Modern Pathology (2009) 22, 1169–1175
© 2009 USCAP, Inc All rights reserved 0893-3952/09 $32.00
www.modernpathology.org

Clinical validation of an array CGH test for *HER2* status in breast cancer reveals that polysomy 17 is a rare event

I-Tien Yeh[1], Mathew A Martin[1], Ryan S Robetorye[1], Aswani R Bolla[1], Chris McCaskill[2], Rashmi K Shah[2], Mercedes E Gorre[2], Mansoor S Mohammed[2] and Shelly R Gunn[1,2]

[1]*Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA and* [2]*Combimatrix Molecular Diagnostics, Irvine, CA, USA*

The *HER2* gene is an important prognostic and therapeutic marker in newly diagnosed breast cancer. Currently, HER2 status is most frequently determined by immunohistochemical detection of HER2 protein expression on the cellular membrane surface or by fluorescence *in situ* hybridization analysis of *HER2* gene copy number in fixed tissue using locus-specific probes for the *HER2* gene and chromosome 17 centromere. However, these methods are problematic because of issues with intra- and inter-laboratory reproducibility and preanalytic variables, such as fixation time. In addition, the commonly used *HER2*/chromosome 17 ratio presumes that chromosome 17 polysomy is present when the centromere is amplified, even though analysis of the rest of the chromosome is not included in the assay. In this study, 97 frozen samples of invasive lobular and invasive ductal carcinoma, with known immunohistochemistry and fluorescence *in situ* hybridization results for *HER2*, were analyzed by comparative genomic hybridization to a commercially available bacterial artificial chromosome whole-genome array containing 99 probes targeted to chromosome 17 and the *HER2/TOP2* amplicon. Results were 97% concordant for HER2 status, meeting the College of American Pathologists/ American Society of Clinical Oncology's validation requirements for HER2 testing. Surprisingly, not a single case of complete polysomy 17 was detected even though multiple breast cancer cases showed clear polysomies of other chromosomes. We conclude that array comparative genomic hybridization is an accurate and objective DNA-based alternative for clinical evaluation of *HER2* gene copy number, and that polysomy 17 is a rare event in breast cancer.
Modern Pathology (2009) **22,** 1169–1175; doi:10.1038/modpathol.2009.78; published online 15 May 2009

Keywords: HER2; array comparative genomic hybridization; validation; polysomy 17

The human *HER2* gene is present on chromosome 17q11 and encodes a membrane receptor tyrosine kinase protein. It is overexpressed in 15–30% of breast cancer cases and overexpression is most commonly because of gene amplification.[1] Standard testing methods include analysis of gene copy number by fluorescence *in situ* hybridization (FISH), as well as immunohistochemical staining of the HER2 membrane protein. HER2 testing in breast cancer is important for prognosis, and accuracy of testing is crucial for the correct identification of patients who will benefit from trastuzumab (Herceptin) therapy, a humanized monoclonal antibody, and lapatinib (Tykerb), a small molecule tyrosine kinase inhibitor. These therapies are generally only effective in patients with *HER2*-amplified tumors, and may have significant toxicities and costs. Yet, it is estimated by the American Society of Clinical Oncology (ASCO)/ College of American Pathologists (CAP) that approximately 20% of current HER2 testing may be inaccurate owing to multiple preanalytic, analytic, and postanalytic variables.[2] The unreliability of current HER2 testing methods is illustrated by recent studies showing that some patients who tested negative for *HER2* amplification by immunohistochemistry and FISH may still benefit from trastuzumab.[3,4] It has been proposed that this discrepancy is not because of misunderstanding the mechanism of action of the drug, but rather because of the inaccuracy of current testing methods.[5]

Currently available FDA-approved FISH assays for the detection of *HER2* gene amplification include an assay with a single probe for the *HER2* gene and

Correspondence: Dr I-Tien Yeh, MD, Department of Pathology, The University of Texas HSC at San Antonio, Mail Code 7750, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA.
E-mail: yehi@uthscsa.edu
Received 24 December 2008; revised and accepted 21 April 2009; published online 15 May 2009

other assays with dual probes, one for the *HER2* gene and another for the centromere of chromosome 17 (probe CEP 17, D17Z1). At this time, assays with dual probes are more widely used.

Recent papers stress that an important source of HER2 testing inaccuracy may be owing to 'polysomy 17', referring to breast cancer cases in which there are presumed to be multiple copies of chromosome 17. In clinical trials, polysomy is a defined condition when the number of a particular chromosome is greater than diploid, meaning that there may be three or more copies of the chromosome, rather than the expected two copies. Polysomy 17 is considered to be present in breast cancer cases that show ≥ 3 signals in FISH assays with a probe targeted to the centromere of chromosome 17. Chromosomal aneusomy based on this finding has been reported in a significant number of breast carcinomas, and polysomy 17, in addition to *HER2* gene amplification, has been associated with HER2 protein overexpression.[6–9] This has led to the assumption that increased numbers of the chromosome 17 centromere are representative of the presence of additional copies of chromosome 17 in the tumor genome. Correction for chromosome 17 polysomy using the *HER2* to chromosome 17 ratio is, therefore, considered to be critical for distinguishing true *HER2* gene amplification from increased chromosome 17 copy numbers.[10,11] However, it may not be prudent to determine chromosome 17 copy number based on the amplification of a single probe, because amplification of the chromosome 17 centromere probe may actually mask *HER2* gene amplification.[12,13]

Although by using multiple chromosome 17 probes for *HER2*, FISH undoubtedly provides a better representation of the true copy number of the chromosome, this approach is impractical for a clinical assay. Array comparative genomic hybridization (array CGH) is available as an alternative to current, commonly used methods that can provide high-throughput, high-resolution, genome-wide copy number analysis in a single test.[14,15] Array CGH was initially developed as a research tool for evaluation of gene dosage variations in many disorders (including breast cancer),[16–19] and array-based testing is now the standard of care for detection of congenital genomic abnormalities.[20] Array CGH has broad applications for cancer analysis and has recently entered into clinical use for genome scanning in chronic lymphocytic leukemias.[21–24] In this study, we used bacterial artificial chromosome (BAC)-based array CGH to evaluate 97 frozen samples of invasive lobular and invasive ductal breast carcinoma. A commercially available microarray with whole-genome coverage was used for this analysis that included probes specifically targeted to the *HER2/TOP2* amplicon as well as additional probes covering the length of chromosome 17. The breast cancers included in this study had known HER2 results obtained using clinically validated immunohistochemistry and FISH assays. Concordance of HER2 status and frequency of complete gain of chromosome 17 was calculated. A subset of the samples was also evaluated by array CGH using an earlier described fresh tissue preservation protocol for procurement of surgical samples for the clinical laboratory. Microarray analysis results from the processed samples were compared with the results obtained from the non-processed samples.

Materials and methods

Fresh frozen tissues from 97 breast cancer cases were analyzed, including 86 invasive ductal carcinomas, 10 invasive lobular carcinomas, and one case with mixed ductal-lobular features. Optimum cutting temperature (OCT) compound was used to make frozen tissue blocks. H and E stained sections cut from the OCT blocks were reviewed, and a 4 mm punch was used to select 25–50 mg regions of tumor tissue for subsequent DNA extraction. The tissue samples were placed into 1.5 ml microfuge tubes and stored at −80°C until DNA isolation. A minimum of 2 μg DNA was isolated from each case using a Promega Maxwell 16 automated nucleic acid isolation instrument (Madison, WI, USA). Verification of the presence of high-molecular-weight DNA was accomplished by agarose gel electrophoresis. Array CGH analysis of the tumor genomes was carried out in the Combimatrix Molecular Diagnostics Laboratory (Irvine, CA, USA) using a BAC array comprised of up to 1526 clones for whole-genome coverage, which also included three overlapping clones for the *HER2* locus (RP11-62N23, RP11-94L15, RP11-387H17), and 96 clones for coverage of chromosome 17 and the *HER2/TOP2A* amplicon (genes *PPARBP, PPP1R1B, STARD3, TCAP, PNMT, PERLD1, GRB7, GSDML, PSMD3, CASC3, RARA, TOP2A,* and *SMARCE1*).[24,25] Tumor genomic DNA (test DNA) and male reference DNA (as an internal control) were differentially fluorescence-labeled with Alexa Fluor 555 and Alexa Fluor 647 (Invitogren, Carlsbad, CA, USA) in dye-swap reactions and hybridized to the BAC arrays. The hybridized microarray slides were scanned and quantified with GenePix 4000B scanner and GenePix Pro Software (Axon Ins. Inc., Union City, CA, USA). Normalized Alexa Fluor 555 to Alexa Fluor 647 intensity ratios were computed for each of the two reactions and plotted together for each chromosome. A ratio plot was assigned such that gains in DNA copy number at a particular locus are observed as the simultaneous deviation of the ratio plots from a modal value of 1.0, with blue ratio plots representing gain of genetic material, showing a positive deviation (to the right), and red ratio plots representing loss of genetic material, showing a negative deviation at the same locus (to the left). Array CGH results for *HER2* copy number were scored based on the average of the forward reaction fluorescence

Table 1 HER2 status by array CGH correlated to FISH interpretation

HER2 status by array CGH[a]	*FISH result*
Single copy loss (<0.8)	Negative
Normal (0.8 to <1.5)	Negative
Gain (1.5–3.5)	Positive
Amplification (>3.5)	Positive

[a]HER2 status by array CGH is based on the average of the fluorescence intensity ratios for the three RP11-94L15 clone replicates.

intensity ratios for the three replicates of the BAC clone containing the *HER2* gene (clone RP11-94L15) as follows: <0.8, loss; 0.8 to <1.5, normal; 1.5–3.5, gain; >3.5, amplified (Table 1).

All cancers were tested for HER2 using an algorithm recommended by the ASCO/CAP guidelines.[2] The tumors were initially tested by immunohistochemistry using the monoclonal antibody, TAB250 (Zymed Laboratories, South San Francisco, CA, USA), followed by FISH in cases that were equivocal by immunohistochemistry (scored as 2+). FISH analysis was carried out using the Vysis PathVysion HER-2 DNA Probe Kit (Abbott Molecular, Abbott Park, IL, USA), which includes a chromosome 17 centromere probe and a *HER2* probe. Array CGH results were correlated to the FISH results and scored as follows: loss or normal = negative; gain or amplified = positive (Table 1). Cases that showed discordant array CGH and FISH results were retested by FISH. In all, a total of 29 cases were tested by FISH.

A second subset of cases, HER2 positive ($n = 5$) and HER2 negative ($n = 5$), were thawed and stored for at least 48 h in RNA*later* (Applied Biosystems, Foster City, CA, USA) according to an earlier described multidisciplinary tumor bank procurement protocol for tissue and nucleic acid preservation of resection specimens.[25,26] Sections were cut for H and E staining followed by tumor-targeted DNA extraction for array CGH analysis. Results from the RNA*later* processed samples were compared with the results obtained from frozen non-processed samples.

Results

Array CGH results were 97% concordant with immunohistochemistry/FISH results for HER2 status, with a sensitivity and specificity of 91 and 99%, respectively. Positive predictive value was 95% and negative predictive value 97%. Of the 76 cases that were scored as HER2 negative by immunohistochemistry/FISH, 67 showed two copies of the *HER2* gene (HER2 negative) by array CGH, seven showed loss of one copy of the *HER2* gene (HER2 negative), and one tumor sample showed a gain in *HER2* copy number (HER2 positive). A total of 21 cases were positive by immunohistochemistry/FISH, with 16 of

Table 2 Comparison of results of HER2 testing by Immunohistochemistry/FISH versus array CGH

	HER2+ by Immunohistochemistry/ FISH	*HER2− by Immunohistochemistry/ FISH*
HER2+ by array CGH	19	1
HER2− by array CGH	2	75

these cases showing amplification by array CGH, three cases showing gains, and two cases showing normal results (Table 2). Discordant samples consisted of one immunohistochemistry/FISH-negative sample that showed gain of HER2 by array CGH, and two immunohistochemistry/FISH-positive samples that were negative by array CGH. Further review of the 'false positive' case showed the histology to be a mixed *in situ* and invasive ductal carcinoma, with the *in situ* component showing HER2 positivity by immunohistochemistry, but negative HER2 in the invasive component. Of the two 'false negatives', one case was positive by immunohistochemistry (3+); FISH testing showed an equivocal result with a ratio of 2.1. The second case was a borderline immunohistochemistry (2+)/FISH low positive, ratio 2.9 (Table 3). Further analysis of the array CGH results in the second case showed amplification of a nearby region at17q21, but the *HER2* locus at 17q12 was normal.

By FISH, 2 of our 97 cases showed a *HER2* to chromosome 17 centromere ratio of >3 and would have been classified as polysomy 17 by FISH. Of these, one was *HER2* amplified and the other was not. Multiple abnormalities were identified on chromosome 17 by array CGH, including losses and gains of genetic material in both the p and q arms, as well as six cases with gains of the centromeric region and 10 cases with loss (Figure 1a–c). Three of six cases with centromeric gains also showed *HER2* amplification (Figure 1d). Although multiple chromosomes in our study exhibited recurrent whole chromosome polysomy by array CGH, no cases showed complete polysomy of chromosome 17 (Table 4 and Figure 2).

In the subset of cases processed in RNA*later*, tissue architecture was preserved for tumor-targeted DNA extraction, and all 10 samples yielded high-quality high-molecular-weight DNA for subsequent array CGH analysis. *HER2* copy number was concordant with previously determined *HER2* copy number results from the frozen tissue, and similar whole-genome array CGH results were obtained (Figure 3).

Discussion

Analysis of 97 breast carcinoma samples by array CGH revealed 97% concordance with previously

Table 3 Analysis of three discordant cases

Case	*FISH*	*Immuno-histochemistry*	*Array CGH*	*Explanation*
1	Negative, ratio 1.0	2+	Gain	*Tumor heterogeneity*: This sample showed mixed DCIS plus invasive carcinoma. Tumor showed HER2 expression in DCIS areas, but no HER2 expression in invasive areas. Array CGH results were most likely representative of the *in situ* cell population.
2	Low positive, ratio 2.9	2+	Normal	*Amplification of nearby region*: FISH results are low positive; array CGH analysis showed amplification at the 17q21 region, but not at the *HER2* locus at 17q12.
3	Equivocal, ratio 2.1	3+	Normal	*Overexpression without gene amplification*: This case most likely represents the <10% of tumors showing overexpression of HER2 protein in the absence of gene amplification.

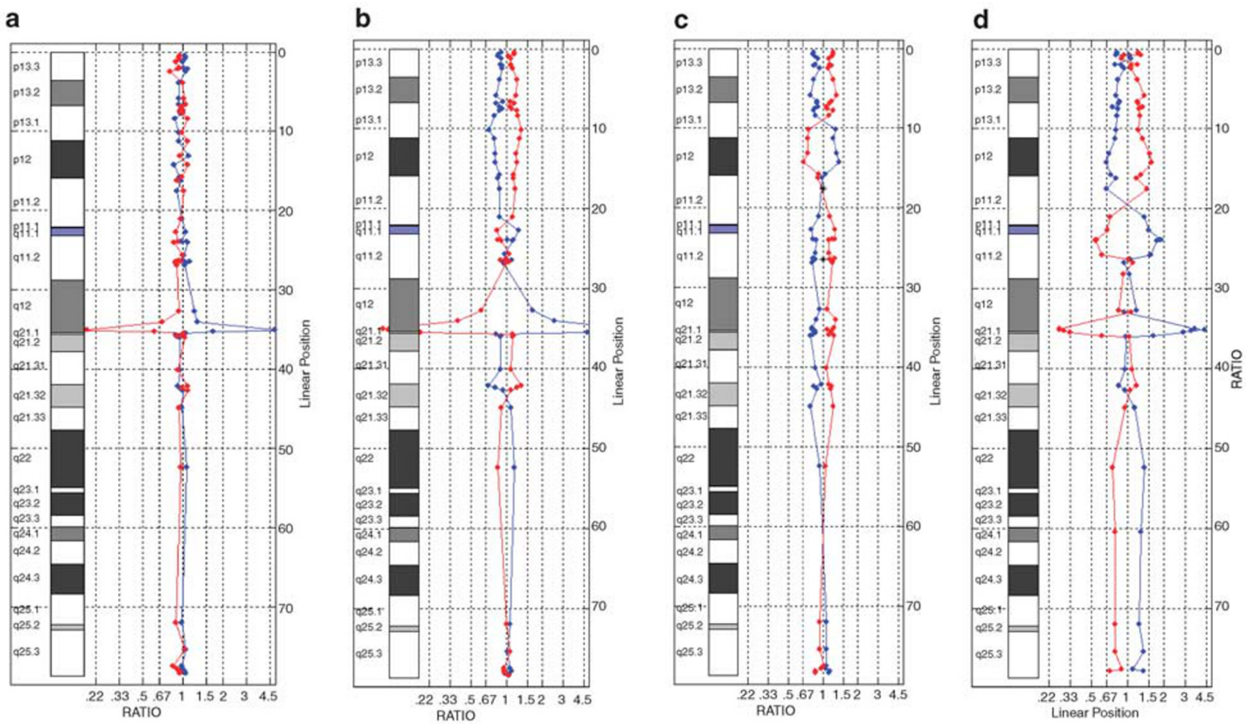

Figure 1 Examples of chromosome 17 complexity. (**a**) Normal chromosome 17 with *HER2* amplification. (**b**) Abnormal chromosome 17 with *HER2* amplification. (**c**) Abnormal chromosome 17 with single copy loss of *HER2* and other centromeric loci. (**d**) Gain of centromeric loci and *HER2* amplification.

Table 4 Incidence of whole chromosome polysomy[a] in 97 breast cancer cases analyzed by array CGH

Chromosome	*4*	*5*	*7*	*8*	*12*	*14*	*16*	*17*	*18*	*20*	*21*	*22*
Incidence	1	1	2	1	1	2	1	0	1	5	4	3

[a]Chromosome polysomy is defined as a gain of all of the BAC clones covering a chromosome. However, some of these polysomy cases have isolated single clones that do not show gain. Isolated single clone changes generally represent polymorphic regions of the genome, so chromosomes in our study with gains of all but a single clone are described as showing polysomy, and considered to represent complete single copy gain of DNA content.

validated methods, meeting the ASCO/CAP validation criteria for HER2 testing. The discordant cases included one false-positive case with a mixed DCIS-invasive carcinoma, in which the invasive component was negative and the *in situ* component was positive for HER2 expression and amplification by immunohistochemistry and FISH, respectively. This result could have been avoided by submission of only invasive carcinoma for analysis. There were also two false-negative cases that most likely

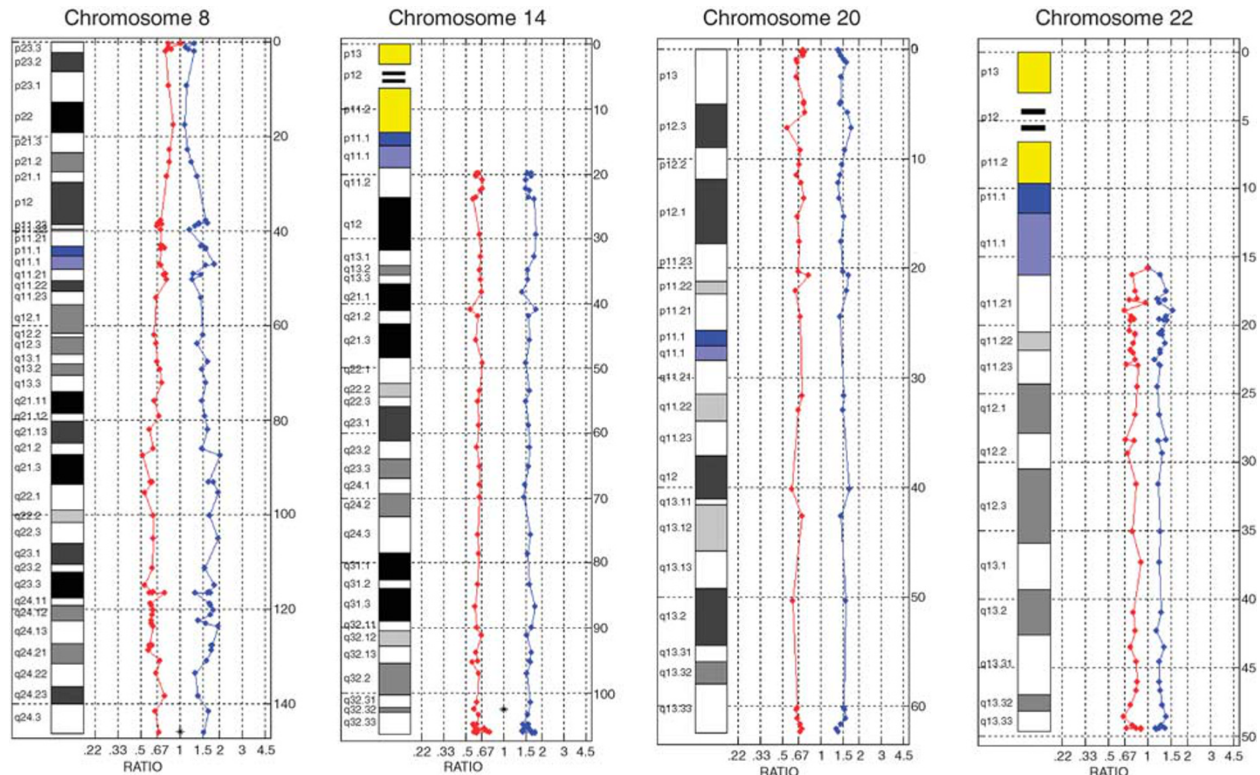

Figure 2 Examples of chromosomes showing complete polysomy. A total of 22 examples of complete chromosome gain were identified in this study, with chromosome 20 showing the highest incidence ($n = 5$). Examples of polysomy are shown for chromosomes 8, 14, 20, and 22.

represent one case in which the *HER2* FISH probe may have picked up amplification of a nearby genomic region, and another case showing HER2 protein overexpression without gene amplification (Table 3).

Array CGH showed reproducible results with high sensitivity and specificity in analyses of frozen tissue samples as well as in tissue samples that had been stored in RNA*later* for at least 48 hours.

Surprisingly, these analyses did not identify a single breast cancer case with complete polysomy of chromosome 17, including two cases that were classified as polysomy 17 by FISH analysis. Polysomy of chromosome 17 in breast cancer has been widely reported and is presumed to be present when FISH analysis detects amplification of a chromosome 17 centromere probe. However, in our array CGH study that included 99 BAC clones for chromosome 17, we found that the term ‘polysomy 17’ is a misnomer and propose the alternate term ‘centromere amplified’ to describe cases with ≥ 3 copies of the chromosome 17 centromere. Although polysomy of chromosome 17 was not observed in our study, complex rearrangements of chromosome 17 and amplification of genes other than *HER2* were relatively common (detected in 52 (55%) cases). It is clear that array CGH can accurately assess true chromosome 17 status, and therefore, the lack of detection of such cases in our study suggests that gain of whole chromosome 17 is a rare event in breast cancer. However, polysomy of other chromosomes is not uncommon, as evidenced by finding 22 instances of complete chromosome gain in a total of 97 cases. Interestingly, only one breast cancer case showed multiple polysomy, with gains of chromosomes 4, 7, and 21. In the other 21 cases, no more than one whole chromosome gain was found per tumor genome.

Array CGH has many advantages over FISH for gene copy number determination, including the fact that it is a DNA-based test with the ability to both subjectively (by visualization of chromosome 17 ratio plots) and objectively (by calculation of fluorescence intensity ratios for *HER2* probes) interpret HER2 status. Objective analysis can eliminate errors caused by reliance on interpretation of purely subjective results, as is the case with current HER2 testing.[27,28] Although immunohistochemical staining for the HER2 protein will remain a necessary part of the breast cancer workup because of a small percentage of cases that show protein overexpression without gene amplification, we have shown that array CGH is a more reliable confirmatory method than FISH for determination of HER2 status, because it can provide whole-chromosome 17 analysis as well as a whole-genome view in a single test.

Optimal array CGH results require adequate high-molecular-weight DNA from fresh or frozen tumor tissue. Although array CGH can be carried out on

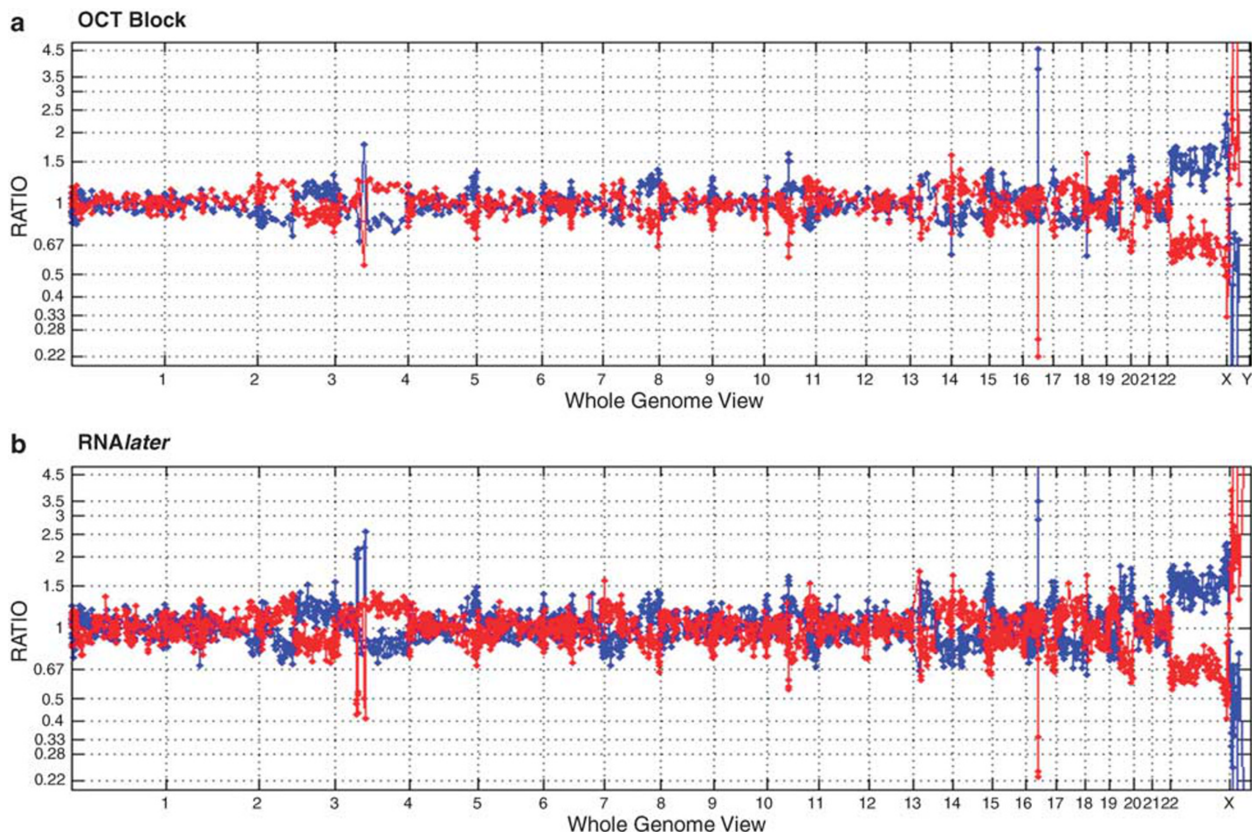

Figure 3 Whole-genome view comparison of array CGH results obtained from analysis of DNA samples isolated from a single breast cancer case that was either frozen in an OCT block (**a**) or processed for 48 h in RNA*later* (**b**). Both samples exhibit similar results and clearly show amplification of *HER2* on chromosome 17 (arrows).

formalin-fixed paraffin-embedded tissue (FFPE), significant differences are observed in the results obtained from FFPE tissue compared with fresh tissue owing to differences in fixation times and processing procedures at various institutions. We have developed two clinically validated breast cancer tissue procurement procedures for determination of HER2 status. These procedures consist of making an OCT block of fresh tissue or placing 25–50 mg of tissue into a tube of *RNAlater*, for subsequent sectioning, H and E staining, and tumor-targeted DNA isolation. Array CGH is a DNA-based test; hence, if adequate tissue is available post-operatively for the assay (almost always the case at the time of lumpectomy or mastectomy, and less frequently for core biopsies and cytology specimens), then DNA from the original tumor can be obtained, analyzed by array CGH, and stored for current studies, as well as for future comparative studies, should the patient have a recurrence or metastatic event.

In conclusion, we have shown that array CGH can be used as a clinical tool for the determination of HER2 and chromosome 17 status in breast cancer samples. In addition, our analysis has clearly shown that polysomy 17 is a rare occurrence in breast cancer, as we were unable to show any cases of polysomy 17 in the evaluation of 97 breast cancer cases. This finding has important implications for currently used FISH methods to determine HER2 status, as it suggests that chromosome 17 copy number assessment methods that use a chromosome 17 centromere probe do not accurately assess chromosome 17 copy number. As array CGH becomes more widely available for clinical breast cancer testing in the future, patients will benefit from the improved prognosis, risk stratification, and treatment planning that results from an accurate assessment of not only of *HER2* gene status but also the entirety of chromosome 17 and the rest of the tumor genome as well.

Acknowledgements

We wish to thank Jamie Parra, tumor bank technician for the Cancer Research Therapy Center at UTHSCSA Pathology Core Laboratory, for her assistance. We also acknowledge the contribution by the Cancer Center Support Grant (P30 CA54174) in this work.

Conflict of interest

SRG, MSM, MEG, CM, and RS are employees of Combimatrix Molecular Diagnostics (CMDX). RSR is

a clinical consultant for CMDX. IY, ARB, and MAM declare no conflicts of interest.

References

1 Ross JS, Fletcher JA. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. Stem Cells 1998;16:413–428.
2 Wolff AC, Hammond ME, Schwartz JN, *et al.* American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007;25:118–145.
3 Barrett C, Magee H, O'Toole D, *et al.* Amplification of the HER2 gene in breast cancers testing 2+ weak positive by HercepTest immunohistochemistry: false-positive or false-negative immunohistochemistry? J Clin Pathol 2007;60:690–693.
4 Hofmann M, Stoss O, Gaiser T, *et al.* Central HER2 IHC and FISH analysis in a trastuzumab (Herceptin) phase II monotherapy study: assessment of test sensitivity and impact of chromosome 17 polysomy. J Clin Pathol 2008;61:89–94.
5 Tuma RS. Inconsistency of HER2 test raises questions. J Natl Cancer Inst 2007;99:1064–1065.
6 Vanden Bempt I, Van Loo P, Drijkoningen M, *et al.* Polysomy 17 in breast cancer: clinicopathologic significance and impact on HER-2 testing. J Clin Oncol 2008;26:4869–4874.
7 Watters AD, Going JJ, Cooke TG, *et al.* Chromosome 17 aneusomy is associated with poor prognostic factors in invasive breast carcinoma. Breast Cancer Res Treat 2003;77:109–114.
8 Wang S, Hossein Saboorian M, Frenkel EP, *et al.* Aneusomy 17 in breast cancer: its role in HER-2/neu protein expression and implication for clinical assessment of HER-2/neu status. Mod Pathol 2002;15:137–145.
9 Bose S, Mohammed M, Shintaku P, *et al.* Her-2/neu gene amplification in low to moderately expressing breast cancers: possible role of chromosome 17/Her-2/neu polysomy. Breast J 2001;7:337–344.
10 Dal Lago L, Durbecq V, Desmedt C, *et al.* Correction for chromosome-17 is critical for the determination of true Her-2/neu gene amplification status in breast cancer. Mol Cancer Ther 2006;5:2572–2579.
11 Salido M, Tusquets I, Corominas JM, *et al.* Polysomy of chromosome 17 in breast cancer tumors showing an overexpression of ERBB2: a study of 175 cases using fluorescence *in situ* hybridization and immunohistochemistry. Breast Cancer Res 2005;7:R267–R273.
12 Press MF. How is Her-2/neu status established when Her-2/neu and chromosome 17 centromere are both amplified? Am J Clin Pathol 2006;126:673–674.
13 Troxell ML, Bangs CD, Lawce HJ, *et al.* Evaluation of Her-2/neu status in carcinomas with amplified chromosome 17 centromere locus. Am J Clin Pathol 2006;126:709–716.
14 Solinas-Toldo S, Lampel S, Stilgenbauer S, *et al.* Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer 1997;20:399–407.
15 Pinkel D, Segraves R, Sudar D, *et al.* High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 1998;20:207–211.
16 Albertson DG. Profiling breast cancer by array CGH. Breast Cancer Res Treat 2003;78:289–298.
17 Naylor TL, Greshock J, Wang Y, *et al.* High resolution genomic analysis of sporadic breast cancer using array-based comparative genomic hybridization. Breast Cancer Res 2005;7:R1186–R1198.
18 van Beers EH, Nederlof PM. Array-CGH and breast cancer. Breast Cancer Res 2006;8:210.
19 Climent J, Garcia JL, Mao JH, *et al.* Characterization of breast cancer by array comparative genomic hybridization. Biochem Cell Biol 2007;85:497–508.
20 Bejjani BA, Shaffer LG. Application of array-based comparative genomic hybridization to clinical diagnostics. J Mol Diagn 2006;8:528–533.
21 Schwaenen C, Nessling M, Wessendorf S, *et al.* Automated array-based genomic profiling in chronic lymphocytic leukemia: development of a clinical tool and discovery of recurrent genomic alterations. Proc Natl Acad Sci USA 2004;101:1039–1044.
22 Patel A, Kang SH, Lennon PA, *et al.* Validation of a targeted DNA microarray for the clinical evaluation of recurrent abnormalities in chronic lymphocytic leukemia. Am J Hematol 2008;83:540–546.
23 Gunn SR, Mohammed MS, Gorre ME, *et al.* Whole-genome scanning by array comparative genomic hybridization as a clinical tool for risk assessment in chronic lymphocytic leukemia. J Mol Diagn 2008;10:442–451.
24 Arriola E, Marchio C, Tan DS, *et al.* Genomic analysis of the HER2/TOP2A amplicon in breast cancer and breast cancer cell lines. Lab Invest 2008;88:491–503.
25 Florell SR, Coffin CM, Holden JA, *et al.* Preservation of RNA for functional genomic studies: a multidisciplinary tumor bank protocol. Mod Pathol 2001;14:116–128.
26 Robetorye RS, Gunn SR, Yeh I. A Tumor-Targeted DNA Extraction Method for Array CGH Analysis of HER2 Gene Copy Number Status in Newly Diagnosed Breast Cancer. United States and Canadian Academy of Pathology. Nature Publishing Group: Boston, MA, 2009.
27 Paik S, Bryant J, Tan-Chiu E, *et al.* Real-world performance of HER2 testing–National Surgical Adjuvant Breast and Bowel Project experience. J Natl Cancer Inst 2002;94:852–854.
28 Roche PC, Suman VJ, Jenkins RB, *et al.* Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. J Natl Cancer Inst 2002;94:855–857.