



## UvA-DARE (Digital Academic Repository)

### HER2 gene amplification in patients with breast cancer with equivocal IHC results

Meijer, S.L.; Wesseling, J.; Smit, V.T.; Nederlof, P.M.; Hooijer, G.K.J.; Ruijter, H.; Arends, J.W.; Kliffen, M.; van Gorp, J.M.; Sterk, L.; van de Vijver, M.J.

**DOI**

[10.1136/jclinpath-2011-200019](https://doi.org/10.1136/jclinpath-2011-200019)

**Publication date**

2011

**Document Version**

Final published version

**Published in**

Journal of clinical pathology

[Link to publication](#)

**Citation for published version (APA):**

Meijer, S. L., Wesseling, J., Smit, V. T., Nederlof, P. M., Hooijer, G. K. J., Ruijter, H., Arends, J. W., Kliffen, M., van Gorp, J. M., Sterk, L., & van de Vijver, M. J. (2011). HER2 gene amplification in patients with breast cancer with equivocal IHC results. *Journal of clinical pathology*, 64(12), 1069-1072. <https://doi.org/10.1136/jclinpath-2011-200019>

**General rights**

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

*UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)*

# HER2 gene amplification in patients with breast cancer with equivocal IHC results

Sybren L Meijer,<sup>1</sup> Jelle Wesseling,<sup>2,3</sup> Vincent T Smit,<sup>4</sup> Petra M Nederlof,<sup>2</sup> Gerrit K J Hooijer,<sup>1</sup> Henrique Ruijter,<sup>2</sup> Jan Willem Arends,<sup>5</sup> Mike Kliffen,<sup>6</sup> Joost M van Gorp,<sup>7</sup> Lotus Sterk,<sup>8</sup> Marc J van de Vijver<sup>1</sup>

<sup>1</sup>Departments of Pathology of the Academic Medical Center, Amsterdam, The Netherlands

<sup>2</sup>The Netherlands Cancer Institute, Amsterdam, The Netherlands

<sup>3</sup>University Medical Center, Groningen, The Netherlands

<sup>4</sup>Leiden University Medical Center, Leiden, The Netherlands

<sup>5</sup>Deventer Hospital, Deventer, The Netherlands

<sup>6</sup>Erasmus University Medical Center, Rotterdam, The Netherlands

<sup>7</sup>Diakonessenhuis Utrecht, Utrecht, The Netherlands

<sup>8</sup>Laboratory of Pathology, East Netherland, Enschede, The Netherlands

## Correspondence to

Marc J van de Vijver, Department of Pathology, Academic Medical Center Meibergdreef 9, Amsterdam 1105 AZ, Netherlands; [m.j.vandevijver@amc.uva.nl](mailto:m.j.vandevijver@amc.uva.nl)

Accepted 30 June 2011

Published Online First

11 August 2011

## ABSTRACT

**Aims** Equivocal human epidermal growth factor receptor 2 protein (HER2) (2+) immunohistochemistry (IHC) is subject to significant interobserver variation and poses a challenge in obtaining a definitive positive or negative test result. This equivocal test result group accounts for approximately 15% of all tumours, and for optimal guidance of HER2 targeted therapy, a further analysis of quantification of gene copy number and amplification status is needed for patients with early or metastatic breast cancer.

**Methods** 553 breast-cancer specimens with equivocal HER2 IHC(2+) test results were collected and subsequently centrally retested by chromogenic in situ hybridisation (CISH), and HER2 gene copy numbers per tumour cell nucleus were determined.

**Results** Using CISH, 77 of 553 equivocal HER2 IHC(2+) test result cases (13.9% of total) showed high levels of HER2 gene amplification ( $\geq 10.0$  gene copies per nucleus), and 41 of 553 (7.4% of total) showed low-level HER2 gene amplification (6.0–9.9 gene copies per nucleus). In 73.6% of cases, no amplification of the HER2 gene was shown, and in only 4.9% of cases was an equivocal test result by CISH observed (4.0–5.9 gene copies per nucleus).

**Conclusions** Testing by CISH of all equivocal HER2 IHC (2+) test result provides a definitive guidance in HER2 targeted therapy in 95.1% of cases. A significant proportion (21.3%) of patients with equivocal IHC(2+) test results show amplification of the HER2 gene.

## INTRODUCTION

Human epidermal growth factor receptor 2 protein (HER2) is a transmembrane receptor protein with tyrosine kinase activity, and signalling through HER2 activation promotes cell proliferation. HER2 is amplified and overexpressed in approximately 15 to 20% of breast carcinomas,<sup>1,2</sup> is associated with poor prognosis and is a predictor of response to chemotherapy and hormonal therapy.<sup>3–5</sup> By specifically targeting the extracellular domain of HER2 with a humanised monoclonal anti-HER2 antibody, trastuzumab (Herceptin; Genentech, South San Francisco, California) in combination with chemotherapy, progression-free and overall survival of patients with HER2-positive breast cancer is improved.<sup>6</sup> Because trastuzumab is only effective for patients with HER2 gene amplified and overexpressing breast carcinomas, reliable determination of the HER2 status is of great importance. HER2 status is most commonly determined by assessment of the amount of cell surface HER2

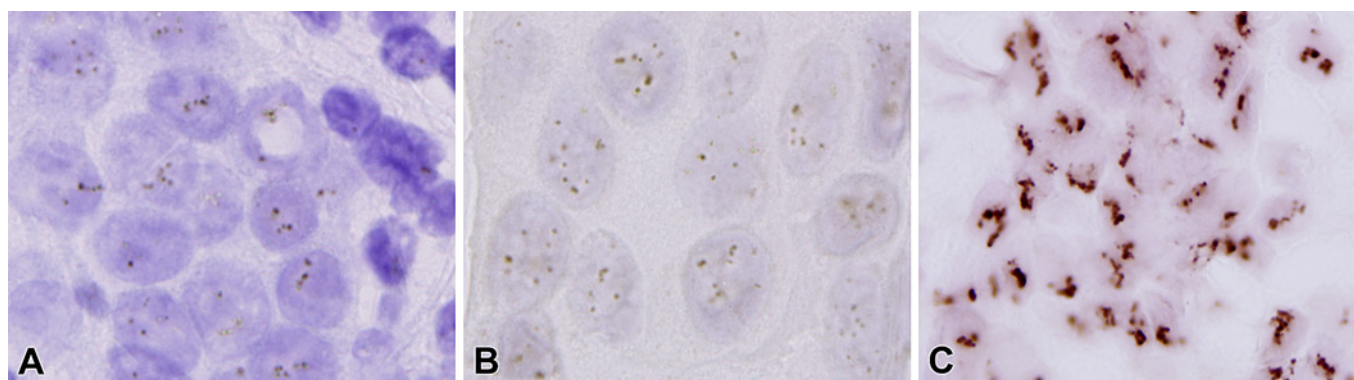
protein expression and/or quantification of the number of HER2 gene copies in the nucleus. Cell-surface HER2 protein expression is usually detected by immunohistochemistry (IHC) using monoclonal or polyclonal antibodies, and results are generally scored as 0, 1+, 2+ or 3+. Scoring criteria have evolved over time, are semiquantitative and comprise the percentage and quality of membrane staining of tumour cells. Tumours with HER2 staining scores of 0 and 1+ are categorised as HER2-negative, 2+ as equivocal and 3+ as HER2-positive. The number of copies of the HER2 gene can be quantified by in situ hybridisation (ISH) techniques using DNA probes, analysed by either chromogenic in situ hybridisation (CISH; or silver enhanced in situ hybridisation, SISH) or fluorescent (fluorescent in situ hybridisation, FISH) detection methods. Concordance between IHC analysis and CISH,<sup>7,8</sup> FISH and CISH,<sup>9–12</sup> and CISH, IHC analysis and FISH<sup>13,14</sup> has been demonstrated in many studies to be high (>85%). Several studies have indicated that HER2 testing in the clinical setting is not optimal, and it is estimated that up to 20% of tumours reported as HER2-positive are in fact HER2-negative.<sup>15,16</sup> Equivocal IHC (2+) results are an important part of the ongoing discussion on optimising methods to assess HER2 status and comprise about 15% of samples in routine practice.<sup>17,18</sup> All guidelines require additional testing by FISH or CISH in equivocal IHC (2+) cases. The exact determination of HER2-status in the equivocal group is further complicated by the frequent occurrence of low-level HER2 gene amplification and polysomy in this subgroup. Polysomy 17 indicates the presence of more than two copies of chromosome 17 and is reported in 13–46% of breast carcinomas.<sup>19–23</sup>

In view of the importance of obtaining optimal HER2 testing results, we selected samples of 553 patients with breast cancer with equivocal HER2 test results by immunohistochemical staining (IHC2+, defined as weak to moderate complete membrane staining in more than 10% of malignant cells, according to the FDA guidelines). Subsequently CISH was performed to analyse and determine the distribution of HER2 gene copy numbers and frequency of amplification in the immunohistochemistry equivocal cases.

## MATERIAL AND METHODS

### Patient selection

Tumours from 553 patients with invasive breast cancer with equivocal HER2 immunohistochemistry



**Figure 1** In situ hybridisation test results in equivocal immunohistochemistry showing (A) equivocal, (B) low amplification and (C) high amplification score according to the American Society of Clinical Oncology/College of American Pathologists guidelines.

results (IHC2+) were collected in 32 community-based hospitals and academic hospitals. The immunohistochemical tests were performed and analysed in the laboratory of origin of the specimen, and material was sent to and analysed by one of seven referral centres by CISH; the Departments of Pathology of the University Medical Centre Groningen (18 patients), Diakonessenhuis Utrecht (27 patients), Deventer Hospital (seven patients), Erasmus University Medical Centre (77 patients), Leiden University Medical Centre (175 patients), The Netherlands Cancer Institute (140 patients) and the laboratory of pathology East Netherland (109 patients).

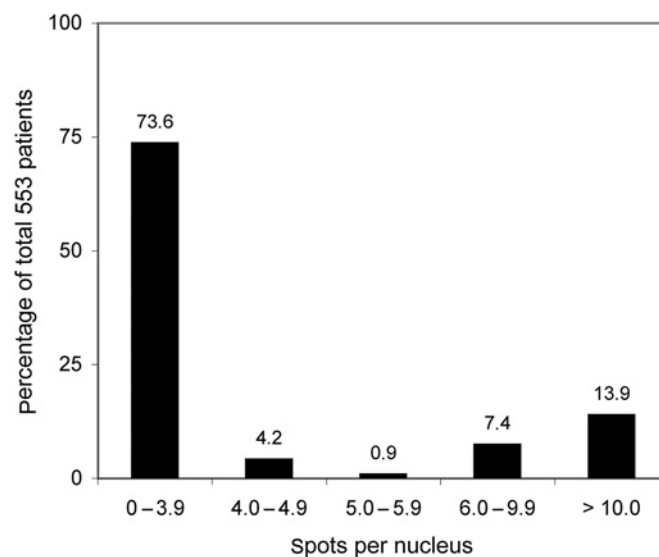
#### Assays and scoring of results

The HercepTest (DakoCytomation, Glostrup, Denmark) was used for IHC testing in most of the participating centres and performed exactly according to the manufacturer's instructions; where alternative assays were used, these had been rigorously evaluated using control cases. All HER2 immunoreactivity was scored from 0 to 3+ locally participating centres, exactly according to the criteria specified by DAKO for the interpretation of the Herceptest and approved by the US Food and Drug Administration. For CISH testing, the SpoT-LIGHT CISH polymer detection kit (Invitrogen, Carlsbad, California) was used and conducted according to the manufacturer's instructions. The scoring system was adapted from the protocol information from Invitrogen. The number of HER2 signals per nucleus was counted (at 400 $\times$  magnification) and categorised as non-amplified (<4.0 spots per nucleus), equivocal (4.0 to 5.9 spots per nucleus), low amplification (6.0 to 9.9 per nucleus) and high amplification ( $\geq$ 10.0 spots per nucleus or clusters of spots in each nucleus). At least 30 tumour cells from each specimen were counted. For dual-colour SISH testing, the INFORM HER2 DNA and chromosome 17 probes and ultraView SISH Detection Kit (Ventana Medical Systems, Tucson) was used and conducted according to the manufacturer's instructions. A single copy of the HER2 gene was visualised as a black dot and a single copy of chromosome 17 as a red dot. At least 30 tumour cells from each specimen were counted and the HER2/CEP17 ratio determined according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. Cases with a HER2/CEP17 ratio  $\leq$ 1.8 were considered negative for HER2 gene amplification, whereas those with a HER2/CEP17 ratio  $\geq$ 2.2 were considered positive for HER2 gene amplification.

#### RESULTS

Samples of a total of 553 breast cancer specimens with equivocal (2+) HER2 immunohistochemical test results were collected in

2005 and 2006, and subsequently retested by chromogenic in situ hybridisation (CISH) (figure 1). Using CISH, 77 cases (13.9% of total) showed high levels of HER2 gene amplification ( $\geq$ 10.0 gene copies per nucleus), 69 cases (12.5% of total) showed 4.0–9.9 of the HER2 gene, and 407 cases (73.6%) did not show HER2 gene amplification ( $\leq$ 3.9 gene copies per nucleus) (figure 2). Of the 69 patients with 4.0–9.9 HER2 gene copies, 23 cases (4.2% of total) showed HER2 gene copy numbers of 4.0 to 4.9 per nucleus, five cases (0.9% of total) showed HER2 gene copy numbers of 5.0 to 5.9 per nucleus, and 41 cases (7.4% of total) showed gene copy numbers of 6.0 to 9.9 per nucleus (figure 1). According to the ASCO/CAP guidelines, the interpretation of these results is that 28 (5.1% of total) showed an equivocal result (4.0–5.9 gene copies per nucleus), and 41 (7.4% of total) showed low-level HER2 gene amplification (6.0–9.9 gene copies per nucleus).



**Figure 2** Samples of 553 patients with breast cancer with equivocal human epidermal growth factor receptor 2 protein test results by immunohistochemistry, retested by chromogenic in situ hybridisation. Results were divided into five categories based on human epidermal growth factor receptor 2 protein gene copy numbers per nucleus: 0–3.9 spots per nucleus (no amplification), 4.0–4.9 spots per nucleus (equivocal test result), 5.0–5.9 spots per nucleus (equivocal test result), 6.0–9.9 spots per nucleus (low-level amplification) and  $\geq$ 10.0 spots per nucleus (high-level amplification). The numbers above the bar represent the percentage of the total of 553 patients.

In the analysis of this series of 553 cases, seven pathology laboratories participated, and each analysed between seven and 175 samples (table 1); the percentage of HER2 gene amplified cases ranged from 0 to 48.2%. In the four centres responsible for the majority of CISH retesting (between 77 and 175 specimens), HER2 gene amplification ( $\geq 6.0$  gene copies per nucleus) varied between 11.7% and 25.7%. High-level HER2 gene amplification ( $\geq 10.0$  gene copies per nucleus) varied between 5.7% and 16.6%, and low-level HER2 gene amplification (6.0–9.9 HER2 gene copies) varied between 2.6% and 12.1%.

## DISCUSSION

Reliable HER2 testing with results that can be categorised as positive or negative play an important role in guiding the treatment of patients with early or metastatic breast cancer. In a large proportion of hospitals worldwide, HER2 testing is performed using immunohistochemistry, and a 3+ score is considered HER2-positive; a 0 or 1+ score is considered HER2-negative. Equivocal HER2(2+) immunohistochemistry test results account for approximately 15% of all tumours and pose a challenge in obtaining a reliable test result. Here we have studied the CISH results for a series of 553 breast carcinomas tested as 2+ using immunohistochemistry, and 118 (21.3%) showed amplification of the HER2 gene ( $\geq 6.0$  gene copies per nucleus) by CISH. These numbers are in concordance with Chibon *et al*,<sup>24</sup> who analysed a selected group of 108 HER2 IHC (2+) invasive patients with breast cancer by FISH, and amplification of the HER2 gene was shown in 24% ( $>6$  gene copies per nucleus). Recently Lebeau *et al* determined the HER2 status of both 500 core needle biopsies and the corresponding surgical specimen by IHC and FISH. In the HER2 equivocal IHC group, a higher percentage of gene amplification (defined by a HER2/CEP17 ratio  $\geq 2.2$ ) was found in core needle biopsies (six of 45, 13.3%) as compared with the corresponding resection specimen (one of 26, 3.8%).<sup>25</sup> In an unpublished single institution series (Antoni van Leeuwenhoek Ziekenhuis/Nederlands Kanker Instituut (AVL/NKI), data not shown) of 128 core needle biopsies with an equivocal IHC test result (IHC2+), we found HER2 amplification in 20.1% when analysed by subsequent ISH. Low amplification was shown in 11.7% and high amplification in 7.4%. These numbers are similar to the outcome of this multicentre series and show no difference in HER2 status in CNB when compared with this study of resection specimens.

In situ hybridisation techniques are considered to be the gold standard for the assessment of HER2 status, as amplification can be quantified as an exact gene copy number. In this study, CISH analyses were performed in seven different pathology laboratories on tumours originated from their own practice and on tumours referred to them by other pathology laboratories (table 1). Of the 119 samples showing HER2 gene amplification by

CISH, 41 (7.4% of total) showed low levels of amplification (6.0–9.9 gene copies per nucleus), and 77 (13.9% of total) showed high levels of amplification ( $\geq 10.0$  gene copies per nucleus). Three laboratories performed only a limited number of tests (between seven and 27 tests); in the four centres responsible for the majority of CISH testing (between 77 and 175 specimens), HER2 gene amplification ( $\geq 6.0$  gene copies per nucleus) varied between 11.7% and 26.3%. High amplification varied between 5.7% and 16.6%, and low amplification varied between 2.6% and 12.1%. This variation in the amplification rate between centres could be due to the performance of the in situ hybridisation techniques and/or the interpretation of the selection criteria of an equivocal HER2 IHC(2+) score, and both techniques could further be complicated by polysomy. We analysed all four equivocal and 13 of the 16 low amplification tumour samples (data not shown) from one of the participating study centres (LUMC) by dual-colour SISH. Polysomy, defined as chromosome 17 copy number  $\geq 3$ , was observed in six of these tumour specimens (35%). When the HER2/CEP17 ratio was calculated according to the ASCO/CAP guidelines, a discrepancy in amplification status according to HER2 gene copy numbers per nucleus and the HER2/CEP17 ratio was observed in four of these 17 tested samples (24%). Of the three tumours with HER2 gene copy numbers of between 6.0 and 7.0 per nucleus (low amplification), two showed a HER2/CEP17 ratio of 2.0, rendering an equivocal test result defined by both dual color ISH and the initial IHC test result. In these isolated cases evaluation of more tumor cells and possible of more tumour blocks is indicated in order to come to a test result with a definite conclusion, if possible, about the true HER2 amplification status. According to the FDA guidelines, these tumours would still be rendered as HER2-amplified. The third tumour with between 6.0 and 7.0 HER2 copy numbers showed 4.5 copies of CEP17 per nucleus, resulting in a HER2/CEP17 ratio of 1.4. This patient, according to the ASCO/CAP guidelines of absolute HER2 gene copy numbers of  $\geq 6.0$ , would be eligible for anti-HER2 therapy, but based on the HER2/CEP17 ratio, no treatment would be indicated. The fourth discrepancy was observed in a tumour with 4.6 HER2 gene copies (equivocal test result) and eusomic numbers of chromosome 17 resulting in a HER2/CEP17 ratio of 2.3. Solely on the basis of the HER2/CEP17 ratio, this tumour could be wrongfully rendered HER2-positive. In some cases of true HER2 amplification (HER2 gene copy number  $\geq 6$ ), the centromere of chromosome 17 is also located within the amplicon, and when HER2 status is determined on the basis of the HER2/CEP17 ratio this may lead to false-negative HER2 status assessment. This all indicates that in the assessment of HER2 status, not only a ratio but also absolute HER2 gene copy numbers should be analysed in order to minimise false-positive and -negative test results. The most important issue is how HER2 test results predict response to HER2-directed therapy.

**Table 1** Subdivision of the chromogenic in situ hybridisation results by spots per nucleus according to the pathology laboratory that performed the chromogenic in situ hybridisation testing

Spots per nucleus	Deventer	University Medical Center, Groningen	Diakonessenhuis Utrecht	Erasmus	Enschede	AVL/NKI	Leiden University Medical Center	Total
0–3.9	7 (100)	12 (66.7)	10 (37.0)	64 (83.1)	78 (71.3)	110 (78.6)	126 (72.0)	407 (73.6)
4.0–4.9	0	0	0	4 (5.2)	11 (10.1)	5 (3.6)	3 (1.7)	23 (4.2)
5.0–5.9	0	0	1 (3.7)	0	3 (2.8)	0	1 (0.6)	5 (0.9)
6.0–9.9	0	0	3 (11.1)	2 (2.6)	3 (2.8)	17 (12.1)	16 (9.1)	41 (7.4)
$>10.0$	0	6 (33.3)	13 (48.2)	7 (9.1)	14 (12.8)	8 (5.7)	29 (16.6)	77 (13.9)
	7	18	27	77	109	140	175	553

Numbers in parentheses represent the percentage of total of cases per participating hospital.

## Take-home message

For breast carcinomas with immunohistochemical HER2 2+ staining, HER2 CISH identifies a low level of amplification in 7.4% and a high level of amplification in 13.9% of patients.

In our study of 553 patients, 146 cases (26.4%) show >4.0 HER2 gene copy numbers, 123 cases (22.2%) show >5.0 HER2 gene copy numbers, and 118 cases (21.3%) show >6.0 HER2 gene copy numbers. In the complicated pathobiology of HER2 amplified tumours, it is difficult to indicate the lower threshold of HER2 gene copy number that can make a therapeutic difference. Furthermore, it is not clear whether low (6.0–9.9 gene copies) or high amplification ( $\geq 10.0$  and more copies) is associated with similar response to anti-HER2 directed therapy. Dowsett *et al* studied the degree of HER2 amplification and disease-free survival in patients treated with adjuvant chemotherapy with or without 1 year of anti-HER2 therapy.<sup>26</sup> They show similar prognosis or benefit from adjuvant anti-HER2 therapy in subgroups of patients with either low ( $4 \leq$  HER2 gene copy number  $\leq 9$ ) or high ( $>9$  HER2 gene copy number) HER2 gene copy numbers. These included IHC(2+) patients with HER2 amplification by FISH (ratio  $\geq 2.0$ ). In this study, a trend was shown only towards increasing effectiveness of trastuzumab with increasing FISH ratio, but this was not statistically significant. HER2 targeted therapy in combination with chemotherapy improves disease-free survival for patients with HER2-positive breast cancer, and reliable HER2 testing is crucial in guiding this treatment.<sup>27 28</sup> The equivocal HER2 IHC(2+) result is responsible for high levels of interobserver variability. Our study contributes to the HER2 testing algorithm showing HER2 gene amplification status in a large series of HER2 2+ staining tumours (n=553) and definitive guidance of therapy after reflex testing by CISH in 95% of patients.

**Funding** The study was supported by an unrestricted educational grant from F Hoffmann-La Roche AG.

**Competing interests** MvdV is a member of the F Hoffmann-La Roche AG Herceptin Pathology Advisory Board and has received honoraria for attendance at these meetings.

**Contributors** MJvdV designed data and collection tools and is guarantor; JW, VTS, JWA, MK, JvG, LS and MJvdV monitored and analysed data collection in participating hospitals; GHJH and HR were responsible for performing part of the experiments; JW, VTS, PMN, GHJH, HR, JWA, MK, JvG, LS and MJvdV revised the draft paper; SLM analysed the data and drafted and revised the paper.

**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

- Hynes NE, Stern DF. The biology of erbB-2/neu/HER-2 and its role in cancer. *Biochim Biophys Acta* 1994;**1198**:165–84.
- Owens MA, Horten BC, Da Silva MM. HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. *Clin Breast Cancer* 2004;**5**:63–9.
- Press MF, Pike MC, Chazin VR, *et al*. Her-2/neu expression in node-negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. *Cancer Res* 1993;**53**:4960–70.
- Press MF, Bernstein L, Thomas PA, *et al*. HER-2/neu gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. *J Clin Oncol* 1997;**15**:2894–904.
- Yamauchi H, Stearns V, Hayes DF. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. *J Clin Oncol* 2001;**19**:2334–56.
- Slamon DJ, Leyland-Jones B, Shak S, *et al*. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;**344**:783–92.
- Kounelis S, Kapranos N, Malamos N, *et al*. Evaluation of HER2 gene status in breast cancer by chromogenic in situ hybridization: comparison with immunohistochemistry. *Anticancer Res* 2005;**25**:939–46.
- Bilous M, Dowsett M, Hanna W, *et al*. Current perspectives on HER2 testing: a review of national testing guidelines. *Mod Pathol* 2003;**16**:173–82.
- Arnould L, Denoux Y, MacGrogan G, *et al*. Agreement between chromogenic in situ hybridisation (CISH) and FISH in the determination of HER2 status in breast cancer. *Br J Cancer* 2003;**88**:1587–91.
- Gupta D, Middleton LP, Whitaker MJ, *et al*. Comparison of fluorescence and chromogenic in situ hybridization for detection of HER-2/neu oncogene in breast cancer. *Am J Clin Pathol* 2003;**119**:381–7.
- Isola J, Tanner M, Forsyth A, *et al*. Interlaboratory comparison of HER-2 oncogene amplification as detected by chromogenic and fluorescence in situ hybridization. *Clin Cancer Res* 2004;**10**:4793–8.
- Gong Y, Gilcrease M, Sneige N. Reliability of chromogenic in situ hybridization for detecting HER-2 gene status in breast cancer: comparison with fluorescence in situ hybridization and assessment of interobserver reproducibility. *Mod Pathol* 2005;**18**:1015–21.
- Hanna WM, Kwok K. Chromogenic in-situ hybridization: a viable alternative to fluorescence in-situ hybridization in the HER2 testing algorithm. *Mod Pathol* 2006;**19**:481–7.
- 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.
- 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**:855–7.
- 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**:852–4.
- Sawaki M, Ito Y, Akiyama F, *et al*. High prevalence of HER-2/neu and p53 overexpression in inflammatory breast cancer. *Breast Cancer* 2006;**13**:172–8.
- Dowsett M, Bartlett J, Ellis IO, *et al*. Correlation between immunohistochemistry (HerceptTest) and fluorescence in situ hybridization (FISH) for HER-2 in 426 breast carcinomas from 37 centres. *J Pathol* 2003;**199**:418–23.
- Hyun CL, Lee HE, Kim KS, *et al*. The effect of chromosome 17 polysomy on HER-2/neu status in breast cancer. *J Clin Pathol* 2008;**61**:317–21.
- Merola R, Mottolese M, Orlandi G, *et al*. Analysis of aneusomy level and HER-2 gene copy number and their effect on amplification rate in breast cancer specimens read as 2+ in immunohistochemical analysis. *Eur J Cancer* 2006;**42**:1501–6.
- 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**:R267–73.
- Torrisi R, Rotmensz N, Bagnardi V, *et al*. HER2 status in early breast cancer: relevance of cell staining patterns, gene amplification and polysomy 17. *Eur J Cancer* 2007;**43**:2339–44.
- Ma Y, Lespagnard L, Durbecq V, *et al*. Polysomy 17 in HER-2/neu status elaboration in breast cancer: effect on daily practice. *Clin Cancer Res* 2005;**11**:4393–9.
- Chibon F, de Mascarel I, Sierankowski G, *et al*. Prediction of HER2 gene status in Her2 2+ invasive breast cancer: a study of 108 cases comparing ASCO/CAP and FDA recommendations. *Mod Pathol* 2009;**22**:403–9.
- Lebeau A, Turzynski A, Braun S, *et al*. Reliability of human epidermal growth factor receptor 2 immunohistochemistry in breast core needle biopsies. *J Clin Oncol* 2010;**28**:3264–70.
- Dowsett M, Procter M, McCaskill-Stevens W, *et al*. Disease-free survival according to degree of HER2 amplification for patients treated with adjuvant chemotherapy with or without 1 year of trastuzumab: the HERA Trial. *J Clin Oncol* 2009;**27**:2962–9.
- Piccari-Gebhart MJ, Procter M, Leyland-Jones B, *et al*. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;**353**:1659–72.
- Paik S, Kim C, Wolmark N. HER2 status and benefit from adjuvant trastuzumab in breast cancer. *N Engl J Med* 2008;**358**:1409–11.



## HER2 gene amplification in patients with breast cancer with equivocal IHC results

Sybren L Meijer, Jelle Wesseling, Vincent T Smit, et al.

*J Clin Pathol* 2011 64: 1069-1072 originally published online August 11, 2011

doi: 10.1136/jclinpath-2011-200019

---

Updated information and services can be found at:

<http://jcp.bmj.com/content/64/12/1069.full.html>

---

*These include:*

### References

This article cites 28 articles, 13 of which can be accessed free at:  
<http://jcp.bmj.com/content/64/12/1069.full.html#ref-list-1>

### Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

---

### Topic Collections

Articles on similar topics can be found in the following collections

[Molecular genetics](#) (273 articles)  
[Breast cancer](#) (407 articles)

---

### Notes

---

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>