PERGAMON

European Journal of Cancer 37 (2001) S11–S17

European Journal of Cancer

www.ejconline.com

Assessment of the need and appropriate method for testing for the human epidermal growth factor receptor-2 (HER2)

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Received 3 July 2000; received in revised form 7 September 2000; accepted 11 September 2000

Abstract

The human epidermal growth factor receptor-2 (*HER2*) gene (also known as c-*erb*B-2 or *neu*) is amplified in 20–30% of breast cancers. *HER2* gene amplification and HER2 overexpression occur early in the development of breast cancers and are found in a high proportion of ductal carcinomas *in situ* (DCIS), non-invasive cancers that generally do not give rise to metastases. In DCIS, HER2 overexpression is found specifically in poorly histologically differentiated disease and not in well differentiated cancers. Various methods have been used to analyse the HER2 status of a tumour. These either measure the degree of *HER2* gene amplification, receptor overexpression or the amount of circulating HER2 protein. In practice, immunohistochemistry is the most frequently used method, being available as a standard technique in all pathology laboratories. It is of critical importance to standardise the methods used for staining and to apply common interpretation criteria to enable direct comparison of results between laboratories.

Keywords: Breast cancer; HER2; *erb*B-2; Trastuzumab

1. Introduction

The human epidermal growth factor receptor-2 (HER2) is one of a family of four closely related transmembrane growth factor receptors, designated HER1 to HER4 (c-*erb*B-1 to c-*erb*B-4), respectively, which exhibit a high degree of homology to each other [1–3]. Transmembrane HER molecules exist as inactive monomers on the cell surface, but form receptor dimers that are stabilised by ligand binding to their extracellular domain: dimerisation can occur between the same receptor (a homodimer) or between different members of the family (a heterodimer) [4,5].

The interaction between the HER monomers and various ligands (e.g. epidermal growth factor (EGF), transforming growth factor α, amphiregulin, heparin-binding EGF, betacellulin, epiregulin, heregulins), and the ensuing diversity of signal transduction from the intracellular tyrosine kinase domain, gives rise to a complexity which explains the key role played by this type 1 growth factor receptor family in regulating cell growth, survival and differentiation. It may be that the function of HER2 is to stimulate growth after the formation of heterodimers with other members of the HER family.

HER2 gene amplification results in the overexpression of *HER2* mRNA and HER2 receptor protein. *HER2* gene amplification and/or protein overexpression is found in a high percentage of some tumour types [2], including approximately 20–30% of human breast carcinomas [6,7]. In breast cancers with normal *HER2* gene copy numbers, expression of HER2 protein may be variable, but is usually expressed at, low levels (in the range of tens of thousands of monomers) and is only very rarely as highly expressed as in tumours with *HER2*-gene amplification (usually 10- to 100-fold higher and equivalent to millions of monomers) [8]. Various animal and *in vitro* studies strongly indicate that this genotypic change of *HER2* amplification plays a pivotal role in oncogenic transformation, tumorigenesis and metastasis [9–11]. This is further supported by the finding that the growth of HER2-positive human breast cancer cell lines and tumour xenografts is inhibited by anti-HER2 monoclonal antibodies [12]. This has opened

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0959-8049/01/$ - see front matter
PII: S0959-8049(00)00403-2

a new approach to HER2-targeted monoclonal antibody therapy of breast cancer with trastuzumab [13].

2. The need for HER2 testing

Numerous studies have investigated the relationship between HER2 status and clinicopathological characteristics in breast cancer [14]. Most of these studies to date have focused on the role of *HER2* protein expression, while only a limited number of studies have investigated the association between HER2 gene amplification and clinicopathological parameters. To exemplify the need for testing HER2 status in breast cancer, it is appropriate to expand on some personal investigations and describe some important examples from the literature. The main examples I wish to cover show that a HER2-positive status: (1) occurs frequently in ductal carcinoma *in situ* (DCIS), especially DCIS of a poorly-differentiated type; (2) is associated with invasive breast carcinomas of a poorly-differentiated type, and/or are oestrogen-receptor (ER)-negative, and have a poor prognosis, especially in node-positive disease; (3) may be predictive of tumour sensitivity to hormonal therapy and chemotherapy.

2.1. DCIS

Our group has developed an immunohistochemical (IHC) assay of HER2 status utilising the 3B5 monoclonal antibody, which detects an intracellular epitope of the HER2 protein [15]. We have found a high degree of correlation between a positive HER2 status using this IHC test and *HER2* gene amplification. For example, of 90 breast carcinoma samples with normal *HER2* gene copy numbers, only four samples (4%) showed strong membrane staining for HER2 receptor overexpression using this IHC test. Of 25 samples that demonstrated *HER2* gene amplification, only two (8%) showed a negative result for HER2 receptor overexpression using the 3B5 monoclonal antibody IHC test.

We have used the 3B5 IHC test to determine the HER2 status of 113 DCIS samples and to relate this to the clinicopathological characteristics of DCIS. The results of this analysis are shown in Table 1. As can be seen, a positive HER2 status was significantly associated with poorly-differentiated DCIS, as indicated by the higher nuclear grade and mitotic activity index, and with a comedo or solid growth pattern as opposed to cribiform, papillary or clinging patterns. A similar association between HER2-protein overexpression with high nuclear grade, high proliferation and the comedo subtype has been noted in a large number of other studies in DCIS [16–23]. Poorly-differentiated DCIS nearly always overexpresses HER2 and is probably indicative of tumours with greater invasive potential [15,24]. For optimal treatment planning in DCIS, it would be of great benefit to stratify patients according to their risk of local recurrence and progression to invasive breast cancer, and assessment of HER2 status may be of benefit in achieving this.

Table 1
HER2 status determined by IHC using the 3B5 monoclonal antibody in relation to histological characteristics of 113 DCIS samples

Characteristic	Membrane staining (*n* (%) of patients)		*P* value
	Absent	Present	
Dominant growth pattern			
Comedo	1 (4)	24 (96)	
Solid	14 (36)	25 (64)	<0.001
Cribiform	22 (100)	0	
Micro(papillary)	18 (86)	3 (14)	
Clinging	5 (83)	1 (17)	
Nuclear grade			
I	32 (100)	0	<0.001
II	25 (74)	9 (26)	
III	3 (6)	44 (94)	
MAI (mitoses/400 cells)[a]			
<2	23 (92)	2 (8)	
2–3	27 (77)	8 (23)	<0.001
4–6	5 (33)	10 (67)	
>6	3 (11)	24 (89)	

DCIS, ductal carcinoma *in situ*; MAI, mitotic activity index.
[a] MAI status was unknown for 11 samples.

2.2. Invasive breast carcinoma

We have also used the 3B5 IHC test to determine the HER2 status of 256 invasive breast cancers and have related this to clinicopathological features of the disease. The results of this analysis are shown in Table 2.

Table 2
HER2 status determined by IHC using the 3B5 monoclonal antibody in relation to clinicopathological characteristics of 256 invasive breast cancer samples[a]

Characteristic	Membrane staining (*n* (%) of patients)		*P* value
	Absent	Present	
Histological grade			
I	22 (100)	0	<0.001
II	88 (94)	6 (6)	
III	110 (79)	30 (21)	
ER status			
Positive	138 (91)	13 (9)	<0.001
Negative	43 (68)	20 (32)	

ER, oestrogen receptor.
[a] For 214 cases, ER status (determined using biochemical ligand binding assay) was available.

As for DCIS, a HER2-positive status in stage I/II invasive breast cancer was significantly associated with grade III (poorly-differentiated) histology ($P < 0.001$). Similar results have been seen in other studies [25,26]. A HER2-positive status was also significantly ($P < 0.001$) associated with a negative ER status, which is also corroborated by other studies [7,26].

It is now generally well accepted that HER2 overexpression is indicative of a poor prognosis in invasive breast cancer, particularly in node-positive disease. Since the key study by Slamon and colleagues in 1987 [7], which showed that HER2 positivity independently predicted overall and disease-free survival in a multivariate analysis, most other large studies have confirmed HER2 status as an independent predictor of prognosis in node-positive patients [6,27–32]. Correlation between HER2 status and prognosis in node-negative patients has yielded conflicting results, with some studies showing a positive correlation when considering all patients or subgroups selected according to other clinicohistological factors [28,31,33–36], but others showing no correlation [30,37,38]. The latter negative results are probably explained by the low numbers of patients evaluated, low event rate and/or inadequate duration of follow-up, which limits the power to demonstrate significance. In node-negative breast cancer patients, HER2 positivity is probably associated with poor prognosis. This association is not entirely independent of poorly-differentiated histological type and not sufficiently large enough to be the sole basis for patient selection for adjuvant systemic treatment.

2.3. *Prediction of treatment response*

One of the most practical applications of determination of HER2 status in breast cancer, which strongly supports the need for routine testing, would be the ability to predict response to therapy and therefore guide treatment decision-making. There is a growing body of evidence to support this, although most data are from retrospective studies. HER2-positive tumours may be less responsive to hormonal therapy: some studies have indicated a resistance to [39–42], or even worse outcome on [43], hormonal therapy, mainly tamoxifen, but other studies have indicated no significant association or even a trend towards and association between HER2 status and response to hormonal therapy [44,45]. It may be that some studies failed to rigorously exclude ER-negative patients, who are unlikely to respond to hormonal therapy and more likely to be HER2 positive.

Some studies have also indicated a reduced benefit from CMF (cyclophosphamide, methotrexate 5-fluorouracil (5-FU) therapy in HER2-positive compared with HER2-negative patients [27,33,39,46,47], although no such association has been found in recent studies [48,49]. There is increasing evidence to suggest that HER2-positive patients exhibit an increased response to optimal versus suboptimal anthracycline dosage when receiving anthracycline-based chemotherapy, while this differential response is not seen in HER2-negative patients [50–53]. Anti-HER2 monoclonal antibody therapy with trastuzumab is licensed for the treatment of metastatic breast cancer in the USA and this is one situation where there is an absolute requirement for a HER2-positive result using an IHC test before appropriate implementation of therapy.

3. Methods available for the detection of *HER2* status

A wide range of assay methods have been used to analyse tumour HER2 status. They can be broadly classified according to the target molecule detected by the assay: DNA, mRNA or protein (Fig. 1). Protein can be measured as intact HER2 receptor on the cell membrane or extracellular domain (ECD^{HER2}) shed from the cell surface. Most tests measure either protein or DNA; mRNA has been rarely used as a target detection molecule, although a reverse transcription-polymerase chain reaction (RT-PCR) method has been recently described [54].

3.1. HER2 *gene amplification*

Various techniques have been used for the detection of *HER2* gene amplification, including Southern blot analysis, fluorescence *in situ* hybridisation (FISH) or bright field *in situ* hybridisation (BRISH) on isolated nuclei or on tissue sections, and polymerase chain reaction (PCR).

There are some drawbacks associated with each of these techniques. Southern blot analysis requires a large amount of high quality DNA, is very labour-intensive and time-consuming, and the results are unreliable if the percentage of tumour cells is low in a sample [14]. These factors therefore preclude the application of Southern blot analysis for routine clinical application in the determination of HER2 status. PCR has a number of advantages: it can be performed using small amounts of tumour cells, DNA from formalin-fixed, paraffin-embedded tumours can be used, and it is easy to automate and standardise the assay. However, the major limitation to its practical clinical application is that it is not optimally suited for carrying out quantitative analysis, although quantitative PCR techniques are currently being assessed for their clinical utility in *HER2* DNA testing [55,56].

FISH is a widely used assay and utilises the visualisation of *HER2* DNA in individual cells using a specific fluorescence-labelled probe. The detection of gene amplification in individual tumour cells is one of its

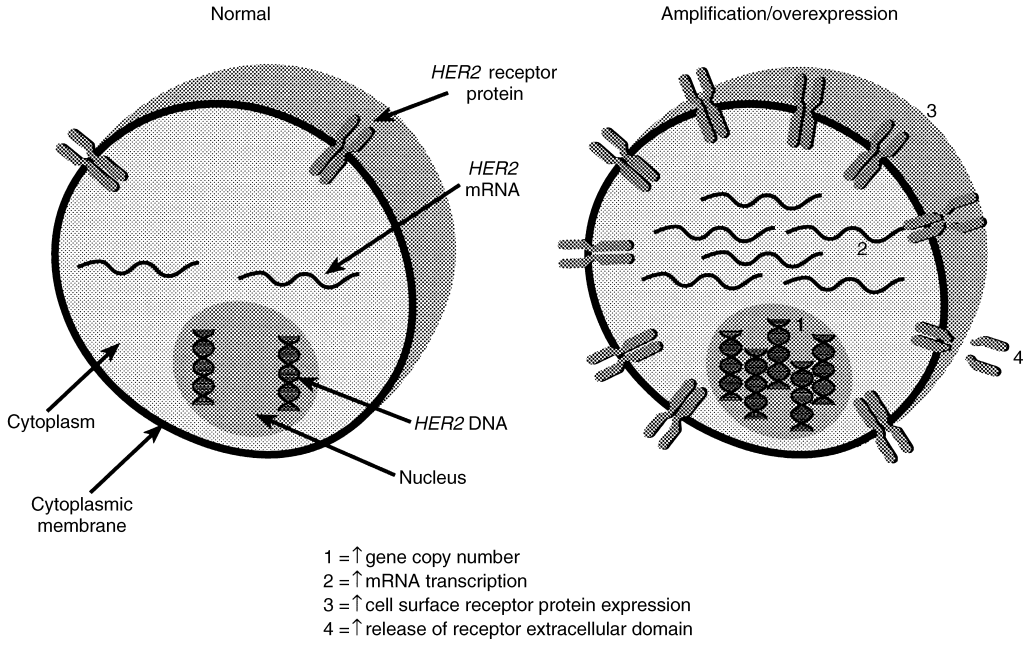

Fig. 1. Indicators of HER2 status: DNA or gene amplification, and mRNA or protein overexpression.

great advantages. However, results may differ considerably if methods are not standardised.

3.2. HER2 protein overexpression

Various techniques have been used for the detection of HER2 protein overexpression, including western blot analysis, enzyme-linked immunosorption assay (ELISA), and IHC.

Western blot analysis has generally been restricted to research rather than routine clinical analysis of HER2 status, since it requires the use of fresh tumour homogenates. The use of tumour homogenates results in large variations depending on the amount of stromal and other non-tumour cells present, i.e. a potential dilution effect [14].

ELISA can be used to measure HER2 protein in tissue homogenates or ECD^{HER2} in serum. It is a relatively simple technique and is well suited to automation. If tumour cytosolic fractions are used, it has the disadvantage that homogenisation of the tumour sample results in the loss of histological information and also in a dilution effect. ELISA measurement in serum has the potential advantage of being able to rapidly determine serial changes in circulating HER2 antigen in response to physiological or therapeutic changes. However, circulating ECD^{HER2} does not necessarily correlate to tumour load and is probably only released into the circulation by certain HER2-positive tumours [57,58], a process regulated by specific metalloproteases [59]. Thus, the ELISA assay of serum cannot be used for the routine determination of HER2 status.

In practice, IHC staining is the most frequently used method for the assessment of HER2 status, being available as a standard technique in all pathology laboratories. IHC allows the identification of HER2 protein overexpression in individual tumour cells. However, results can be affected by a number of variables, including the antibody used, the detection technique used and the use of frozen sections or paraffin-embedded tissue. In the latter case, the use of paraffin-embedded tissues can be influenced by the time between surgical removal of the tumour and fixation, the fixative used and whether or not pre-treatment of the section is performed prior to IHC staining ('antigen retrieval'). It also relies on subjective interpretation of staining results, which can be highly variable. An example of the variability of results can be seen from the dependence of HER2 positivity, ranging from 2 to 30%, on the particular antibody used (Table 3) [60].

Table 3
Incidence of breast tumours determined to be HER2-positive dependent on the particular antibody used in IHC testing[a]

Antibody	HER2 positivity (%)
9C2	30
TAB250	26
4D5	21
CB11	19
3B5	20
TA-1	9
PAB	2

HER2, human epidermal growth factor receptor-2.
[a] Adapted with permission from Press and colleagues [60].

Table 4
Scores for HER2 overexpression using the DAKO HercepTest® kit range from 0 to 3+ [a]

Score	HER2 overexpression assessment	Staining pattern
0	Negative	No staining is observed or membrane staining is observed in < 10% of tumour cells
1+	Negative	A faint/barely perceptible membrane staining is detected in > 10% of tumour cells; the cells are only stained in part of their membrane
2+	Weak positive	A weak to moderate complete membrane staining is observed in > 10% of tumour cells
3+	Strong positive	A strong complete membrane staining is observed in > 10% of tumour cells

HER2, human epidermal growth factor receptor-2.
[a] Reproduced with permission from DAKO Corporation.

One way to standardise IHC results is to consistently use a single commercial test kit and this has been achieved with the HercepTest® (DAKO). This method has been approved by the US Food and Drug Administration (FDA) for routine selection of metastatic breast cancer patients with HER2-positive tumours who are appropriate candidates for treatment with trastuzumab. The assay arbitrarily classifies the degree of membrane staining using a four-point scale (Table 4). It provides a standardised procedure and inclusion of a set of controls against which scoring can be judged. There has been some discussion about the sensitivity and interlaboratory standardisation of results [61–64], and it has to be determined in the future whether this test kit is the most appropriate assay to determine HER2 status.

4. Conclusion

The assessment of HER2 status in breast carcinomas is of great importance for clinical decision-making since it provides valuable prognostic and predictive information, particularly when used alongside routine breast cancer markers. More studies are required to strengthen the predictive ability of HER2 status determination in guiding therapeutic decision making. IHC is the cornerstone of assessing HER2 status in breast cancer. However, it is of critical importance to standardise the methods used for staining and to apply common interpretation criteria to enable direct comparison of results between laboratories. Other methods of determining HER2 status, such as FISH and maybe PCR, can be used in conjunction with IHC, possibly as a back-up test in patients in whom IHC results are believed to be equivocal. With further study, such tests may prove more suitable in the future. Assessment of HER2 status is essential for the selection of patients who are likely candidates for specific anti-HER2 therapy, e.g. trastuzmab.

References

1. Coussens L, Yang-Feng TL, Liao Y-C, *et al*. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science* 1985, **230**, 1132–1139.
2. Hynes NE, Stern DF. The biology of *erb*B-2/*neu*/*HER-2* and its role in cancer. *Biochem Biophys Acta* 1994, **1198**, 165–184.
3. Schechter AL, Stern DF, Vaidyanathan L, *et al*. The *neu* oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. *Nature* 1984, **213**, 513–516.
4. Alroy I, Yarden Y. The ErbB signaling network in embryogenesis and oncogenesis: signal diversification through combinatorial ligand-receptor interactions. *FEBS Lett* 1997, **410**, 83–86.
5. Lemmon MA, Schlessinger J. Regulation of signal transduction and signal diversity by receptor oligomerization. *Trends Biochem Sci* 1994, **19**, 459–463.
6. Slamon D, Godolphin W, Jones LA, *et al*. Studies of the HER-2/neu protooncogene in human breast and ovarian cancer. *Science* 1989, **244**, 707–712.
7. Slamon DJ, Clark GM, Wong SG, *et al*. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science* 1987, **235**, 177–182.
8. Venter DJ, Tuzi NL, Kumar S, Gullick WJ. Overexpression of the c-*erb*B-2 oncoprotein in human breast carcinomas: immunohistological assessment correlates with gene amplification. *Lancet* 1987, **ii**, 69–72.
9. Benz CC, Scott GK, Sarup JC, *et al*. Estrogen-dependent, tamoxifen-resistant tumorigenic growth of MCF-7 cells transfected with HER2/*neu*. *Breast Cancer Res Treat* 1992, **24**, 85–95.
10. Chazin VR, Kaleko M, Miller AD, Slamon DJ. Transformation mediated by the human HER-2 gene independent of epidermal growth factor receptor. *Oncogene* 1992, **7**, 1859–1866.
11. Hudziak RM, Schlessinger J, Ullrich A. Increased expression of the putative growth factor receptor $p185^{HER2}$ causes transformation and tumorigenesis of NIH 3T3 cells. *Proc Natl Acad Sci USA* 1987, **84**, 7159–7163.
12. Harwerth IM, Wels W, Schlegel J, *et al*. Monoclonal antibodies directed to the *erb*B-2 receptor inhibit *in vivo* tumour cell growth. *Br J Cancer* 1993, **68**, 1140–1145.
13. Baselga J, Tripathy D, Mendelsohn J, *et al*. Phase II study of weekly intravenous recombinant humanized anti-$p185^{HER2}$ monoclonal antibody in patients with HER2/*neu*-overexpressing metastatic breast cancer. *J Clin Oncol* 1996, **14**, 737–744.
14. 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.
15. van de Vijver MJ, Peterse JL, Mooi WJ, *et al*. Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma and limited prognostic value in stage II breast cancer. *N Engl J Med* 1988, **319**, 1239–1245.
16. Albonico G, Querzoli P, Ferretti S, *et al*. Biophenotypes of breast carcinoma in-situ defined by image analysis of biological parameters. *Pathol Res Pract* 1996, **192**, 117–123.
17. Bobrow LG, Happerfield LC, Gregory WM, Millis RR. Ductal carcinoma in-situ: assessment of necrosis in nuclear morphology

and their association with biological markers. *J Pathol* 1995, **176**, 333–341.
18. Bose S, Lesser ML, Norton L, Rosen PP. Immunophenotype of intraductal carcinoma. *Arch Pathol Lab Med* 1996, **120**, 81–85.
19. Brower ST, Ahmed S, Tartter PI, *et al*. Prognostic variables in invasive breast cancer: contribution of comedo versus non-comedo in situ component. *Ann Surg Oncol* 1995, **2**, 440–444.
20. Inaji H, Koyama H, Motomura K, Noguchi S. Differential distribution of erbB-2 and pS2 proteins in ductal carcinoma in-situ of the breast. *Breast Cancer Res Treat* 1996, **37**, 89–92.
21. Lodato RF, Maguire Jr HC, Greene MI, *et al*. Immunohistochemical evaluation of c-erb-B2 oncogene expression in ductal carcinoma *in situ* an atypical ductal hyperplasia of the breast. *Mod Pathol* 1990, **3**, 449–454.
22. Moreno A, Lloveras B, Figueras A, *et al*. Ductal carcinoma in-situ of the breast: correlation between histologic classification and biologic markers. *Mod Pathol* 1997, **10**, 1088–1092.
23. Zafrani B, Leroyer A, Fourquet A, *et al*. Mammographically-detected ductal in-situ carcinoma of the breast analyzed with a new classification. A study of 127 cases: correlation with estrogen and progesterone receptors, p53 and c-erbB-2 proteins, and proliferative activity. *Semin Diagn Pathol* 1994, **11**, 208–214.
24. Barnes DM, Bartkova J, Camplejohn RS, *et al*. Overexpression of the c-*erb*B-2 oncogene: why does this occur more frequently in ductal carcinoma in situ than in invasive mammary carcinoma and is this of prognostic significance. *Eur J Cancer* 1992, **28A**, 644–648.
25. Berger MS, Locher GW, Saurer S, *et al*. Correlation of c-*erb*B2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. *Cancer Res* 1988, **48**, 1238–1243.
26. Heintz NH, Peterse JL, Mooi WJ, *et al*. Amplification of the c-erb B-2 oncogene in prognosis of breast adenocarcinoma. *Arch Pathol Lab Med* 1990, **114**, 160–163.
27. Gusterson BA, Gelber RD, Goldhirsch KN, *et al*. Prognostic importance of c-*erb*B-2 expression in breast cancer. *J Clin Oncol* 1992, **10**, 1049–1056.
28. McCann AH, Dervan PA, O'Regan M, *et al*. Prognostic significance of c-*erb*B-2 and estrogen receptor status in human breast cancer. *Cancer Res* 1991, **51**, 3296–3303.
29. Paik S, Hazan R, Fisher ER, *et al*. Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: prognostic significance of erbB-2 protein overexpression in breast cancer. *J Clin Oncol* 1990, **8**, 103–112.
30. Rilke F, Colnaghi MI, Cascinelli N, *et al*. Prognostic significance of HER-2/*neu* expression in breast cancer and its relationship to other prognostic factors. *Int J Cancer* 1991, **49**, 44–49.
31. Seshadri R, Firgaira FA, Horsfall DJ, *et al*. for the South Australian Breast Cancer Study Group. Clinical significance of HER-2/*neu* oncogene amplification in primary breast cancer. *J Clin Oncol* 1993, **11**, 1936–1942.
32. Toikkanen S, Helin H, Isola J, Joensuu H. Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. *J Clin Oncol* 1992, **10**, 1044–1048.
33. Allred DC, Clark GM, Tandon AK, *et al*. HER-2/*neu* in node-negative breast cancer: prognostic significance of overexpression influenced by the presence of *in situ* carcinoma. *J Clin Oncol* 1992, **10**, 599–605.
34. Dykins R, Corbett IP, Henrey JA, *et al*. Long-term survival in breast cancer related to overexpression in the c-*erb*B-2 oncoprotein: an immunohistochemical study using monoclonal antibody NCL-CB11. *J Pathol* 1991, **163**, 105–110.
35. Gullick WJ, Love SB, Wright C, *et al*. c-*erb*B-2 protein overexpression in breast cancer is a risk factor in patients with involved and uninvolved lymph nodes. *Br J Cancer* 1991, **63**, 434–438.
36. Press MF, Pike MC, Chazin VR, *et al*. HER-2/*neu* expression in node-negative breast cancer: direct tissue quantification by computerized image analysis and association of overexpression with increased risk of recurrent disease. *Cancer Res* 1993, **53**, 4960–4970.
37. Bianchi S, Paglierani M, Zampi G, *et al*. Prognostic significance of c-*erb*B-2 expression in node negative breast cancer. *Br J Cancer* 1993, **67**, 625–629.
38. Clark GM, McGuire WL. Follow-up study of HER-2/*neu* amplification in primary breast cancer. *Cancer Res* 1991, **51**, 944–948.
39. Berns EMJJ, Foekens JA, van Staveren IL, *et al*. Oncogene amplification and prognosis in breast cancer: relationship with systemic treatment. *Gene* 1995, **159**, 11–18.
40. Leitzel K, Teramoto Y, Konrad K, *et al*. Elevated serum c-*erb*B-2 antigen levels and decreased response to hormone therapy of breast cancer. *J Clin Oncol* 1995, **13**, 1129–1135.
41. Newby JC, Johnston SRD, Smith IE, Dowsett M. Expression of epidermal growth factor receptor and c-*erb* B2 during the development of tamoxifen resistance in human breast cancer. *Clin Cancer Res* 1997, **3**, 1643–1651.
42. Yamauchi H, O'Neill A, Gelman R, *et al*. Prediction of response to antiestrogen therapy in advanced breast cancer patients by pretreatment circulating levels of extracellular domain of the HER-2/c-*neu* protein. *J Clin Oncol* 1997, **15**, 2518–2525.
43. Bianco AR, De Laurentis M, Carlomagno C, *et al*. 20 year update of the Naples GUN trial of adjuvant breast cancer therapy: evidence of interaction between c-*erb*-B2 expression and tamoxifen efficacy. *Proc Am Soc Clin Oncol* 1998, **17**, 97a (abstract 373).
44. Elledge RM, Green S, Ciocca D, *et al*. HER-2 expression and response to tamoxifen in estrogen receptor-positive breast cancer: a Southwest Oncology Group Study. *Clin Cancer Res* 1998, **4**, 7–12.
45. Muss H, Berry D, Thor A, *et al*. Lack of interaction of tamoxifen (T) use and ErbB-2/HER-2/neu (H) expression in CALGB 8541: a randomized adjuvant trial of three different doses of cyclophosphamide, doxorubicin and fluorouracil (CAF) in node-positive primary breast cancer (BC). *Proc Am Soc Clin Oncol* 1999, **18**, 68a (abstract 256).
46. Giai M, Roagna R, Ponzone R, *et al*. Prognostic and predictive relevance of c-*erbB*-2 and *ras* expression in node positive and negative breast cancer. *Anticancer Res* 1994, **14**, 1441–1450.
47. Stål O, Sullivan S, Wingren S, *et al*. c-erbB2 expression and benefit from adjuvant chemotherapy and radiotherapy of breast cancer. *Eur J Cancer* 1995, **31A**, 2185–2190.
48. Ménard S, Valagussa S, Pilotti E, *et al*. Benefit of CMF treatment in lymph-node positive breast cancer overexpressing HER2. *Proc Am Soc Clin Oncol* 1999, **18**, 69a (abstract 257).
49. Miles DW, Harris WH, Gillett CE, *et al*. Effect of c-*erb*B2 and estrogen receptor status on survival of women with primary breast cancer treated with adjuvant cyclophosphamide/methotrexate/fluorouracil. *Int J Cancer* 1999, **84**, 354–359.
50. Budman DR, Berry DA, Cirrincione CT, *et al*. Dose and dose-intensity as determinants of outcome in the adjuvant treatment of breast cancer: The Cancer and Leukemia Group B. *J Natl Cancer Inst* 1998, **90**, 1205–1211.
51. Muss HB, Thor AD, Berry DA, *et al*. c-*erb*B-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. *N Engl J Med* 1994, **330**, 1260–1266.
52. Paik S, Bryant J, Park C, *et al*. *erb*B-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. *J Natl Cancer Inst* 1998, **90**, 1361–1370.
53. Thor AD, Berry DA, Budman DR, *et al*. erbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. *J Natl Cancer Inst* 1998, **90**, 1346–1360.
54. Bièche I, Onody P, Laurendeau I, *et al*. Real-time reverse transcription-PCR assay for future management of *ERBB2*-based clinical applications. *Clin Chem* 1999, **45**, 1148–1156.

55. Okuyama N, Hatano Y, Park Y, *et al*. Quantitation of c-*erb*B-2 gene amplification in breast cancer tissue by competitive PCR. *Tumor Biol* 1999, **20**, 153–161.
56. Vona G, Caldini A, Sestini R, *et al*. The c-erbB-2 oncogene amplification by competitive PCR in aneuploid cell clones sorted from breast cancer samples. *Clin Chem Lab Med* 1999, **37**, 649–654.
57. Leitzel K, Teramoto Y, Sampson E, *et al*. Elevated soluble c-erbB-2 antigen levels in the serum and effusions of a proportion of breast cancer patients. *J Clin Oncol* 1992, **10**, 1436–1443.
58. Stearns V, Yamauchi H, Hayes DF. Circulating tumor markers in breast cancer: accepted utilities and novel prospects. *Breast Cancer Res Treat* 1998, **52**, 239259.
59. Codony-Servat J, Albanell J, Lopez-Talavera JC, *et al*. Cleavage of the HER2 ectodomain is a pervanadate-activable process that is inhibited by the tissue inhibitor of metalloproteases-1 in breast cancer cells. *Cancer Res* 1999, **59**, 1196–1201.
60. Press MF, Hung G, Godolphin W, Slamon DJ. Sensitivity of HER-2/*neu* antibodies in archival tissue samples: potential source of error in immunohistochemical studies of oncogene expression. *Cancer Res* 1994, **54**, 27712777.
61. Espinoza F, Anguiano A. The HercepTest assay: another perspective. *J Clin Oncol* 1999, **17**, 2293–2294.
62. Jacobs TW, Gown AM, Yaziji H, *et al*. Specificity of HercepTest in determining *HER-2/neu* status of breast cancers using the United States Food and Drug Administration-approved scoring system. *J Clin Oncol* 1999, **17**, 1983–1987.
63. Maia DM. Immunohistochemical assays for HER2 overexpression. *J Clin Oncol* 1999, **17**, 1650.
64. Roche PC, Ingle JN. Increased HER2 with U.S. Food and Drug Administration-approved antibody. *J Clin Oncol* 1999, **17**, 434.