Human Epidermal Growth Factor Receptor 2 Assessment in a Case-Control Study: Comparison of Fluorescence In Situ Hybridization and Quantitative Reverse Transcription Polymerase Chain Reaction Performed by Central Laboratories

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See accompanying editorials on pages 4289 and 4293 and article on page 4307

ABSTRACT

Purpose
The optimal method to assess human epidermal growth factor receptor 2 (HER2) status remains highly controversial. Before reporting patient HER2 results, American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines mandate that laboratories demonstrate ≥ 95% concordance to another approved laboratory or methodology. Here, we compare central laboratory HER2 assessed by fluorescence in situ hybridization (FISH) and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) using Onco type DX in lymph node–negative, chemotherapy-untreated patients from a large Kaiser Permanente case-control study.

Patients and Methods
Breast cancer specimens from the Kaiser–Genomic Health study were examined. Central FISH assessment of HER2 amplification and polysomy 17 was conducted by PhenoPath Laboratories (ratios ≥ 2.2, 1.8 to 2.2, and < 1.8 define HER2 positive, HER2 equivocal, and HER2 negative, respectively). HER2 expression by RT-PCR was conducted using Onco type DX by Genomic Health (normalized expression units ≥ 11.5, 10.7 to < 11.5, and < 10.7 define HER2 positive, HER2 equivocal, and HER2 negative, respectively). Concordance analyses followed ASCO/CAP guidelines.

Results
HER2 concordance by central FISH and central RT-PCR was 97% (95% CI, 96% to 99%). Twelve percent (67 of 568 patients) and 11% (60 of 568 patients) of patients were HER2 positive by RT-PCR and FISH, respectively. HER2-positive patients had increased odds of dying from breast cancer compared with HER2-negative patients. Polysomy 17 was demonstrated in 12.5% of all patients and 33% of FISH-positive patients. Nineteen of 20 FISH-positive patients with polysomy 17 were also RT-PCR HER2 positive. Although not statistically significantly different, HER2-positive/polysomy 17 patients tended to have the worst prognosis, followed by HER2-positive/eusomic, HER2-negative/polysomy 17, and HER2-negative/eusomic patients.

Conclusion
There is a high degree of concordance between central FISH and quantitative RT-PCR using Onco type DX for HER2 status, and the assay warrants additional study in a trastuzumab-treated population.

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INTRODUCTION

The human epidermal growth factor receptor 2 gene (HER2) is reportedly amplified in 20% to 25% of human breast cancers; however, recent data suggest that it may be amplified in 10% to 11% of lymph node–negative disease.1,2 HER2 gene amplification and protein overexpression is a prognostic marker for aggressive disease and an important predictive marker for specific therapies, including trastuzumab (Herceptin; Genentech, South San Francisco, CA) and lapatinib (Tykerb; GlaxoSmithKline, London, United Kingdom).3,4 The US Food and Drug Administration (FDA) has approved two immunohistochemical (IHC) assays and three fluorescent in situ hybridization (FISH) assays for HER2 assessment; however, considerable debate persists about which assay is best for establishing HER2 status.5
The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) developed guidelines for laboratory evaluation of HER2 status. These enumerate requirements for HER2 analyses and recommend either using IHC assays for initial evaluation of HER2 status followed by reflex testing by FISH of some IHC categories or the primary use of FISH in initial testing. Even with these guidelines, concern remains about the accuracy of HER2 testing. Two independent cooperative groups reported that up to 20% of locally performed HER2 assays reported as positive could not be confirmed by central laboratories.

HER2 gene amplification is highly associated with mRNA overexpression and increased protein levels, and small studies have compared mRNA expression by reverse transcriptase polymerase chain reaction (RT-PCR) with FISH. Quantitative RT-PCR by the Oncotype DX assay (Genomic Health, Redwood City, CA) quantifies gene expression using RNA extracted from fixed paraffin-embedded tumor tissue and has been shown to be 95% concordant with IHC.

Herein, we compare central laboratory HER2 assessment by FISH and quantitative RT-PCR using Oncotype DX in patients from a large Kaiser Permanente case-control study according to the ASCO/CAP guidelines and explore the association between HER2 assessed by both assays and chromosome 17 polysomy status with risk of breast cancer death.

**Tumor Samples and Patient Clinical Data**

This is a case-control study nested within a cohort of women diagnosed with lymph node–negative breast cancer who were not treated with chemotherapy. Eligible women, identified using the Kaiser Permanente Northern California Cancer Registry, were diagnosed from 1985 to 1994 and were younger than age 75 years at diagnosis. The study was approved by the Kaiser Permanente Institutional Review Board. Patients were excluded for inflammatory carcinoma, bilateral breast carcinoma, evidence of metastasis (including lymph nodes) at initial diagnosis, prior invasive cancer at diagnosis, or unknown/unconfirmed tamoxifen treatment. Cohort eligibility was confirmed by medical record review, and members were observed until breast cancer death, contralateral breast cancer, departure from health plan (including any death cause), or December 2002, whichever came first.

Patient cases were patients whose first event was breast cancer death. At each death, up to three controls were randomly selected from patients who were alive and under follow-up for at least as long as the patient case’s time to breast cancer death (incidence density sampling). A patient could be both a control (up until time of death from breast cancer) and patient case. Controls were matched to their patient case on age, race, calendar year of diagnosis, and treatment of index breast cancer with tamoxifen. Eligibility and case-control selection were performed before laboratory analyses. Tamoxifen treatment was defined as treatment for ≥ 3 months within 2 years of surgery and before breast cancer recurrence. Matching on tamoxifen treatment facilitated analyses stratified by tamoxifen treatment.

Pathology was conducted blinded to clinical outcome using the Nottingham system. Microscopic tumor size was from pathology reports. Tumors with less than 5% carcinoma were excluded.

**Central Laboratory IHC and FISH**

PhenoPath Laboratories (Seattle, WA) performed and interpreted IHC for estrogen receptor (ER) and progesterone receptor (PR) and FISH for HER2. ER (SP1; 1:250; LabVision, Fremont, CA) and PR (636; 1:200; Dako, Carpinteria, CA) were performed and scored by two pathologists as published. For FISH, deparaffinized tissue sections were pretreated using a modification of the vendor’s standard protocol and then incubated with the FDA-approved Vysis PathVysion probe set (Abbott Diagnostics, Chicago, IL). Morphometric analysis used Metafer software with extended focus/tile sampling (MetaSystems, Altlussheim, Germany). Manual counting was performed when autolucency and/or artifact prevented sufficient cell counting. All samples with ratios of HER2/CEP17 between 1.5 and 2.5 by morphometric analysis were scored manually by counting more than 60 nonoverlapping cells. A ratio of more than 2.2, 1.8 to 2.2, or less than 1.8 is positive, equivocal, or negative for amplification, respectively. For primary concordance analysis, equivocal and negative groups were combined. Polysony 17 was defined as a CEP17 signal ≥ three copies.

**Sample Preparation and Gene Expression Analysis Using Quantitative RT-PCR by Oncotype DX**

Gene expression used whole tumor sections and the Oncotype DX assay. RNA was extracted from 3 × 10 μm or 6 × 10 μm whole sections when manual microdissection was performed when tumor constituted less than 50% of the total epithelium or when ductal carcinoma in situ grade and invasive carcinoma nuclear grade differed. After DNase I treatment, total RNA content was measured, and absence of DNA contamination was verified. Gene-specific reverse transcription was performed followed by quantitative PCR (TaQMan) using Prism 7900HT instruments (Applied Biosystems, Foster City, CA). The expression of 16 cancer-related genes, including ER (ESR1) and PR (PGR), were normalized relative to five reference genes (ACTB, GAPDH, GUSB, RPLP0, and TFRC); reference normalized expression ranged from 2 to 16 units (each 1-unit increase reflects approximately a two-fold increase in RNA). HER2 status was classified as negative (< 10.7 normalized expression units), equivocal (10.7 to < 11.5 units), and positive (≥ 11.5 units); ER status was classified as negative (< 6.5 units) and positive (≥ 6.5 units), and PR status was classified as negative (< 5.5 units) and positive (≥ 5.5 units; expression relative to reference genes; log10). All cut points were prespecified.

**Statistical Analyses**

Analyses were conducted by Kaiser Permanente. For concordance analysis, only unique patients were included. Equivocal samples from both methods were excluded (ASCO/CAP guidelines). Measures of agreement include overall concordance (number of samples that agree divided by the total number of samples) and κ statistics (agreement adjusted for chance).

Exact 95% CIs for the concordance statistic were calculated using the F-distribution method using the FREQ procedure in SAS (SAS Institute, Cary, NC). Percent positive agreement was calculated as the number of samples positive by both assays divided by the number of samples positive by the FISH assay. Percent negative agreement was calculated similarly.

Outcome analyses were performed on all patients and separately in patients who were and were not treated with tamoxifen. Odds ratios (ORs) for breast cancer death associated with HER2 status and breast cancer subtypes were calculated by conditional logistic regression using maximum likelihood estimation. HER2 status by FISH and hormone receptor (HR) status by IHC were compared with HER2 and HR status by RT-PCR. P values using the likelihood ratio test and 95% Wald CIs for the OR were calculated. All statistical tests were performed at the two-sided α = .05 significance level.

**RESULTS**

**Patient Characteristics**

Of the 402 patient cases and 989 controls identified as eligible, 234 patient cases and 631 controls who were eligible after chart review met matching case-control criteria and had tumor blocks. Two hundred twenty patient cases and 570 controls (647 unique patients) were RT-PCR evaluable; a subset of 203 patient cases and 490 controls (568 unique patients) had sufficient tumor for FISH. Data are presented for the evaluable 568 unique patients. Patient demographics and clinical characteristics are listed in Table 1. Patient cases and controls, as
expected, were similar by matching factors. One third of patients were treated with adjuvant tamoxifen (not all ER-positive patients received tamoxifen because this cohort includes patients from the 1980s). Compared with controls, patient cases more commonly had larger/more poorly differentiated tumors. HER2-positive tumors were more commonly moderately or poorly differentiated than HER2-equivocal and HER2-negative tumors by both methods (Table 1).

### Table 1. Patient Demographics and Baseline Clinical Characteristics

<table>
<thead>
<tr>
<th>Demographic or Clinical Characteristic</th>
<th>Patient Cases (n = 203)</th>
<th>Controls (n = 365)</th>
<th>Total Patients for Concordance Analysis (n = 568)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ER status and Tam treatment strata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER positive, Tam = yes</td>
<td>50</td>
<td>25</td>
<td>175</td>
</tr>
<tr>
<td>ER positive, Tam = no</td>
<td>105</td>
<td>52</td>
<td>259</td>
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<tr>
<td>ER negative, Tam = yes</td>
<td>9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>ER negative, Tam = no</td>
<td>39</td>
<td>19</td>
<td>72</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 40</td>
<td>16</td>
<td>8</td>
<td>29</td>
</tr>
<tr>
<td>40-49</td>
<td>40</td>
<td>20</td>
<td>62</td>
</tr>
<tr>
<td>50-59</td>
<td>56</td>
<td>28</td>
<td>84</td>
</tr>
<tr>
<td>≥ 60</td>
<td>91</td>
<td>45</td>
<td>175</td>
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<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White (non-Hispanic)</td>
<td>159</td>
<td>78</td>
<td>237</td>
</tr>
<tr>
<td>White (Hispanic)</td>
<td>5</td>
<td>3</td>
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<td>Black</td>
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<td><strong>Adjuvant Tam</strong></td>
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<td></td>
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<td>No</td>
<td>144</td>
<td>71</td>
<td>215</td>
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<tr>
<td>Yes</td>
<td>59</td>
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<td>88</td>
</tr>
<tr>
<td><strong>Tumor size, cm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1.0</td>
<td>46</td>
<td>23</td>
<td>69</td>
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<tr>
<td>&gt; 1.0-2.0</td>
<td>87</td>
<td>43</td>
<td>130</td>
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<tr>
<td>&gt; 2.0-4.0</td>
<td>67</td>
<td>33</td>
<td>100</td>
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<tr>
<td>&gt; 4.0</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><strong>Tumor grade† (all patients)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>23</td>
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<td>Moderate</td>
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<td>41</td>
<td>125</td>
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<tr>
<td>Poor</td>
<td>96</td>
<td>47</td>
<td>143</td>
</tr>
<tr>
<td><strong>Tumor grade† (HER2 positive by FISH)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Moderate</td>
<td>12</td>
<td>34</td>
<td>46</td>
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<tr>
<td>Poor</td>
<td>22</td>
<td>63</td>
<td>85</td>
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<tr>
<td><strong>Tumor grade† (HER2 positive by RT-PCR)</strong></td>
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<td></td>
</tr>
<tr>
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<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Moderate</td>
<td>15</td>
<td>42</td>
<td>57</td>
</tr>
<tr>
<td>Poor</td>
<td>19</td>
<td>53</td>
<td>72</td>
</tr>
</tbody>
</table>

NOTE. Percentages may not sum to 100% exactly as a result of rounding. Abbreviations: ER, estrogen receptor; Tam, tamoxifen; HER2, human epidermal growth factor receptor 2; FISH, fluorescence in situ hybridization; RT-PCR, reverse transcriptase polymerase chain reaction.†Tumor grade as assessed by pathologist 1.

### Table 2. HER2 Concordance Excluding Equivocal Results (2007 ASCO/CAP Guidelines)

<table>
<thead>
<tr>
<th>Oncotype DX Status</th>
<th>Central FISH Positive</th>
<th>Central FISH Negative</th>
<th>Total No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onco type DX positive</td>
<td>55</td>
<td>98</td>
<td>11</td>
</tr>
<tr>
<td>Onco type DX negative</td>
<td>1</td>
<td>2</td>
<td>408</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>419</td>
<td>66</td>
</tr>
</tbody>
</table>

NOTE. Percentages are column percentages. FISH HER2 status is used as the denominator. Concordance is 97% (95% CI, 96% to 99%); k is 89% (95% CI, 83% to 95%).

Abbreviations: HER2, human epidermal growth factor receptor 2; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; FISH, fluorescence in situ hybridization.

#### Distribution of HER2

Among the 568 evaluable patients, 12% and 11% were HER2 positive by quantitative RT-PCR and FISH, respectively (Appendix Fig A1, online only). There were more equivocal results by RT-PCR than FISH (15% v 2%, respectively). The majority (87%) of the 88 RT-PCR–equivocal samples were FISH negative (Appendix Fig A2, online only). This relatively high number of RT-PCR–equivocal samples has been noted among ER-positive patients. Appendix Table A1 (online only) presents cross-tabulated results for HER2 status by FISH and RT-PCR.

#### Concordance Between FISH and RT-PCR

Central FISH and RT-PCR concordance was 97% (95% CI, 96% to 99%), percent positive agreement was 98% (95% CI, 90% to 99%), and percent negative agreement was 97% (95% CI, 95% to 99%; Table 2). Lacking a gold standard, the terms percent positive agreement and percent negative agreement are preferred by the FDA over sensitivity and specificity. We conducted additional analyses of concordance excluding FISH-equivocal results, treating equivocal results by RT-PCR as negative, and excluding equivocal results by both assays. In all cases, concordance for these comparisons was 97% (95% CI, 95% to 98%; Appendix Tables A2 and A3, online only).

The distribution of FISH HER2/CEP17 ratio compared with central RT-PCR HER2 is shown in Figure 1. Spearman rank correlation of the relationship between the two assays is 0.45. One observation was FISH HER2 positive but RT-PCR HER2 negative.

#### HER2 Expression and Breast Cancer Death

Among all patients, by FISH (OR = 1.95; 95% CI, 1.19 to 3.19) and RT-PCR (OR = 1.72; 95% CI, 1.04 to 2.84), HER2-positive patients had increased odds of breast cancer death compared with HER2-negative patients. OR estimates and overlapping 95% CIs for the comparisons by FISH and RT-PCR are shown in Figure 2. By both assays, equivocal patients did not have significantly different odds of dying from breast cancer compared with HER2-negative patients (FISH: OR = 0.84; 95% CI, 0.22 to 3.13; RT-PCR: OR = 0.66; 95% CI, 0.40 to 1.09). Similar results were obtained in the groups that did and did not receive tamoxifen (Fig 2).

We explored the relationship between breast cancer death and breast cancer subtypes defined as combinations of HER2 and HR
status (assessed by RT-PCR or FISH/IHC). HR-positive, HER2-negative status is the reference category for all comparisons. Although the 95% CIs are wide and overlap, the point estimates trend toward HR-negative/HER2-positive patients having the worst prognosis (OR = 4.21 by IHC/FISH and 3.91 by RT-PCR), followed by HR-negative/HER2-negative patients (OR = 2.13 by IHC/FISH and 2.42 by RT-PCR), HR-positive/HER2-positive patients (OR = 1.58 by IHC/FISH and 1.79 by RT-PCR), and HR-positive/HER2-negative patients.

#### Chromosome 17 Polysomy Status and HER2 Amplification

Among all patients, seventy-one patients (12.5%) showed chromosome 17 polysomy (polysomy 17; Table 3). Among FISH HER2-positive patients, 33% showed polysomy 17. In FISH-negative patients, 10% showed polysomy 17. Among FISH-positive patients with polysomy 17, 19 of 20 were RT-PCR HER2 positive. The majority of FISH-negative polysomy 17 patients were RT-PCR HER2 negative (32 of 49 patients; 65%), but rare patients (four of 49 patients; 8%) were RT-PCR HER2 positive.

#### Breast Cancer Death: Chromosome 17 Polysomy Status and HER2 Amplification

By FISH, we evaluated polysomy status and breast cancer death (Fig 3). Compared with HER2-negative, chromosome 17 eusomic patients, HER2-positive patients with polysomy 17 had 2.77 times (95% CI, 1.21 to 6.33 times) the odds of dying from breast cancer; HER2-positive, chromosome 17 eusomic patients had 1.78 times (95% CI, 0.97 to 3.25 times) the odds of dying from breast cancer; and HER2-negative, polysomy 17 patients had 1.50 times (95% CI, 0.83 to 2.72 times) the odds of dying from breast cancer. Similar results were obtained when HER2 status was measured by RT-PCR, although the results were not statistically significant (OR = 2.14, 1.41, and 1.22 for HER2-positive, polysomy 17; HER2-positive, chromosome 17 eusomic; and HER2-negative, polysomy 17 patients, respectively, compared with HER2-negative, chromosome 17 eusomic patients).

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### DISCUSSION

HER2 positivity in breast cancer has a significantly worse prognosis with an increased risk of recurrence, and large randomized trials have established trastuzumab as the standard of care in patients with early HER2-positive breast cancer, who are also suitable for treatment with chemotherapy. Accurate and precise measurement of HER2 status is of marked therapeutic importance, and new methodologies for HER2 assessment must be rigorously compared with the current measurement standards. The primary objective of this study was to determine the HER2 concordance using central quantitative RT-PCR by Oncotype DX and central FISH in a large cohort of patients with early breast cancer with long follow-up (> 8 years).

Overall, there was a high level of concordance between quantitative RT-PCR and FISH (97%; 95% CI, 96% to 99%), and the number of discordant samples was small. There was an imbalance in the distribution of the HER2-discordant tumors between central FISH and central RT-PCR. FISH-negative but RT-PCR–positive tumors were more common than FISH-positive but RT-PCR–negative tumors. Importantly, there was only a single tumor that was FISH positive and RT-PCR negative.

The variability in RNA extraction, reverse transcription, PCR protocols, instruments, primer/probe selection, and reagent manufacturing can contribute to assay variation. The Oncotype DX assay uses controls, calibrators, reference ranges (for quantitative single-gene ER, PR, and HER2 results), and normalization to address differences in RNA quality. The variability in RNA extraction, reverse transcription, PCR protocols, instruments, primer/probe selection, and reagent manufacturing can contribute to assay variation. The Oncotype DX assay uses controls, calibrators, reference ranges (for quantitative single-gene ER, PR, and HER2 results), and normalization to address differences in RNA quality.
This study suggests that RT-PCR HER2-equivocal patients are similar to HER2-negative patients with respect to grade and clinical outcomes; hence, it would be reasonable to consider them as HER2 negative. Comparing FISH to RT-PCR, when RT-PCR–equivocal patients are considered negative, the concordance remains high at 97% (Appendix Tables A2 and A3). Of note, ASCO/CAP guidelines recommend use of FISH for determination of HER2 status in equivocal patients.

The significance of HER2 status should be considered in terms of prognosis and prediction of treatment benefit. As previously reported for patients not treated with trastuzumab, HER2 positivity by both methods was a weakly prognostic factor in the present chemotherapy- and trastuzumab-naïve cohort; HER2-positive patients had increased odds of dying from breast cancer compared with HER2-negative patients.44-46 By both assays, the HER2-equivocal patients did not have significantly different odds of dying from breast cancer compared with HER2-negative patients. These results were independent of tamoxifen treatment. Although HER2 positivity has been associated with tamoxifen resistance in HR-positive patients, we could not directly examine this question because patient cases and controls were matched on tamoxifen therapy.41 We observed a slightly stronger association between HER2 status and risk of breast cancer death among patients treated with tamoxifen compared with those not treated with tamoxifen (Fig 2). Consistent with reported observations regarding the intrinsic subtypes of breast cancer, in this cohort, HR-negative/HER2-positive patients tended to have the worst prognosis, followed by HR-negative/HER2-negative (triple-negative) patients, HR-positive/HER2-positive patients, and finally, HR-positive/HER2-negative patients.47 This relationship was similar between patients who were and were not treated with tamoxifen.

Depending on study population and polysomy 17 definition, the frequency of polysomy 17 in breast cancer ranges from 13% to 46%.48-52 Using the common definition of ≥ three CEP17 copies, 12.5% of tumors showed polysomy 17 by FISH.22 Consistent with reports that patients with polysomy 17 and HER2 amplification have clinical outcomes similar to HER2-positive patients, one third of HER2-positive patients showed polysomy 17 and had the worst prognosis. Patients with polysomy 17 without HER2 amplification (HER2 negative), often equivocal by IHC, are reported to have outcomes similar to HER2-negative, chromosome 17 eusomic patients, and this was observed in our cohort.23,53,54 In contrast to reports that polysomy 17 tumors without HER2 amplification invariably show no increase in HER2 RNA levels, in this study, 9% (four of 49 tumors) did show expression levels consistent with HER2 amplification, as previously

### Table 3. Incidence of Polysomy 17 by HER2 Status (FISH and RT-PCR)

<table>
<thead>
<tr>
<th>HER2 Status</th>
<th>Central FISH Positive</th>
<th>Central FISH Equivocal</th>
<th>Central FISH Negative</th>
<th>Total RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Polysomy-</td>
<td>% of Polysomy-</td>
<td>No. of Polysomy-</td>
<td>% of Polysomy-</td>
</tr>
<tr>
<td></td>
<td>Positive Patients*</td>
<td>Positive Patients</td>
<td>Equivocal Patients</td>
<td>Positive Patients</td>
</tr>
<tr>
<td>RT-PCR positive</td>
<td>19/55 35%</td>
<td>1/1 100%</td>
<td>4/11 36%</td>
<td>24/87 36%</td>
</tr>
<tr>
<td>RT-PCR equivocal</td>
<td>0/4 0%</td>
<td>0/4 0%</td>
<td>2/408 8%</td>
<td>32/413 8%</td>
</tr>
<tr>
<td>RT-PCR negative</td>
<td>1/1 100%</td>
<td>2/10 20%</td>
<td>49/498 10%</td>
<td>71/568 13%</td>
</tr>
<tr>
<td>Total FISH</td>
<td>20/60 33%</td>
<td>2/10 20%</td>
<td>49/498 10%</td>
<td>71/568 13%</td>
</tr>
</tbody>
</table>

Abbreviations: HER2, human epidermal growth factor receptor 2; FISH, fluorescence in situ hybridization; RT-PCR, reverse transcriptase polymerase chain reaction.

*Patients show both polysomy and chromosome 17 amplification by FISH.
†Patients show polysomy but no chromosome 17 amplification.
described by IHC. This may support recent reports that standard FISH probes may misclassify rare HER2-amplified tumors as polysomy 17 when the amplicon extends into the centromeric region of chromosome 17.

The strengths of this study include the large study cohort of lymph node–negative, chemotherapy- and trastuzumab-naïve patients and the use of central laboratories with standardized methods for FISH and RT-PCR assays. Not all FISH or all RT-PCR assays are the same. There are also some limitations to consider when interpreting these study results. Because of the cohort age, only approximately 30% of patients were treated with tamoxifen, although this is consistent with other reported populations during this period. HER2 IHC was not available for review. Finally, the cohort is chemotherapy and trastuzumab naive and thus shows prognosis with respect to HER2 status but not prediction of trastuzumab benefit. Assessment of HER2 status by IHC and/or FISH should continue as the approach for making trastuzumab or lapatinib treatment decisions. Although with regard to trastuzumab benefit, recent quantitation of HER2 gene amplification by FISH did not correlate with trastuzumab benefit, HER2 gene expression in a trastuzumab-treated population has not been studied with quantitative RT-PCR and warrants further study.

Recent reports that patients with discordant local and central laboratory HER2 results responded to trastuzumab highlight the need for standardized, robust methods that are less affected by sources of variability. These data show that RT-PCR is highly concordant with high-quality, central laboratory–performed FISH and may be of use to clinicians and pathologists who are uncertain about select HER2 assay results obtained by FISH and IHC. Furthermore, these standardized HER2 results may prove useful for laboratories performing mandatory ASCO/CAP concordance studies.

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