁹ Indeed, tumors featuring an increased chromosome 17 copy number will contain more copies of the *HER2* gene, which could result in elevated HER-2 expression. At present, it remains unknown to what extent polysomy 17 obscures the interpretation of HER-2 testing results and whether polysomy 17 tumors share biologic characteristics with true HER-2-positive breast cancers. In the present study, we aimed to clarify this issue.

PATIENTS AND METHODS

Patients

Since 2002, routine HER-2 FISH analysis (PathVysion; Vysis, Downers Grove, IL) has been performed at the Pathology Department of the University Hospital Gasthuisberg in Leuven, Belgium, on patients with breast cancer showing an equivocal or positive HER-2 result on IHC (score 2+/3+). From this series of 751 patients, patients with noninvasive breast carcinomas or metastatic lesions and patients lacking clinical or pathologic data, as well as referral patients, were excluded. The remaining 171 patients as well as a control series of 55 consecutive patients with a negative HER-2 result on IHC were recruited into the present study. Table 1 lists the clinicopathologic characteristics of the 226 patients included in the study. Patients underwent mastectomy or local wide excision of their primary breast tumor with an axillary lymph node dissection at least at level I and II. Histopathologic examination was performed on hematoxylin and eosin–stained sections, and tumors were classified and graded according to the WHO Classification and the Elston and Ellis grading system, respectively.^{20,21} Disease-free survival was defined as the time period (in months) between the date of surgery and the date of recurrence or distant metastasis. All patients had the best standard of care for local and systemic treatment; trastuzumab was not yet standard of care in the adjuvant or neoadjuvant setting. This study was approved by the Local Commission for Medical Ethics and Clinical Studies.

IHC

IHC HER-2 assessment was performed using the CB11 mouse monoclonal antibody (1/40 diluted; Novocastra Laboratories, Newcastle-on-Tyne, United Kingdom). Staining results were scored as described previously.²² For the present study, we validated our IHC assay against HercepTest (DakoCytomation, Glostrup, Denmark) in a subset of 50 patients (35 patients with score 0/1+; 15 patients with score 3+) according to the ASCO/CAP guidelines for HER-2 testing,¹⁶ revealing 98% concordance between both IHC assays. For all cases, IHC data were available on the estrogen receptor (ER) and progesterone receptor (PR) status (mouse monoclonal antibodies NCL-ER-6F11, 1/30 diluted, and NCL-PgR-312, 1/40 diluted; Novocastra Laboratories).

FISH

FISH analysis (PathVysion, Vysis) was performed manually according to the manufacturer's recommendations. Using a Zeiss Axioplan 2 epifluorescence microscope (Carl Zeiss, Göttingen, Germany), we counted signals in at least 100 tumor nuclei in two or more separate regions of the tissue section. Averages of *HER2* gene and chromosome 17 copy number counts were rounded off to the nearest whole number; in case *HER2* gene amplification appeared as clusters of uncountable *HER2* signals, we estimated the average *HER2* gene copy number. FISH results were interpreted according to two different scoring methods: (1) based on absolute *HER2* gene copy number (*HER2* absolute) or (2) based on the ratio *HER2* gene/chromosome 17 copy number (*HER2*/Chr17 ratio). Actually, these scoring methods were developed to be used in combination with, respectively, the Oncor INFORM *HER-2/neu* test kit (Ventana Medical Systems Inc, Tucson, AZ) or the PathVysion test kit.

Table 1. Clinicopathologic Characteristics of Patients With Invasive Breast Carcinoma Included in Our Study (N = 226)

Characteristic	No. of Patients	%
Age, years		
≤ 50	75	33.2
> 50	151	66.8
Tumor grade		
1	12	5.3
2	75	33.2
3	139	61.5
Tumor size, cm		
≤ 2	103	45.6
> 2	123	54.4
Nottingham Prognostic Index		
I	44	19.5
II	102	45.1
III	80	35.4
Lymph node status		
Negative	120	53.1
Positive	106	46.9
Lymphovascular invasion		
Negative	180	79.6
Positive	46	20.4
Estrogen receptor status		
Negative	47	20.8
Positive	179	79.2
Progesterone receptor status		
Negative	90	39.8
Positive	136	60.2
HER-2 status on immunohistochemistry		
Negative, 0/1+	55	24.3
Equivocal, 2+	99	43.8
Positive, 3+	72	31.8
Follow-up		
Mean, months	42.2	
Metastasis	33	14.6

We have previously compared both FISH test kits in a series of 20 patients with breast cancer and found that the count of absolute *HER2* gene copy number was nearly identical for both kits.²² Therefore, we applied both scoring methods in combination with the PathVysion test kit. As proposed by the ASCO/CAP guidelines,¹⁶ an absolute *HER2* gene copy number lower than four or an *HER2*/Chr17 ratio of less than 1.8 was considered HER-2 negative; an absolute *HER2* copy number between four and six or an *HER2*/Chr17 ratio between 1.8 and 2.2 was considered HER-2 equivocal, and an absolute *HER2* gene copy number greater than six or an *HER2*/Chr17 ratio higher than 2.2 was considered HER-2 positive. Polysomy 17 was defined as an average chromosome 17 copy number ≥ 3 .^{19,23} Lymphocytes, (myo)fibroblasts, and normal epithelial cells served as internal control.

Quantitative Reverse Transcriptase Polymerase Chain Reaction

In 157 of 226 patients, representative frozen tumor tissue was available for quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analysis. For each patient, total RNA was extracted from 20- μ m sections using the RNeasy mini kit (Qiagen, Hilden, Germany). RNA purity and concentration were checked spectrophotometrically (Nanodrop Technologies, Wilmington, DE). One μ g of total RNA was reverse transcribed and PCR reactions on the resulting cDNA were performed in the ABI-Prism 7900 HT Sequence Detector (Applied Biosystems, Lennik, Belgium). PCR primers and probes for *HER2* and housekeeping gene *GAPDH* were obtained from Applied Biosystems (TaqMan Gene Expression Assays). Each sample was analyzed in triplicate in a MicroAmp optical 96-well reaction plate (Applied Biosystems). A

sample of normal breast tissue was used as a calibrator and the $\Delta\Delta C_t$ -method was applied to determine relative gene expression levels.²⁴

Statistical Analysis

Differences in *HER2* mRNA expression levels between different subgroups were assayed by a Wilcoxon rank sum test. Differences in clinicopathologic variables between subgroups were checked using χ^2 tests. The Bonferroni method was used for multiple testing correction. Survival analysis was performed using the Kaplan-Meier method. Survival differences between subgroups were assayed by log-rank tests. A *P* value of less than .05 was considered statistically significant.

RESULTS

Comparison Between IHC and FISH for HER-2 Testing

Comparison between IHC and FISH for HER-2 testing is outlined in Table 2. An equivocal HER-2 status by FISH was found in 44 (19.5%) of 226 patients based on absolute *HER2* gene copy number and in three (1.3%) of 226 patients based on the ratio *HER2*/Chr17. Note that none of these patients showed overexpression on IHC (score 3+). Remarkably, all patients with an equivocal HER-2 status by FISH as well as those cases showing discordant HER-2 testing results displayed polysomy 17.

Impact of Polysomy 17 on HER-2 Testing Results by IHC and FISH

Polysomy 17 was observed in 104 (46.0%) of 226 patients, either on its own (62 of 104 patients) or in combination with *HER2* gene amplification (42 of 104 patients). As shown in Table 3, polysomy 17 did not affect the interpretation of HER-2 testing results by FISH when it was accompanied by *HER2* gene amplification. Furthermore, most of these patients showed overexpression on IHC (78.6% with score 3+). By contrast, a score 3+ on IHC was not found in tumors displaying polysomy 17 in the absence of *HER2* gene amplification. Moreover, in cases where polysomy 17 on its own resulted in an absolute *HER2* gene copy number greater than three, the interpretation of HER-2 FISH results was obscured. Indeed, tissue from 44 patients showed a modest increase in *HER2* gene copy number as a result of polysomy 17 (four to six copies) and were interpreted as equivocal by FISH if only *HER2* copies were counted. However, when chromosome 17 copy number was taken into account (*HER2*/Chr17 ratio), all these patients turned out to be HER-2 negative. Five patients showed a relatively high increase in *HER2* gene copy number as a result of polysomy 17 (seven to 10 copies) and were interpreted as positive based on absolute *HER2* gene copy number. According to the *HER2*/

Table 3. Polysomy 17 in Relation to HER-2 Testing Results

Variable	PS 17 + <i>HER2</i> GA (n = 42)		PS 17 - <i>HER2</i> GA (n = 62)	
	No.	%	No.	%
HER-2 status by IHC				
Positive, score 3+	33	78.6	0	0
Equivocal, score 2+	9	21.4	46	74.2
Negative, score 0/1+	0	0	16	25.8
HER-2 status by FISH, <i>HER2</i>				
Positive, <i>HER2</i> > 6	42	100	5	8.1
Equivocal, $4 \leq \text{HER2} \leq 6$	0	0	44	71.0
Negative, <i>HER2</i> < 4	0	0	13	20.9
HER-2 status by FISH, R				
Positive, R > 2.2	42	100	0	0
Equivocal, $1.8 \leq R \leq 2.2$	0	0	3	4.8
Negative, R < 1.8	0	0	59	95.2

Abbreviations: PS 17 + *HER2* GA, polysomy 17 accompanied by *HER2* gene amplification; PS 17 - *HER2* GA, polysomy 17 on its own, in the absence of *HER2* gene amplification; IHC, immunohistochemical determination of HER-2 protein expression scored on a 0 to 3+ scale; FISH, fluorescence in situ hybridization to determine the HER-2 status based on either absolute *HER2* gene copy number (*HER2*) or the ratio of *HER2*/chromosome 17 copy number (R).

Chr17 ratio however, two of these patient cases were interpreted as HER-2 negative (ratio 7/4 and 7/5, both < 1.8), whereas three patient cases were in the equivocal range (ratio 10/5 = 2.0). These data illustrate how polysomy 17 can be interpreted as HER-2 positive or HER-2 negative, depending on which scoring method is applied to interpret HER-2 FISH results.

Stratification

To investigate whether tumors displaying polysomy 17 in the absence of *HER2* gene amplification should be regarded as HER-2 negative or HER-2 positive, we compared *HER2* mRNA levels and clinicopathologic characteristics in the following three groups: HER-2-negative tumors (normal *HER2* gene and chromosome 17 copy number, n = 67), polysomy 17 tumors (polysomy 17 in the absence of *HER2* gene amplification, n = 62), and HER-2-positive tumors (*HER2* gene amplification defined as a ratio *HER2*/Chr17 ≥ 2.2 , n = 97).

HER2 mRNA Expression by Quantitative RT-PCR

As illustrated in Figure 1, polysomy 17 tumors had low relative *HER2* mRNA expression values comparable to those found in the

Table 2. Comparison Between IHC and FISH for Determination of the HER-2 Status in Breast Cancer

IHC	FISH															
	Positive								Negative							
	<i>HER2</i> > 6, No.	Total No. Tested	R > 2.2, No.	Total No. Tested	$4 \leq \text{HER2} \leq 6$, No.	Total No. Tested	$1.8 \leq R \leq 2.2$, No.	Total No. Tested	<i>HER2</i> < 4, No.	Total No. Tested	R < 1.8, No.	Total No. Tested	<i>HER2</i> > 6, No.	Total No. Tested	R > 2.2, No.	Total No. Tested
Positive, 3+	72	72	100	72	72	100	0	72	0	0	72	0	0	72	0	0
Equivocal, 2+	27	99	27.3	25	99	25.3	37	99	37.4	1	99	1.0	35	99	35.3	73
Negative, 0/1+	3	55	5.5	0	55	0	7	55	12.7	2	55	3.6	45	55	81.8	53
Total	102	226	45.1	97	226	42.9	44	226	19.5	3	226	1.3	80	226	35.4	126

Abbreviations: IHC, immunohistochemical determination of HER-2 protein expression scored on a 0 to 3+ scale; FISH, fluorescence in situ hybridization to determine the HER-2 status based on either absolute *HER2* gene copy number (*HER2*) or the ratio of *HER2*/chromosome 17 copy number (R).

Fig 1. *HER2* mRNA expression by quantitative reverse transcriptase polymerase chain reaction.

HER-2-negative group (mean expression, 0.914 ± 0.912 ; $P = .1865$). By contrast, HER-2-positive tumors generally had increased relative expression values, with most cases showing at least a five-fold increase in *HER2* mRNA expression compared with normal breast tissue. In HER-2-positive cases, *HER2* mRNA expression levels were significantly higher than those in HER-2-negative (mean, 7.831 ± 0.912 ; $P < 10^{-15}$) and polysomy 17 tumors (mean, 7.831 ± 0.914 ; $P < 10^{-16}$).

Clinicopathologic Characteristics of Polysomy 17 Tumors

Table 4 shows the distribution of clinicopathologic parameters in HER-2-negative, polysomy 17, and HER-2-positive tumors. Compared with HER-2-negative tumors, HER-2-positive tumors showed higher tumor grade ($P < 10^{-8}$) and higher Nottingham Prognostic Index risk group ($P = .030$) and were more frequently ER negative ($P < .001$) and PR negative ($P = .0062$). Polysomy 17 tumors were more similar to HER-2-negative than HER-2-positive tumors. Although tumor grade ($P = .0031$), ER status ($P < .001$), and PR status ($P = .024$) differed significantly between polysomy 17 tumors and HER-2-positive tumors, no differences were found between polysomy 17 tumors and HER-2-negative tumors in any of the clinicopathologic parameters investigated. Kaplan-Meier survival curves (Fig 2) illustrate shorter disease-free survival in patients with HER-2-positive tumors as compared with patients with HER-2-negative tumors ($P < .001$). Survival in patients with polysomy 17 tumors was intermediate between HER-2-negative (not significant, $P = .056$) and HER-2-positive patients ($P = .031$).

DISCUSSION

Polysomy 17 is common in breast cancer, with reported frequencies ranging from 13% to 46% depending on the study population and the definition of polysomy 17.^{17-19,23,25} In our series, including 171 patients with an IHC score 2+/3+ and 55 patients with a score 0/1+, polysomy 17 was found in 46.0%. Because polysomy 17 implies extra copies of the *HER2* gene, it is conceivable that polysomy 17 might lead to increased HER-2 expression levels. However, it remains unclear

whether polysomy 17 results in HER-2 overexpression in a way similar to *HER2* gene amplification and whether polysomy 17 tumors should be regarded as HER-2 positive.^{17,18,25-29}

In the present study, we provide evidence that polysomy 17 and *HER2* gene amplification are two distinct genetic aberrations with a different clinicopathologic significance in breast cancer. First, we show that polysomy 17 on its own does not result in HER-2 overexpression, neither at the protein nor at the mRNA level. Indeed, we did not encounter any breast tumor showing HER-2 overexpression on IHC (score 3+) and polysomy 17 in the absence of *HER2* gene amplification. Moreover, and in accordance with Dal Lago et al,²⁶ we did not find increased *HER2* mRNA expression by quantitative RT-PCR in polysomy 17 tumors, not even in those tumors showing up to 10 *HER2* gene copies. Second, we could demonstrate that *HER2* gene amplification and polysomy 17 have a different clinicopathologic impact in breast cancer. Whereas *HER2* gene amplification was clearly associated with high tumor grade, hormone receptor negativity, and reduced disease-free survival, polysomy 17 did not show any significant association with adverse clinicopathologic parameters. Nevertheless, a trend toward shorter disease-free survival was observed in polysomy 17 tumors. Because polysomy 17 may reflect aneuploidy and increased chromosomal instability, it can be expected that tumors harboring this anomaly will behave more aggressively than those without it.³⁰⁻³² Still, our data suggest that the clinicopathologic impact of polysomy 17 is not as strong as that of *HER2* gene amplification in breast cancer and that tumors displaying polysomy 17 in the absence of *HER2* gene amplification behave more similar to HER-2-negative than to HER-2-positive tumors.

Our current findings could have important clinical implications. Because polysomy 17 on its own is not associated with HER-2 overexpression and because it does not have the same clinicopathologic significance as true *HER2* gene amplification, one may wonder whether polysomy 17 tumors benefit from HER-2-targeted therapy such as trastuzumab, which targets the HER-2 protein at the tumor cell membrane. Indeed, the best therapeutic response rates have been observed in breast cancers showing HER-2 overexpression by IHC.^{28,33} Recently, trastuzumab response has been reported in two patients showing polysomy 17 in the absence of *HER2* gene amplification and in one patient showing neither polysomy 17 nor *HER2* gene amplification. Of interest, all three patients showed HER-2 overexpression on IHC.²⁸ We speculate that in such rare cases, HER-2 overexpression might result from deregulated gene transcription. Further phase III trials are needed to elucidate whether polysomy 17 tumors benefit from HER-2-targeted therapy.

It is important to realize that polysomy 17 has a substantial impact on the interpretation of HER-2 testing results, especially in those patients with an equivocal HER-2 status on IHC (score 2+). Indeed, in patients in whom polysomy 17 results in a moderate increase in *HER2* gene copy number (four to six), HER-2 FISH results could be interpreted as equivocal if only absolute *HER2* copies are counted. As such, we found that 37 (37.4%) of 99 tumors with an IHC score of 2+ were still considered equivocal after FISH analysis based on absolute *HER2* copy number, whereas only one tumor (1.0%) remained equivocal based on the *HER2*/chromosome 17 ratio. On the basis of these data, and given that approximately 15% of newly diagnosed patients with breast cancer show an IHC score 2+,¹⁶ we estimate that 5.6% and 0.15% of breast carcinomas remain equivocal after FISH testing, depending on whether a control probe for chromosome

Table 4. Distribution of Clinicopathologic Features in Polysomy 17 Tumors Compared With HER-2-Negative and HER-2-Positive Tumors

Characteristic	HER-2 Negative (n = 67)		<i>P</i> *	Polysomy 17 (n = 62)		<i>P</i> †	HER-2 Positive (n = 97)		<i>P</i> ‡
	No.	%		No.	%		No.	%	
Age, years			.99			.18			.99
≤ 50	21	31.3		14	22.6		42	43.3	
> 50	46	68.7		48	77.4		55	56.7	
Tumor grade			.99			3.1×10^{-3}			7.5×10^{-9}
1	6	8.9		5	8.1		1	1.0	
2	38	56.7		23	37.1		14	14.5	
3	23	34.4		34	54.8		82	84.5	
Tumor size, cm			.99			.99			.99
≤ 2	29	43.3		24	38.7		41	42.3	
> 2	38	56.7		38	61.3		56	57.7	
Nottingham Prognostic Index			.99			.71			.030
I	20	29.8		15	24.2		9	9.3	
II	30	44.8		27	43.5		45	46.4	
III	17	25.4		20	32.3		43	44.3	
Lymph node status			.99			.99			.99
Negative	41	61.2		35	56.5		44	45.4	
Positive	26	38.8		27	43.5		53	54.6	
Lymphovascular invasion			.99			.99			.99
Negative	55	82.1		53	85.5		72	74.2	
Positive	12	17.9		9	14.5		25	25.8	
Estrogen receptor status			.99			1.2×10^{-4}			1.4×10^{-4}
Negative	5	7.5		4	6.5		38	39.2	
Positive	62	92.5		58	93.5		59	60.8	
Progesterone receptor status			.99			.024			6.2×10^{-3}
Negative	18	26.9		18	29.0		54	55.7	
Positive	49	73.1		44	71.0		43	44.3	
Follow-up			NA			NA			NA
Mean, months		47.7			48.6			33.3	
Metastasis	3	4.5		9	14.5		21	21.6	

NOTE. All *P* values given are the result of a χ^2 test, corrected for multiple testing by the Bonferroni method.

Abbreviation: NA, not applicable.

*HER-2 negative versus polysomy 17.

†Polysomy 17 versus HER-2 positive.

‡HER-2 positive versus HER-2 negative.

17 is used. Remarkably, the one tumor (0.15%) showing an equivocal HER-2 status on both IHC and FISH, even after correction for chromosome 17 copy number, also displayed polysomy 17, with a mean ratio of 10/5 or 2.0. In this particular case, quantitative RT-PCR

indicated no increase in *HER2* mRNA expression, suggesting a negative HER-2 status after all. In the end, one may wonder whether quantitative RT-PCR could be a valuable alternative for HER-2 testing in routine clinical practice. Still, the need for representative fresh or frozen breast cancer tissue for optimal RT-PCR testing results, as well as inevitable dilution of invasive tumor cells with normal and stromal cell populations or noninvasive breast lesions, limits the use of RT-PCR for routine HER-2 testing.

In conclusion, polysomy 17 is a major cause of equivocal HER-2 testing results by FISH. We provide evidence that polysomy 17 and *HER2* gene amplification have a distinct impact on the clinicopathologic parameters in breast cancer and that polysomy 17 tumors should be regarded HER-2 negative. Indeed, *HER2* gene amplification usually results in excessive HER-2 expression levels and defines a distinct clinicopathologic breast cancer entity characterized by high tumor grade, reduced hormone receptor expression, and poor prognosis. By contrast, polysomy 17 is not related to HER-2 overexpression or adverse clinicopathologic features but may rather reflect increased chromosomal instability in breast cancer. These findings underscore the importance of using dual-color systems for HER-2 FISH testing and urge the need for clinical trials to investigate whether polysomy 17 tumors benefit from HER-2-targeted therapy.

Fig 2. Kaplan-Meier survival curves illustrate shorter disease-free survival in patients with HER-2-positive tumors as compared with patients with HER-2-negative tumors (*P* < .001).

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Acknowledgment

We thank Peter Vandenberghe, MD, PhD, and Iwona Wlodarska, PhD, from the Centre of Human Genetics for helpful collaboration and Miet Vanherck, Johan Van Even, Helga Van Den Bosch, and Lieve Ophalvens for excellent technical assistance.