Polysomy 17 in HER-2/neu Status Elaboration in Breast Cancer: Effect on Daily Practice
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Abstract

Purpose: To assess the effect of chromosome 17 copy number on HER-2/neu status determination in breast cancers.

Experimental Design: HER-2/neu gene copy and chromosome 17 centromere numbers were evaluated on 893 breast carcinomas using double color fluorescence in situ hybridization (FISH). The net and chromosome 17 corrected (ratio) HER-2/neu copy numbers were compared and related to immunohistochemistry done according to the Food and Drug Administration (FDA)–approved scoring system (0, 1+, 2+, and 3+) as a first screening step in 584 cases.

Results: When a ratio ≥2 was considered as criterion for FISH positivity, 49.3% (440 of 893) of cases showed amplification versus 56.2% (502 of 893) by using a net HER-2/neu gene copy number >4 as an alternative criterion; 14.8% (67 of 453) of cases having a ratio <2 had a net HER-2/neu gene copy number >4 and 11% (5 of 440) with a ratio ≥2 had a net HER-2/neu gene copy number ≤4. Among discordant cases, 88.8% (64 of 72) were polysomic (>2.25 chromosomes 17/cell) and among polysomic cases, 12.8% (40 of 312) of the low polysomic (2.26–3.75 chromosomes 17/cell) and 36.9% (24 of 65) of the highly polysomic (>3.75 chromosomes 17/cell) cases showed discordance. In cases with a ratio <2, polysomy 17 incidences were 85.7% (6 of 7) in IHC 3+, 42.4% (79 of 186) in IHC 2+, 33.3% (15 of 45) in IHC 1+, and 29.1% (16 of 55) in IHC 0.

Conclusion: A net increase in HER-2/neu gene copy number consecutive to polysomy 17 in the absence of specific gene amplification might lead to a strong protein overexpression in a small subset of breast carcinomas. HER-2/neu status determination by FISH is dependent on the criterion considered for positivity in clinical practice.

Located on the long arm of chromosome 17 (17q12-21.32), the HER-2/neu oncogene encodes for a type 1 growth factor membrane receptor that is commonly overexpressed in several types of cancer, mainly in breast cancer where its overexpression is closely related to gene amplification. HER-2/neu overexpression and/or amplification is seen in ~20% to 30% of breast cancers and has been associated with a poor prognosis (1–5).

The demonstration of HER-2/neu overexpression/amplification has become clinically important in the current management of metastatic breast cancer patients given to the availability of a humanized specific monoclonal antibody, trastuzumab (Herceptin, Roche, Basel, Switzerland), which has significant antitumor activity in these patients both as a single agent and in combination with chemotherapy (6–8). Moreover, HER-2/neu is suspected to play a role in hormonal resistance or chemosensitivity of breast cancer (5, 9–14). Therefore, laboratory assessment of HER-2/neu status is becoming a key step in the optimal management of patients with advanced breast cancer.

For clinical HER-2/neu status determination, methods that can be done on formalin-fixed paraffin-embedded tissue have now been implemented in several laboratories. Immunohistochemistry according to the FDA-approved scoring system (0, 1+, 2+, and 3+) detects HER-2/neu protein overexpression whereas fluorescence in situ hybridization (FISH) assesses HER-2/neu gene copy amplification. Both methods are recommended by national and international guidelines (15–17). Considering their relative costs and the growing body of data showing a very high correlation between the two methods in the 0, 1+, and 3+ categories (18–25), immunohistochemistry has emerged as a method of choice for screening, whereas FISH is often viewed as a confirmatory test particularly in borderline immunohistochemistry cases (26–ref. 26). With respect to FISH assays, two FDA-approved kits can be used: (a) the PathVysion kit (Vysis, Downers Grove, IL) which includes a dual probe system for simultaneous detection of the HER-2/neu gene and...
the chromosome 17 centromere (centro-17), considering the HER-2/neu amplification when the ratio of HER-2/neu gene copies per centro-17 reaches the value 2; and \( b \) the Inform kit (Ventana Medical Systems, Tucson, AZ) which does not take into account the chromosome 17 copy number and considers the FISH assay positive when the absolute or net HER-2/neu gene copy number is >4. Nevertheless, despite the fact that some authors consider the chromosome 17 copy correction to be essential \((22, 27, 28)\), no definitive consensus exists.

Aberrant numerical changes in chromosome copy or aneusomy 17 are frequently encountered in invasive breast cancer and carry a poor prognosis \((18, 29–31)\). This has led some authors in recent studies to raise the question about the potential effect of aneusomy 17 and particularly of polysomy 17 on HER-2/neu status \((22, 32–36)\).

Having previously shown a very good positive correlation for HER-2/neu gene amplification between a primary occurrence and metastasis in breast cancer \((37)\), in the present study, we did FISH assay using the dual color PathVysion system on 893 cases of invasive breast cancers, either primary or metastasis previously screened by immunohistochemistry, among which 584 cases were first screened in our institution according to the 0, 1+, 2+, and 3+ scoring system. Correlation between the two methods based on the literature data were made to validate our results but, in addition, results were analyzed and discussed with respect to chromosome 17 copy number. The effect of chromosome 17 copy number was measured in terms of the discordance between FISH-FISH results depending on the criterion used (centro-17 corrected or absolute HER-2/neu gene copy number) and between FISH and immunohistochemistry results. Results are discussed in light of the recent literature.

### Materials and Methods

**Case selection.** A series of 893 formalin-fixed paraffin-embedded tissue blocks of infiltrating breast carcinoma from either primary or metastatic sites, which were sent to the Pathology Department of the Jules Bordet Institute, Brussels, Belgium, for FISH testing between April 2001 and June 2004, were included in this study. In this series, 584 cases were issued from a selection process by immunohistochemistry done in a single institution (Jules Bordet Institute). The remaining 309 cases were referred for FISH assay directly from other centers, most often after immunohistochemical screening, but immunohistochemistry results were not included in the present study.

**HER-2/neu status determination by immunohistochemistry.** Overexpression of HER-2/neu was determined immunohistochemically using CB11 monoclonal antibody directly purchased from Novocastra (Newcastle upon Tyne, England). The detection system used was the Ventana Nexes Staining System with 3,3’-diaminobenzidine (DAB) as chromogen purchased from Ventana Medical Systems, which used a biotinylated secondary antibody followed by avidin-horseradish peroxidase as detection kit. Briefly, sections (4 mm thick) from formalin-fixed paraffin-embedded blocks were cut on Superfrost Plus slides (Menzel-Gläser, Braunschweig, Germany). Slides were then dewaxed and rehydrated, and immunoreactivity was enhanced by antigen retrieval treatment that consisted of a 30-minute incubation in 10 mmol/L sodium citrate buffer (pH 6) in a boiling water bath followed by cooling for 20 minutes at room temperature. Slides were subsequently incubated for 30 minutes at 37°C with the primary antibody CB11 at 1:40 dilution, after which sections were incubated for 10 minutes at 37°C with the biotinylated secondary antibody and then with avidin-peroxidase for the same time. 3,3’-Diaminobenzidine was used as chromogen. All the products needed for these steps were included in the DAB Detection Kit provided by the manufacturer (Ventana). The slides were then counterstained with Mayer’s hematoxylin, dehydrated, and mounted. SK-BR-3 cell line and breast cancer known to show amplification and overexpression of HER-2/neu were used as positive external controls, whereas MCF-7 cell line and normal breast tissue were used as external and internal negative controls, respectively.

All immunostained slides were scored according to following criteria: 0, membrane staining is observed in <10% of tumor cells; 1+, a partial and focal staining is observed in <10% of tumor cells; 2+, a weak to moderate complete membrane staining is detected in >10% of tumor cells; 3+, a moderate to strong complete membrane staining is detected in >10% of tumor cells. All cases with positive immunohistochemical staining \((2+\) or \(3+)\) were selected and processed for FISH assay. The cases showing no protein overexpression \((0 \text{ or } 1+)\) were generally excluded from FISH analysis, excepted for control or confirmation when requested by the clinician.

**HER-2/neu status determination by fluorescence in situ hybridization.** The PathVysion (Vysis) assay was done using the recommended protocol of the manufacturer on 4 \(4\mu \text{m}\) sections prepared from formalin-fixed paraffin-embedded tissue. Two directly labeled probes, LSI HER-2/neu SpectrumOrange and CEP 17 SpectrumGreen, were used to detect the distinct fluorescent signals in individual cells for the respective HER-2/neu gene and \(\alpha\) satellite sequences of chromosome 17 centromere.

Four-micrometer-thick sections were placed on Superfrost Plus slides (Menzel-Gläser). Slide-mounted tissue sections were then baked overnight at 37°C and dewaxed in toluol for 10 minutes twice, followed by immersion in 100% ethanol for 5 minutes twice. Air-dried tissue sections were subsequently treated in 0.2 mol/L HCl for 20 minutes at room temperature, washed twice in 2× SSC \((1 \times \text{SSC is } 0.15\text{ mol/mol NaCl, 0.015 mol/L sodium citrate, pH 7})\) for 5 minutes each and then treated in a pretreatment solution of 1 mol/L sodium sulfocyanate for 30 minutes at 80°C. Slides were subsequently washed twice in 2× SSC and treated in a solution of protease for 15 minutes at 37°C. Pretreatment and protease digestion steps were prepared, as recommended by the manufacturer, in a Paraffin Pretreatment Kit I purchased from Vysis. Following protease digestion, tissue sections were washed in 2× SSC and postfixed in fixator \((45 \text{ mL PBS pH 7, } 1 \text{ mol/L MgCl}_2, 2.5 \text{ mL, acid-free formaldehyde }1.25 \text{ mL})\) for 10 minutes. Slides were then washed twice in 2× SSC before denaturation and hybridization. Denaturation was done in 70% formamide/2× SSC \((\text{pH 7.0})\) at 74°C for 5 minutes, after which slides were immersed in graded alcohol \((75\%, 85\%, \text{ and } 100\% \text{ ethanol})\) at room temperature. Slides were then air dried and warmed at 50°C. The dual color probe Spectrum Orange-HER-2/Spectrum Green CEP17 was then applied \((8 \mu\text{L})\) on a selected area of the sample (invasive part of the tumor determined on a serial tissue section stained with H&E). Sections were coverslipped and sealed with rubber cement. Hybridization was carried out overnight at 37°C in a moist chamber. Two 5-minute washes were then done in 2× SSC/0.3% NP40, the first at room temperature and the second at 74°C. After drying, sections were counterstained with 4,6-diamidino-2-phenylindole (Boehringer Mannheim, Germany) anti-fade solution and analyzed under a fluorescence microscope. An Olympus microscope equipped with a 100W mercury-arc lamp and 40× and 100× objectives was used in combination with a Triple bandpass filter for simultaneous detection of Spectrum Green, Spectrum Orange, and 4’,6-diamidino-2-phenylindole (DAPI/FITC/TRITC). In each breast cancer specimen, 60 cells in the invasive part of the tumor were analyzed according to the PathVysion criteria of interpretation of FISH assay. The FISH-defined genetic variables were reported as follows: HER-2/neu gene copy number, chromosome 17 copy number, and the average HER-2/neu gene to chromosome 17 signal ratio. Tumors with HER-2/neu gene to chromosome 17 ratio >2 were designated as having HER-2/neu gene amplification, and the ones with a ratio <2 as having no gene amplification. A ratio ranging
between 2 and 3 was qualified as low; between 3 and 5 as moderate; and >5 as high. As an alternative criterion for FISH interpretation, positivity was declared when the absolute HER-2/neu gene copy number was >4. The cutoff points for chromosome 17 copy number alterations were estimated for each group according to published standards (21, 22, 33, 34, 36, 38, 39) adapted for truncated nuclei on paraffin sections. For the convenience of data analysis, we chose to apply the criteria suggested by Wang et al. (34) with an arbitrary division between low and high polysomy for a better segregation of these tumors. Specimens with a chromosome 17 copy number in the range of 1.5 to 2.25 signals per cell were defined as having disomy 17. The other cases were considered to have aneusomy 17: either hypodisomy 17 (<1.5 signals per cell), low polysomy 17 (2.26-3.75 signals per cell), or high polysomy 17 (>3.76 signals per cell).

**Statistical analysis.** HER-2/neu status was assessed as a binary status for the two FISH used criteria as well as with continuous variables. For binary evaluations, observed rates of positivity were calculated and 95% confidence intervals were obtained using asymptotic normal distributions. Rates of positivity were compared for paired observations using Mac Nemar tests and concordance between the two criteria evaluation was assessed by calculation of the \( \kappa \) coefficient. Association between the binary evaluations and immunohistochemistry score was analyzed using \( \chi^2 \) tests for a trend. The effect of immunohistochemistry score on the continuous evaluations was analyzed with one-way ANOVAs and Fisher-Snedecor tests. When overall heterogeneity was detected, post hoc tests were done to make paired comparisons. All reported significance probabilities were set to two tailed.

**Results**

**Comparison between immunohistochemistry and fluorescence in situ hybridization assay data.** HER-2/neu status was determined by immunohistochemistry according to the 0, 1+, 2+, and 3+ scoring system as a first-step selection process, followed by FISH using a double color HER-2/neu/CEP17 probe set as confirmatory test. Cases previously defined as negative by immunohistochemistry (0, 1+) were excluded from FISH analysis, except for control, or for confirmation when requested by clinicians. All cases with positive immunohistochemical staining (2+ or 3+) were processed with FISH assay.

Both immunohistochemistry and FISH results were compared for 584 cases issued from the selection process for FISH assay (Table 1). Our results showed that among the 110 of 584 cases showing no protein overexpression (scored as 0 or 1+), 90.9% (100 of 110) of cases had a centro-17 corrected HER-2/neu gene copy number <2, 5.4% (6 of 110) had a 2 to 2.99 ratio, 2.7% (3 of 110) had a 3 to 4.99 ratio, and 0.9% (1 of 110) had a ratio >5; whereas 83.6% (92 of 110) of cases showed a net HER-2/neu copy number >4 and 16.4% (18 of 110) of cases a net HER-2/neu copy number ≥4. Of 584 cases, 474 showed moderate or strong protein overexpression (as scored by IHC 2+ or 3+). Among these, 41.3% (196 of 474) had a ratio >5, 13.9% (66 of 474) a 3 to 4.99 ratio, 4.0% (19 of 474) a 2 to 2.99 ratio, and 40.7% (193 of 474) a ratio <2; whereas 66.2% (314 of 474) of cases showed a net HER-2/neu copy number ≥4 and 33.7% (160 of 474) of cases showed a net HER-2/neu copy number <4. The better rate of agreement between immunohistochemistry and both centro-17 corrected and net HER-2/neu gene copy numbers was observed in the IHC 3+ category, with percentage of FISH positive reaching 96.6% (196 of 203 cases) and 98.5% (200 of 203 cases), respectively. It was observed that both centro-17 corrected and net HER-2/neu gene copy numbers were associated with immunohistochemistry score because the frequency of FISH-positive tumors, whatever the criteria considered, significantly increased according to immunohistochemistry score (P < 0.001).

**Fluorescence in situ hybridization assay results in a cohort of 893 cases.** This series included the 584 cases issued from the selection process by immunohistochemistry (i.e., 2+ and 3+) done in the same institution (Jules Bordet Institute) and 309 cases directly referred for FISH assay from other centers. Globally, and by definition, it results in the underrepresentation of the IHC−specimens for the FISH assay.

By considering the centro-17 corrected HER-2/neu gene copy number as a criterion for evaluation of FISH assay, we observed in this selected series the following: 50.7% (453 of 893) of cases had a HER-2/neu/centro-17 ratio of <2; 4.7% (42 of 893) had a low level of amplification with a ratio between 2 and 2.99; 11.3% (101 of 893) had a moderate level of amplification with a ratio between 3 and 4.99; and 33.2% (297 of 893) had a high rate of amplification with a ratio >5. Using this criterion, 49.3% (440 of 893) of cases were then defined as FISH positive (confidence limits: 46.2%, 52.6%). In contrast, by considering a net HER-2 copy number >4/cell as an alternative criterion of FISH positivity (as for the alternative FDA-approved FISH kit), 56.2% (502 of 893) of the cases were positive (confidence limits: 53.0%, 59.5%).

Despite the fact that a very good level of agreement between the two evaluation criteria was observed (\( \kappa \) concordance coefficient = 0.84; 95% confidence interval, 0.80-0.88), the results nevertheless showed a significant difference in positivity rates between the two methods of FISH evaluation for HER-2 gene status (P < 0.001). When using a net HER-2/neu gene copy number >4 as the criterion, 14.8% (67 of 453) of cases having a centro-17 corrected HER-2/neu gene copy number <2 were defined as positive. When using the alternative criterion, 1.1% (5 of 440) of cases with a centro-17 corrected HER-2/neu gene copy number >2 were defined as negative (Table 2).

**Comparison between the two evaluation criteria of fluorescence in situ hybridization assay results in a cohort of chromosome 17 copy number and effect of chromosome 17 copy number on discordance.** Our aim was to investigate how the chromosome 17 copy number might influence FISH assessment. Specimens with a chromosome 17 copy number in the range of 1.5 to 2.25 signals per cell were defined as having disomy 17. The other cases were considered to have aneusomy 17: either hypodisomy 17 (<1.5 signals per cell), low polysomy 17 (2.26-3.75 signals per cell), or high polysomy 17 (>3.75 signals per cell). Analysis of chromosome 17 copy numbers showed 8.9% (80 of 893) hypodisomy, 48.8% (436 of 893) disomy, 34.9% (312 of 893) low polysomy, and 7.3% (65 of 893) high polysomy cases, totaling 51.2% of the aneusomic cases in the series.

Table 3 shows that the average chromosomes 17 copy number influenced both centro-17 corrected and net HER-2/neu gene copy numbers as continuous variables with the observation that, going from monosomy to high polysomy, the mean ratio significantly decreased whereas the mean HER-2/neu gene copy number significantly increased (P < 0.001).

**Polysony 17 in relation with immunohistochemistry and fluorescence in situ hybridization assays.** There were 293 cases showing a centro-17 corrected HER-2/neu gene copy
Table 1. Comparison of immunohistochemistry and FISH assay data on 584 cases

<table>
<thead>
<tr>
<th>HER-2/neu/centro-17 ratio</th>
<th>IHC−</th>
<th>IHC 1+</th>
<th>IHC 2+</th>
<th>IHC 3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 (−)</td>
<td>55 (91.6%)</td>
<td>45 (90.0%)</td>
<td>186 (68.6%)</td>
<td>7 (3.4%)</td>
</tr>
<tr>
<td>2-2.99</td>
<td>2 (3.3%)</td>
<td>4 (8.0%)</td>
<td>11 (4.1%)</td>
<td>8 (3.9%)</td>
</tr>
<tr>
<td>3-4.99</td>
<td>2 (3.3%)</td>
<td>1 (2%)</td>
<td>29 (10.7%)</td>
<td>37 (18.2%)</td>
</tr>
<tr>
<td>&gt;5 (−)</td>
<td>1 (1.7%)</td>
<td>0 (0%)</td>
<td>46 (16.8%)</td>
<td>151 (74.0%)</td>
</tr>
<tr>
<td>Mean ratio†</td>
<td>1.5</td>
<td>1.3</td>
<td>2.6</td>
<td>7.0</td>
</tr>
<tr>
<td>HER-2/neu gene copy number‡</td>
<td>≤4</td>
<td>51 (85.0%)</td>
<td>41 (82.0%)</td>
<td>157 (57.9%)</td>
</tr>
<tr>
<td>&gt;4</td>
<td>9 (15.0%)</td>
<td>9 (18.0%)</td>
<td>114 (42.1%)</td>
<td>200 (98.5%)</td>
</tr>
<tr>
<td>Mean HER-2/neu gene copy number§</td>
<td>3.0</td>
<td>2.8</td>
<td>6.1</td>
<td>15.4</td>
</tr>
<tr>
<td>Total (n)</td>
<td>60</td>
<td>50</td>
<td>271</td>
<td>203</td>
</tr>
</tbody>
</table>

NOTE: n, number of cases included in each immunohistochemistry category.
*Ratio of HER-2/neu/centro-17 copy number in 60 cells: value <2 is nonamplified, 2-2.99 is low amplified, 3-4.99 is moderately amplified, and >5 is highly amplified.
†Mean of HER-2/neu/centro-17 copy number ratio in n cases.
‡Absolute HER-2/neu copy number per nucleus evaluated in 60 cells: value ≤4 is nonamplified; >4 is amplified.
§Mean of absolute HER-2/neu copy number per nucleus in n cases.

Table 2. Comparison between two criteria (HER-2/neu/centro-17 ratio and HER-2/neu gene copy number) of FISH analysis on 893 cases

<table>
<thead>
<tr>
<th>HER-2/neu/centro-17 ratio</th>
<th>HER-2/neu gene copy number</th>
<th>Total copy number</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 (−)</td>
<td>≤4 (−)</td>
<td>386</td>
</tr>
<tr>
<td>≥2 (+)</td>
<td>&gt;4 (+)</td>
<td>67</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>453</td>
</tr>
</tbody>
</table>

NOTE: Data in boldface designate cases with discordant FISH results according to the criteria considered for analysis.
*Ratio of HER-2/neu/centro-17 copy number in 60 cells: value <2 is nonamplified or negative (−); ≥2 is amplified or positive (+).
†Absolute HER-2/neu copy number per nucleus evaluated in 60 cells: value ≤4 is nonamplified or negative (−); >4 is amplified or positive (+).

Discussion

In the present study, HER-2/neu status was determined by FISH assay in a cohort of 893 cases of infiltrating breast cancers by using the FDA-approved PathVysion FISH kit assay, which includes a dual probe set for HER-2/neu gene and centro-17 and has been suggested to be the best approach for HER-2/neu evaluation by FISH (22, 27, 28, 31). Although we did not compare the two commercial FDA-approved FISH assay kits, we compared the two methods of FISH analysis (i.e., the centro-17 corrected and the net HER-2/neu gene copy number criteria) and observed a very good level of concordance between the two methods. Nevertheless, we also observed a statistically significant difference in positivity rates (P < 0.001) depending on the criteria considered.

Numerical aberrations in specific chromosomes have been shown to occur in a high proportion of breast carcinomas. In particular, aneusomy 17 has been frequently reported at different rates depending on the series considered (18, 29–36, 40) and shown to correlate to lymph node metastasis (29) as well as other poor prognostic factors (31).

In the present series, the cutoff points for chromosome 17 copy number alterations were estimated for each group according to published standards (21, 22, 33, 34, 36, 38, 39) adapted for truncated nuclei on paraffin sections. For the convenience of data analysis and comparison with recent literature, we applied the criteria suggested by Wang et al. (34) with an arbitrary division between low and high polyosmy for a better segregation of these tumors. Aneusomy 17, including hypodisomy and polysomy, was observed in 51.2% of cases. This value agrees with data reported on paraffin sections (31, 33) as well as on cytologic samples (18, 29). Despite the fact that on paraffin sections only truncated nuclei are analyzed, the percentages of aneusomy 17 reported do not differ greatly. Of note, Grushko et al. (38) addressed the comparison between breast cancer cell lines and revealed that cells embedded in paraffin had only ~8% fewer chromosome 17 than cells in suspension.
Moreover, according to Wang et al. (34), we observed aneusomy 17 mainly in the form of low polysomy 17 (34.9%), but also occasionally in the form of high polysomy 17 (7.3%). Our results showed that polysomy 17 could be a factor of discordance between the two criteria for FISH analysis because 88.8% (64 of 72) of FISH-FISH discordant cases showed polysomy 17 and that these 64 cases represented 16.9% (34 of 377) of polysomic 17 cases.

What is the implication of polysomy 17 in the evaluation of HER-2/neu status? In contrast to the statement by some authors that polysomy 17 alone might not significantly contribute to HER-2/neu gene copy number and to HER-2/neu protein overexpression (34), we show that its effect could be important in some cases, and hence share the view of others that chromosome 17 copy number should be incorporated in the assessment of HER-2/neu status (31).

Similarly to other studies, our results showed that both centro-17 corrected and net HER-2/neu gene copy numbers are correlated with immunohistochemistry scores. We observed the greatest correlation between FISH and immunohistochemical assays in the IHC−, 1+, and 3+ categories, with a >90% correspondence, whereas only one third of IHC 2+ cases showed HER-2/neu amplification by FISH regardless of criteria considered for FISH analysis. In this series, only 1.7% of highly amplified cases (ratio > 5) were IHC−. This is in the same range as reported in a huge series where 2.8% IHC− cases showed high amplification (ratio > 5; ref. 26). Our results are supported by the literature (20–27) and agree with the testing algorithm for HER-2 determination by using immunohistochemistry as the method of choice for primary testing, with FISH done for cancers with indeterminate results (2+ score) for secondary testing (26), particularly relevant when immunohistochemistry

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Table 3. HER-2/neu/centro-17 ratio and HER-2/neu gene copy number means as a function of chromosome 17 copy number

<table>
<thead>
<tr>
<th>Centro-17 copy number</th>
<th>n</th>
<th>Mean HER-2/neu/centro-17 ratio*</th>
<th>Mean HER-2/neu gene copy number†</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.5†</td>
<td>80</td>
<td>5.5</td>
<td>6.8</td>
</tr>
<tr>
<td>1.5–2.25†</td>
<td>496</td>
<td>4.0</td>
<td>7.6</td>
</tr>
<tr>
<td>2.26–3.75†</td>
<td>312</td>
<td>3.4</td>
<td>9.5</td>
</tr>
<tr>
<td>&gt;3.75†</td>
<td>65</td>
<td>2.6</td>
<td>13.2</td>
</tr>
</tbody>
</table>

NOTE: n, number of cases included in each category according to centro-17 copy number.
*Mean of HER-2/neu/centro-17 copy number ratio in 60 cells in n cases.
†Mean of absolute HER-2/neu copy number per nucleus evaluated in 60 cells in n cases.
‡Cases with values of centro-17 copy number per cell <1.5 were arbitrarily defined as hypodisomic, 1.5–2.25 as disomic, 2.26–3.75 as low polysomic, and >3.75 as highly polysomic.

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Fig. 1. A, polysomy 17 incidences in nonamplified cases (HER-2/neu/centro-17 copy number ratio < 2) in each immunohistochemistry category. Low polysomy 17 is defined as centro-17 copy number per cell between 2.26 and 3.75 and high polysomy 17 as centro-17 copy number per cell >3.75. Total polysomy 17 includes both categories. B, means of HER-2/neu gene copies and centro-17 copy number per nucleus in nonamplified cases (HER-2/neu/centro-17 copy number ratio < 2) in each immunohistochemistry category.
method includes antigen retrieval leading to a potential large number of false positives.

One major concern is whether polysomy 17 could be a factor of discordance between FISH and immunohistochemical assays. We found a greater incidence of polysomy 17, particularly high polysomy 17, in IHC 3+/nonamplified cases than in IHC–/1+/2+/nonamplified ones. We also observed that the mean chromosome 17 copy number increases with immunohistochemistry scoring in these nonamplified cases. Despite the fact that these data should be cautiously interpreted because of the small number of cases in the nonamplified IHC 3+ category, these differences remain striking. Varshney et al. (36) made similar observations in a series of 650 cases among which they found a polysomy 17 incidence in IHC 2+/nonamplified cases (4%) similar to the one found in IHC– cases (5.5%), in contrast to the 47% polysomy found among the IHC 3+/nonamplified cases. These results also suggest that weak overexpression without gene amplification is not secondary to polysomy 17, but that strong overexpression could be due to polysomy 17. Bose et al. (33) reported a significant difference in polysomy 17 incidence between IHC– cases (5%) and IHC 1+/2+ cases (39%). In addition, Lal et al. (35) reported that in a series of 561 cases, IHC 3+/FISH– cases showed a significant increase in chromosome 17 copy number when compared with IHC 0+/1+/2+/FISH– cases. All these authors as well as others (32) draw the conclusion that protein overexpression without gene amplification might indeed be secondary to an increased chromosome 17 copy number associated with an increased total number of HER-2/neu gene copies per tumor cell, particularly for a small subset of infiltrating breast carcinomas that strongly overexpress HER-2/neu protein in the absence of specific gene amplification. Our results confirm and expand on these data.

The clinically relevant question is whether or not those patients with polysomy 17/nonamplified/HER 3+ tumors might also benefit from trastuzumab treatment. Nevertheless, it is important to emphasize that in the clinical setting centro-17 corrected amplification seems to be the best predictor of response to trastuzumab in advanced breast cancer and that no responses to trastuzumab as a single agent were reported among the cohort of HER-2/neu 2+ patients if no concomitant gene amplification was detected (8, 41). These data seem to support the concept that if polysomy 17 is involved in the prediction of response to trastuzumab, it should not play a major role. More recently, data in this field presented at the ESMO 2004 by Hofmann et al. (42) show that all the responders (19 of 86) to trastuzumab had a high level of protein overexpression (3+) detected by immunohistochemistry, but some of these IHC 3+ patients did not have amplification as defined by the ratio HER2/centro-17, but had low amplification by chromogenic in situ hybridization and actually showed polysomy 17. Only a prospective clinical trial could definitely address this issue. The study should be focusing on patients with polysomy 17 and concomitant HER-2/neu overexpression documented by immunohistochemistry.

Our results also raise the issue of the most appropriate criteria for FISH evaluation. For polysomic 17 cases, both immunohistochemistry and FISH assays should be considered for HER-2/neu status determination, and, in case of strong protein overexpression, trastuzumab treatment should be considered until additional clinical studies quantify the benefit of this agent in this subset. There is also a need to pursue research on improved quantitative methods for measuring protein levels in those polysomic 17 cases. Quantitative methods like ELISA might be a valuable alternative in this setting (5).

Whereas FISH is generally considered the most accurate method for HER-2/neu status evaluation compared with immunohistochemistry, which suffers from technical and interpretation variability, particularly when antigen retrieval method is used to overcome to poor sensibility, the FISH technique may also be the subject of delicate interpretation, mostly in cases of polysomy 17. Our results show how HER-2/neu status interpretation is dramatically dependent on the criterion considered (centro-17 corrected or net HER-2/neu gene copy number) for FISH analysis on such cases.

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References

Polysomy 17 Effect on HER-2/neu Status of Breast Cancer


Polysomy 17 in HER-2/neu Status Elaboration in Breast Cancer: Effect on Daily Practice

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