PERGAMON

European Journal of Cancer 37 (2001) S3–S10

European Journal of Cancer

www.ejconline.com

The value of the human epidermal growth factor receptor-2 (HER2) as a prognostic marker

T. Cooke *, J. Reeves, A. Lannigan, P. Stanton

University Department of Surgery, Royal Infirmary, Queen Elizabeth Building, Alexandra Parade, Glasgow G31 2ER, UK

Received 3 July 2000; received in revised form 7 September 2000; accepted 12 September 2000

Abstract

The prognosis for patients with breast cancer is determined by well-established pathological features associated with biological aggressiveness, histological grade, tumour size and nodal involvement. These remain the key determinants, despite the identification of numerous other potential biological markers. The use of prognostic indices, such as the Nottingham Prognostic Index (NPI), which combines and weights these factors, enables clinicians to predict outcome with a certain amount of accuracy. Approximately 20–30% of breast cancers express very high quantities of the human epidermal growth factor receptor-2 (HER2) protein and this is almost always associated with gene amplification. With the use of sensitive techniques, such as the radio-immunohistochemical method (rIHC) described herein, to quantify HER2 protein levels, up to a further 50% of such cancers will be found to express the HER2 receptor at least 4-fold higher than normal breast cells. Adding HER2 expression to the NPI helps to determine more accurately the prognosis for individual patients, particularly those with node-negative disease. Overall, the main value of HER2 measurement is likely to be in the prediction of response to therapies targeting the *HER2* gene and protein.

Keywords: HER2; Breast cancer; Prognosis; Nottingham Prognostic Index

1. Introduction

Factors accepted as being important in the prognosis of breast cancer include the size of the primary tumour, stage of disease at diagnosis, hormone receptor status and the number of axillary lymph nodes involved [1]. The single most reliable prognostic indicator is axillary lymph node involvement [2]. However, combining these factors has been shown to produce more accurate prognostic information. For example, the Nottingham Prognostic Index (NPI) [3] utilises a scoring system that combines tumour grade (1–3), number of positive lymph nodes (1 = no nodes; 2 = 1–3 nodes; 3 = > 3 nodes) and tumour size × 0.2 to classify breast cancer patients into three groups: group 1, score 2–3.4; group 2, score 3.5–5.6; and group 3, score ⩾5.7. This classification system efficiently stratifies patients in terms of mortality risk. Breast cancer patients treated in Glasgow who are classified as NPI group 1 have a 10-year survival rate of 88%, compared with 66% in NPI group 2 and 28% in NPI group 3 (Dr T. Cooke, Royal Infirmary, Glasgow). Thus, standard pathological factors provide accurate and useful prognostic information and these factors are used most commonly in clinical practice.

Other non-pathological prognostic factors include proliferation markers, vessel invasion and markers of angiogenesis, and mitotic index, which have all been shown to have some value in determining prognosis [4]. Furthermore, for many years, molecular factors have also been investigated for their potential prognostic value. Such tumour markers include the human epidermal growth factor receptor (HER) gene family [5,6], hst [7], c-myc [8], p53 [9,10], H-ras [11] and int2 [7]. It has been demonstrated that combining tumour markers with other non-pathological factors identifies two distinct breast cancer phenotypes with differing prognosis [12]. However, one of the best studied molecular factors in breast cancer and one of the factors with greatest statistical prognostic value is the HER2 growth factor receptor.

* Corresponding author. Tel.: +44-141-211-4000; fax: +44-141-211-4943.

E-mail address: t.cooke@clinmed.gla.ac.uk (T. Cooke).

0959-8049/01/$ - see front matter
PII: S0959-8049(00)00402-0

The HER family comprises four homologous receptors named HER1 to HER4 [13]. The members of the HER family are transmembrane tyrosine kinase receptors with growth-stimulating activity and have a recognised role in the normal development and differentiation of mammary tissue [14–17]. The HER2 receptor is a protein comprising 1255 amino acids of molecular weight 185 kD ($p185^{HER2}$) [18]. $p185^{HER2}$ is recognised to have a central role in the HER family, being the preferred heterodimerisation partner for the other members [19] and forming heterodimers that have particularly high ligand-binding and signalling potency [20]. Importantly, both preclinical and clinical studies indicate that *HER2* amplification and overexpression are involved in oncogenic transformation and tumorigenesis in breast cancer [5,6,21–26].

Having identified the critical role of HER2 in both normal and abnormal breast cell growth regulation, determining whether this receptor could be used to determine prognosis, predict response to therapy and as a target for specific therapy in women with breast cancer became interesting. This review focuses on data regarding the prognostic value of HER2 in breast cancer, both at the invasive and pre-invasive stages, and its relationship with other prognostic variables.

2. HER2 status and breast cancer prognosis

As previously stated, combining pathological factors allows a relatively good estimate of breast cancer recurrence and mortality risk to be made. Women with node-positive breast cancer are recognised to be at high risk and are treated accordingly [27,28]. However, within this group patients can be stratified based on factors such as hormone receptor status and location of metastases.

Node-negative breast cancer patients are at lower risk of disease recurrence, but as many as 30% of these women develop recurrent disease after apparently curative surgery [29,30]. Adjuvant chemotherapy can reduce the relative risk of disease recurrence by as much as 40% [31–34]. However, adjuvant therapy is not without risk due to the potential for adverse effects associated with many cytotoxic agents. Therefore, there has been intensive research directed towards identifying factors that can select women with node-negative breast cancer who are at greater risk of relapse and would therefore benefit from adjuvant therapy.

2.1. Identification of HER2 as a prognostic factor

Slamon and colleagues [35] first linked HER2 with poor prognosis in breast cancer. By examining *HER2*-gene amplification in node-positive patients, they were able to demonstrate a significant correlation with reduced time to disease recurrence ($P \leqslant 0.0001$) and decreased survival ($P = 0.0011$). These correlations were independent of other prognostic factors and were as significant as the relationship between nodal status and disease recurrence and survival in this study. Furthermore, the survival duration of women with breast cancer overexpressing HER2 was 3 years on average compared with an average of 6–7 years in those whose disease did not overexpress HER2. Further studies confirmed these results and demonstrated that *HER2*-gene amplification correlates with HER2-receptor overexpression [36]. However, despite the positive data produced by Slamon and colleagues, further studies have produced differing results that have prevented the widespread use of HER2 status as a prognostic factor.

2.2. HER2 status in node-positive and node-negative breast cancer

Since the initial work of Slamon and colleagues [35,36], numerous clinical studies using different HER2 measurement techniques have investigated the relationship between HER2 status and breast cancer prognosis. Forty-seven of these trials, involving 15 248 patients, have been retrospectively analysed by Ross and Fletcher [37]. This analysis showed that HER2 status was an independent predictor of prognosis in 60% of the trials involving 67% of the patients. Most of the large studies (>300 patients) included in this analysis confirmed the correlation between a HER2-positive status and poor clinical outcome in HER-positive breast cancer patients [38–49]. Thus, it is generally accepted that there is a significant correlation between *HER2* amplification/overexpression and poor prognosis in patients with node-positive breast cancer.

The relationship between HER2 status and prognosis in node-negative breast cancer patients is more controversial. Many studies have shown that there is a correlation between outcome and HER2 status in these patients [29,30,47,50–54], although others have not [55,56]. The majority of the data indicate that HER2 overexpression predicts the occurrence of metastases in these patients, although the clinical significance of this association has not been clarified.

Finally, the identification of ductal carcinoma *in situ* (DCIS) is important if the development of invasive breast cancer is to be prevented. This condition is often detected on screening mammography because it is often associated with microcalcification. At this stage in development, DCIS can be effectively treated by complete mastectomy, after which the risk of progression is close to zero [57]. However, increasing numbers of patients are being treated with breast-conserving therapy, for which the risk of recurrence is considerably higher [57]. In this situation, providing patients with an estimate of their risk of recurrence would aid therapeutic decision-making.

HER2 amplification/overexpression has been associated with both the high-grade and comedo subtypes of DCIS [58–60]. The majority of cases of comedo-type DCIS seem to be HER2-positive. In one study, van de Vijver and associates [61] showed that all HER2-positive cases of DCIS were of the comedo type. This result has been supported by other studies [21]. As comedo-type DCIS is a particularly aggressive form of the disease, these data suggest that HER2 overexpression may identify a subset of DCIS patients at risk of progression to invasive disease who should be treated aggressively. Another theory suggests that the development of invasive breast cancer may follow two pathways [62]. According to this hypothesis, invasive tumours developing from DCIS are more aggressive, express markers of poor prognosis and are associated with a higher risk of progression. Other tumours develop from atypical ductal hyperplasia, which do not overexpress HER2, and are lower risk, more slowly progressing tumours.

3. Effect of testing technique on the prognostic value of HER2

The data summarised above appear to show convincingly that HER2 status can be used to make prognostic decisions in breast cancer. However, enough studies have yielded conflicting results, showing either that HER2 status does not have prognostic value or that it has only univariate significance, to indicate that the clinical use of HER2 status for prognosis remains controversial. One of the major causes of variability of data between studies is the different testing methods used in the studies performed.

Two studies have reviewed the effect of the testing technique on the findings of studies examining the association between HER2 status and breast cancer prognosis in some detail [37,63]. In the meta-analysis by Ross and Fletcher [37], only six of 47 studies failed to reveal any association between HER2 status and prognosis; four of these used immunohistochemistry (IHC) to detect HER2-protein overexpression and two used Southern blotting and slot-blot analysis to detect *HER2*-gene amplification. Thus, 41 studies revealed at least a univariate correlation between HER2 status and breast cancer prognosis whatever technique is used. Ross and Fletcher stress that IHC on fresh or frozen sections would be "an ideal method of detection" if fresh or frozen tissue and a standardised testing technique were widely available. They also state that all the studies included in the meta-analysis that used fluorescence *in situ* hybridisation (FISH) to detect *HER2*-gene amplification revealed an association between HER2 status and prognosis, overcoming the problem of DNA dilution that affects Southern and slot-blot analysis.

Mitchell and Press [63] concentrated on five recent studies [54,64–67]. Their conclusions were similar to those of Ross and Fletcher [35]; the IHC techniques used to date vary widely, introducing inconsistency into the results obtained, but generally reveal a correlation between HER2 status and prognosis, whereas FISH consistently identifies a negative association between *HER2* amplification and prognosis.

4. Radio-immunohistochemistry: further evidence for the association between *HER2* abnormalities and poor breast cancer prognosis

Both IHC and FISH produce useful and accurate information if performed correctly. However, IHC can be affected by the qualitative aspect of scoring stained sections and FISH is not widely available. We have investigated the use of a new technique termed radio-immunohistochemistry (rIHC) that enables quantitative analysis of immunohistochemically-stained tumour sections. Briefly, rIHC uses a radiolabelled anti-HER2 antibody that binds to the HER2 protein on the membrane of HER2-overexpressing tumour cells in tissue sections (Fig. 1). The section is then dipped in autoradiographic emulsion, exposed and developed. This produces a tissue section on which grains of stain are visible. Using computerised image analysis, areas of the tumour section of particular interest can be selected and the number of grains per unit area counted. This produces a quantitative estimate of the level of HER2 overexpression.

Using rIHC, we compared HER2 expression levels in tumours and normal breast tissue from the same patients. This demonstrated that approximately 85% of 179 breast tumours studied express HER2 at levels that range from normal to up to 1000-fold of normal [68]. The majority of these overexpress the HER2 protein at levels of up to 15-fold of normal. However, 23% overexpress HER2 at higher levels [68] and these patients also showed *HER2*-gene amplification in FISH analysis (Dr Tim Cooke, Royal Infirmary, Glasgow) (Fig. 2). Thus, we have been able to demonstrate that rIHC produces data linking *HER*2 overexpression to breast cancer prognosis provided that appropriate cut-off levels are established. Furthermore, these overexpression cut-off levels have been shown to correlate with *HER2*-gene amplification as determined using FISH.

We have also investigated the distribution of HER2 expression levels in DCIS ($n=21$), DCIS associated with invasive disease ($n=47$) and invasive breast cancer ($n=179$) using rIHC [22]. This demonstrated that frequency distributions for HER2 expression were similar in DCIS with and without invasion (Mann–Whitney U test, $P=0.19$) and both were comparable with invasive breast cancer (Mann–Whitney U test, $P=0.09$ (without

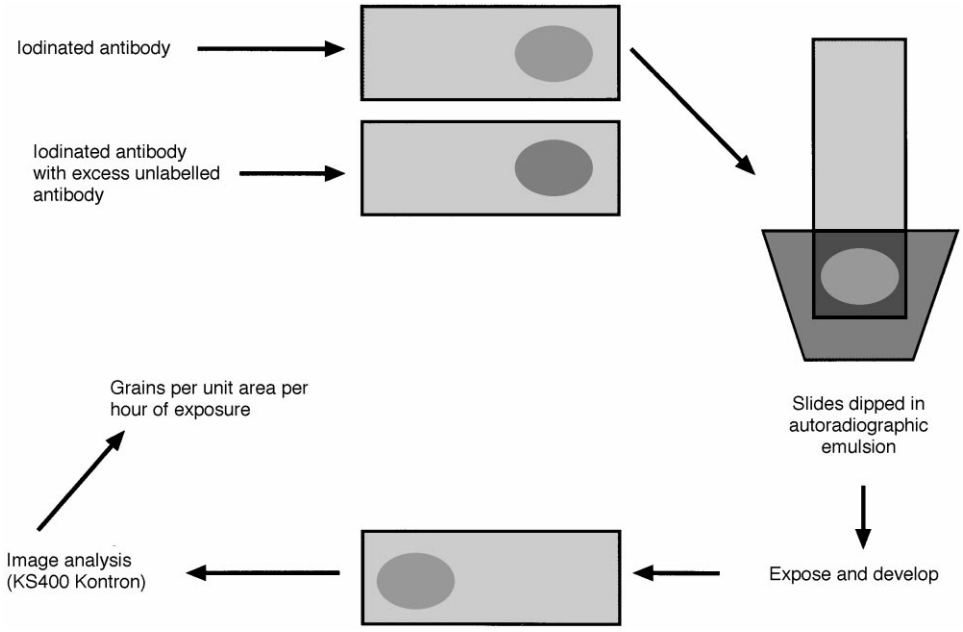

Fig. 1. Description of the rIHC technique used in the quantitative assessment of HER2 expression levels.

invasion) $P = 0.83$ (with invasion) (Fig. 3). Furthermore, in tumours that showed evidence of DCIS and invasion, the expression of HER2 was similar in each component (Wilcoxon Signed Rank Test, $P = 0.108$). Further studies demonstrated that HER2 expression levels were not associated with tumour grade or size, node status or HER1 levels, i.e. HER2 expression is an independent predictor of prognosis.

Further studies revealed that approximately 15% of breast cancers underexpress HER2 and that this is also associated with poor prognosis [68]. When patients in NPI groups 1–3 were subdivided into those with normal HER2 expression levels and those with abnormal levels (overexpressors and underexpressors), HER2 expression abnormalities were shown to be able to identify patients at higher risk of disease progression and death within each NPI group (Table 1) [22].

5. Conclusions

The first suggestion that oestrogen might have a role in breast cancer was provided by Beatson in 1896 [69], who demonstrated that oophorectomy produced breast

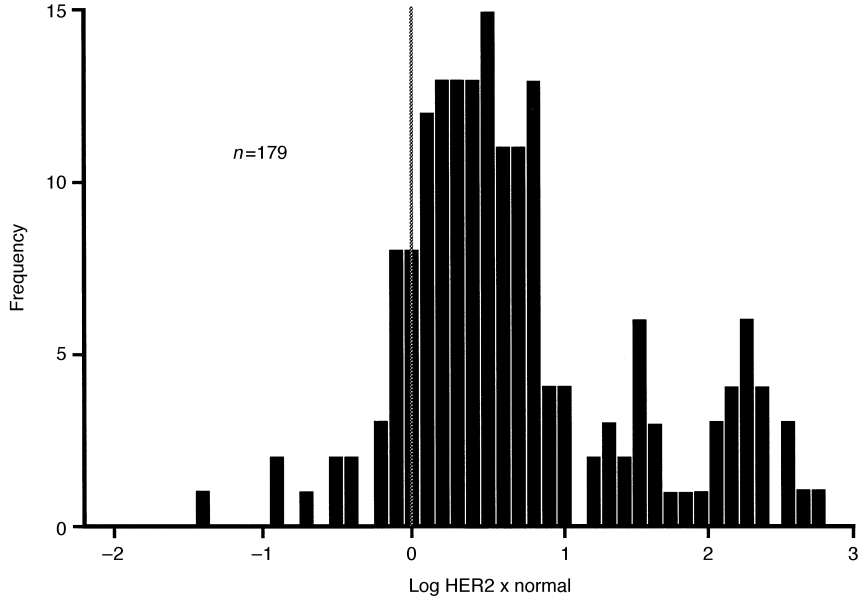

Fig. 2. HER2 expression levels in 179 breast cancer patients and the correlation between high levels of overexpression and *HER2*-gene amplification.

Table 1
Breast cancer survival at 10 years when categorised using the NPI and HER2 status

	HER2[a] status (n = 179)	
NPI[b] group	Normal (%)	Abnormal (%)
1	92	79
2	66	29
3	29	20
	$P < 0.0001$	

[a] HER2, human epidermal growth factor receptor-2.
[b] NPI, Nottingham Prognostic Index.

cancer regression in premenopausal women. This was many years before oestrogen was first identified [70] and the mechanism of action was elucidated with the identification of the oestrogen receptor (ER) in rats [71]. Whether breast tumours express ER and/or progesterone receptors (PgR) is the best recognised molecular prognostic marker for breast cancer, with patients with ER/PgR-positive tumours having a significantly better prognosis than those with ER/PgR-negative tumours, although evidence for this was not produced until the 1970s [72,73]. Furthermore, definitive evidence that targeting the ER with anti-oestrogens produces clinical benefit in ER/PgR-positive, but not ER/PgR-negative women with breast cancer was not published until 1992 [33].

This example illustrates the difficulty of demonstrating that molecular factors have value in determining the prognosis of cancer patients and their response to therapy. Molecular biological techniques have improved, enabling factors with a potential role in cancer to be identified more easily, and many new and more sophisticated tests for identifying such factors in tumour samples have been developed. This has allowed the relatively rapid screening of many molecular factors, but has also increased the potential for conflicting results to be produced.

Despite this, the data available indicate that determining HER2 status has prognostic value in breast cancer, identifying patients who are at high risk of reduced disease-free and overall survival. However, due to the variety of tests of different accuracy used to determine HER2 status over the past 20 years, this association remains controversial. We have used an accurate, quantitative and sensitive technique (rIHC) to study this association and have demonstrated that 85% of breast tumours express HER2 at higher levels than normal. Those tumours with the highest levels of HER2 overexpression (> 15-fold of normal; 23% of tumours) also showed gene amplification and these patients have a poor prognosis. Using this test, the prognostic power of HER2 status is such that the information produced is independent of other prognostic factors. Thus, relative to hormone status, information on the prognostic value HER2 status has become rapidly available.

Full acceptance of HER2 as a prognostic factor will only come as well-designed, prospective studies using quantitative, standardised techniques are performed. However, whether this will prove to be the most widely used application of HER2 status remains open to question. The latest update of the American Society of Clinical Oncology recommendations for the use of tumor markers in breast and colorectal cancer [74,75] recommends the use of ER and PgR status for making prognostic and therapeutic decisions. However, hormone receptor status is probably most widely used

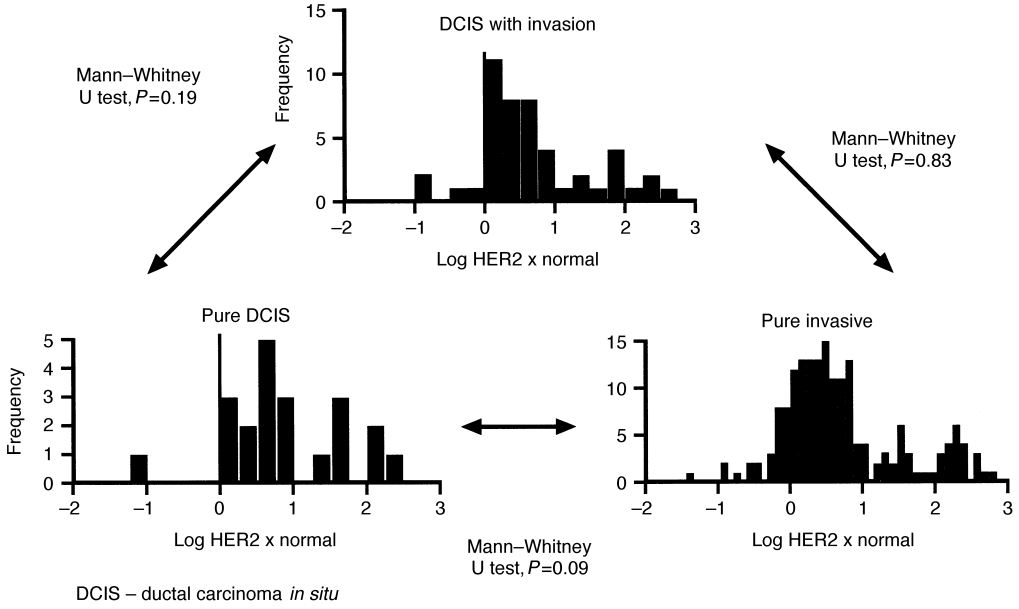

Fig. 3. Frequency distributions of HER2 expression in DCIS, DCIS associated with invasive disease and invasive breast cancer.

clinically to make therapeutic rather than prognostic decisions.

We believe that in future HER2 status will be used similarly. The availability of agents such as Herceptin® (trastuzumab), which targets the HER2 receptor [76] and produces significant survival benefits [77], together with the emerging role of HER2 status in predicting the outcome of hormonal therapy and chemotherapy [37], make knowing HER2 status important when determining the most appropriate treatment for a patient. Furthermore, a single molecular factor is unlikely to provide full prognostic information because breast cancer is characterised by multiple genetic abnormalities. In this regard, good assessment of pathological factors such as tumour grade and node status, which reflect the multiple gene defects, will probably remain the most important measure of the oncogenic potential of tumours.

In summary, HER2 status provides independent prognostic information at all stages of breast cancer from DCIS to invasive disease. Combining HER2 status with more conventional prognostic indicators, such as tumour size and grade and node status, allows high-risk groups to be identified. However, the most important application of HER2 status will be in selecting patients for targeted therapy using agents such as trastuzumab, much as hormone status is used to select patients for anti-oestrogen therapy using agents such as tamoxifen.

References

1. Clark GM, McGuire WL, Hubay CA, Pearson OH, Marshall JS. Progesterone receptors as a prognostic factor in stage II breast cancer. *N Engl J Med* 1983, **309**, 1343–1347.
2. Hynes NE, Stern DF. The biology of *erbB-2/neu/HER-2* and its role in cancer. *Biochim Biophys Acta* 1994, **1198**, 165184.
3. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. *Breast Cancer Res Treat* 1992, **22**, 207–219.
4. Ravaioli A, Bagli L, Zucchini A, Monti F. Prognosis and prediction of response in breast cancer: the current role of the main biological markers. *Cell Prolif* 1998, **31**, 113–126.
5. 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**, 8595.
6. 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.
7. Lidereau R, Callahan R, Dickson E, Peters G, Escot G, Ali I. Amplification of the int-2 gene in primary human breast tumours. *Oncogene Res* 1988, **2**, 285–291.
8. Escot C, Theillet C, Liderau R. Genetic alteration of the c-myc protooncogene (MYC) in human primary breast carcinomas. *Proc Natl Acad Sci USA* 1986, **83**, 4834–4848.
9. Cossman J, Schlegel R. p53 in the diagnosis of human neoplasia. *J Natl Cancer Inst* 1991, **83**, 980–981.
10. Kovach JS, McGovern RM, Cassady JD, *et al.* Direct sequencing from touch preparations of human carcinomas: analysis of p53 mutations in breast carcinoma. *J Natl Cancer Inst* 1991, **83**, 1004–1009.
11. Agnantis NJ, Parissi P, Anagnostakis D, Spandidos DA. Comparative study of Harvey-ras oncogene expression with conventional clinicopathologic parameters of breast cancer. *Oncology* 1986, **43**, 36–39.
12. Menard S, Casalini P, Tomasic G, *et al.* Pathobiologic identifiaction of two distinct breast carcinoma subsets with diverging clinical behaviors. *Breast Cancer Res Treat* 1999, **55**, 169–177.
13. Pinkas-Kramarski R, Eilam R, Alroy I, Levkowitz G, Lonai P, Yarden Y. Differential expression of NDF/neuregulin receptors ErbB-3 and ErbB-4 and involvement in inhibition of neuronal differentiation. *Oncogene* 1997, **15**, 2803–2815.
14. DiAugustine R, Richards RG. EGF-related peptides and their receptors in mammary gland development. *J Mammary Gland Biol Neoplasia* 1997, **2**, 109–118.
15. Normanno N, Ciardiello F. EGF-related peptides in the pathophysiology of the mammary gland. *J Mammary Gland Biol Neoplasia* 1997, **2**, 143–152.
16. Schroeder W, Biesterfeld S, Zillessen R, Rath W. Epidermal growth factor receptor-immunohistochemical detection and clinical significance for treatment of primary breast cancer. *Anticancer Res* 1997, **17**, 2799–2802.
17. Schroeder W, Lee DC. Dynamic expression and activation of ErbB receptors in the developing mouse mammary gland. *Cell Growth Differ* 1998, **9**, 451–464.
18. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science* 1986, **232**, 1644–1646.
19. Graus-Porta D, Beerli RR, Daly JM, Hynes NE. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signalling. *EMBO J* 1997, **16**, 1647–1655.
20. Sliwkowski MX, Schaefer G, Akita RW, *et al.* Coexpression of erbB2 and erbB3 proteins reconstitutes a high affinity receptor for heregulin. *J Biol Chem* 1994, **269**, 14661–14665.
21. Barnes DM, Bartkova J, Camplejohn RS, Gullick WJ, Smith PJ, Millis RR. 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.
22. Chong D, Cooke TG, Reeves JR, *et al.* Quantitation of EGFR and c-erbB-2 expression in preinvasive compared to invasive breast cancer. *Eur J Cancer* 1999, **35**(Suppl. 4), S203 (abstract 792A).
23. Di Fiore PP, Pierce JH, Fleming TP, *et al.* Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells. *Cell* 1987, **51**, 1063–1070.
24. Di Fiore PP, Pierce JH, Kraus MH, Segatto O, King CR, Aaronson SA. *erb*B-2 is a potent oncogene when overexpressed in NIH/3T3 cells. *Science* 1987, **237**, 178–182.
25. 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.
26. Liu E, Thor A, He M, Barcos M, Ljung B-M, Benz C. The *HER2* (c-erbB-2) oncogene is frequently amplified in *in situ* carcinomas of the breast. *Oncogene* 1992, **7**, 1027–1032.
27. Hortobagyi GN. Treatment of breast cancer. *N Engl J Med* 1998, **339**, 974–984.
28. Piccart MJ, Awada A & Hamilton A. Integration of new therapies into management of metastatic breast cancer: a focus on chemotherapy, treatment selection through use of molecular markers, and newly developed biologic therapies in late clinical development. In *American Society of Clinical Oncology 1999 Educational Book*. 1999, 526–538.
29. Allred DC, Clark GM, Tandon AK, *et al.* HER2/*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.

30. Andrulis IL, Bull SB, Blackstein ME, *et al*. *neu/erbB-2* amplification identifies a poor-prognosis group of women with node-negative breast cancer. *J Clin Oncol* 1998, **16**, 1340–1349.
31. Early Breast Cancer Trialists' Collaborative Group. Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer: an overview of 61 randomized trials among 28,896 women. *N Engl J Med* 1988, **319**, 1681–1692.
32. Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomized trials. *Lancet* 1998, **352**, 930–942.
33. Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic or immune therapy: 133 randomized trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. *Lancet* 1992, **339**, 1–15.
34. Early Breast Cancer Trialists' Collaborative Group. *Treatment of Early Breast Cancer, vol. 1: Worldwide Evidence 1985–1990*. Oxford, Oxford University Press, 1990.
35. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science* 1987, **235**, 177–182.
36. Slamon DJ, Godolphin W, Jones LA, *et al*. Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. *Science* 1989, **244**, 707–712.
37. 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.
38. Borg A, Tandon AK, Sigurdsson H, *et al*. HER-2/*neu* amplification predicts poor survival in node-positive breast cancer. *Cancer Res* 1990, **50**, 4332–4337.
39. Gusterson BA, Gelber RD, Goldhirsch A, *et al*. Prognostic importance of c-*erb*B-2 expression in breast cancer. *J Clin Oncol* 1992, **10**, 1049–1056.
40. Hartmann LC, Ingle JN, Wold LE, *et al*. Prognostic value of CerbB2 overexpression in axillary lymph node-positive breast cancer. Results from a randomized adjuvant treatment protocol. *Cancer* 1994, **74**, 2956–2963.
41. Kallioniemi OP, Holli K, Visakorpi T, *et al*. Association of C-erb B2 protein over-expression with high rate of cell proliferation, increased risk of visceral metastases and poor long-term survival in breast cancer. *Int J Cancer* 1991, **49**, 650–655.
42. Lovekin C, Ellis IO, Locker A, *et al*. C-erb B2 oncoprotein expression in primary and advanced breast cancer. *Br J Cancer* 1991, **63**, 439–443.
43. McCann AH, DeDervan TA, O'Regan M, *et al*. Prognostic significance of C-erb B2 and estrogen receptor status in human breast cancer. *Cancer Res* 1991, **51**, 3296–3303.
44. Molina R, Ciocca DR, Candon AK, *et al*. Expression of HER-2/*neu* oncoprotein in breast cancer: a comparison of immunohistochemical and western blot techniques. *Anticancer Res* 1992, **12**, 1965–1991.
45. Quenel N, Wafflart J, Bonichon F, *et al*. The prognostic value of c-erbB2 in primary breast carcinomas: a study on 942 cases. *Breast Cancer Res Treat* 1995, **35**, 283–291.
46. 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.
47. Seshadri R, Firgaira FA, Horsfall DJ, McCaul K, Setlur V, Kitchen P. 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.
48. Tetu B, Brisson J. Prognostic significance of Her-2/neu oncogene expression in node-positive breast cancer. The influence of the pattern of immunostaining and adjuvant therapy. *Cancer* 1994, **73**, 2359–2365.
49. Winstanley J, Cooke T, Murray GD, *et al*. The long term prognostic significance of C-erb B2 in primary breast cancer. *Br J Cancer* 1991, **63**, 447–450.
50. Dykins R, Corbett IP, Henry JA, *et al*. Long-term survival in breast cancer related to overexpression of the c-erbB-2 oncoprotein: an immunohistochemical study using monoclonal antibody NCL-CB11. *J Pathol* 1991, **163**, 105110.
51. Gullick WJ, Love SB, Wright C, *et al*. c-erbB-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.
52. Harbeck N, Ross JS, Yurdseven S, *et al*. HER-2/neu gene amplification by fluorescence in situ hybridization allows risk-group assessment in node-negative breast cancer. *Int J Oncol* 1999, **14**, 663–671.
53. O'Malley FP, Saad Z, Kerkvliet N, *et al*. The predictive power of semiquantitative immunohistochemical assessment of p53 and C-erbB2 in lymph node-negative breast cancer. *Hum Pathol* 1996, **27**, 955–963.
54. Press MF, Bernstein L, Thomas PA, *et al*. HER2/*neu* gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. *J Clin Oncol* 1997, **15**, 2894–2904.
55. 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.
56. Clark GM, McGuire WL. Follow-up study of HER-2/neu amplification in primary breast cancer. *Cancer Res* 1991, **51**, 944–948.
57. Silverstein MJ, Barth A, Poller DN, *et al*. Ten-year results comparing mastectomy to excision and radiation therapy for ductal carcinoma in situ of the breast. *Eur J Cancer* 1995, **31A**, 1425–1427.
58. Allred DC, Clark GM, Molina R. Overexpression of HER-2/neu and its relationship with other prognostic factors changed during progression of in-situ to invasive breast cancer. *Hum Pathol* 1992, **23**, 974–979.
59. Moreno A, Lloveras B, Figueras A, *et al*. Ductal carcinoma in situ of the breast: correlation between histologic classifications and biologic markers. *Mod Pathol* 1997, **10**, 1088–1092.
60. 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.
61. van de Vijver MJ, Peterse JL, Mooi WJ, *et al*. Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. *N Engl J Med* 1988, **319**, 1239–1245.
62. Ménard S, Casalini P, Campiglio M, Pupa S, Agresti R, Tagliabue E. HER2 overexpression in various tumor types. *Ann Oncol* 2001, in press.
63. Mitchell MS, Press MF. The role of immunohistochemistry and fluorescence in situ hybridization for HER-2/*neu* in assessing the prognosis of breast cancer. *Semin Oncol* 1999, **26**(Suppl. 12), 108–116.
64. Elledge RM, Green S, Ciocca D, et al HER. 2 expression and response to tamoxifen in oestrogen receptor positive breast cancer: a Southwest Oncology Study Group study. *Clin Cancer Res* 1998, **4**, 7–12.
65. Haffty BG, Brown F, Carter D, Flynn S. Evaluation of HER-2 neu oncoprotein expression as a prognostic indicator of local recurrence in conservatively treated breast cancer: a case-control study. *Int J Rad Oncol Biol Phys* 1996, **35**, 751–757.
66. MacGrogan G, Mauriac L, Durand M, *et al*. Primary chemotherapy in breast invasive carcinoma: predictive value of the immunohistochemical detection of hormonal receptors, p53, c-erbB-2, MiB1, pS2 and GST pi. *Br J Cancer* 1996, **74**, 1458–1465.

67. Xing WR, Gilchrist KW, Harris CP, Samson W, Meisner LF. FISH detection of HER-2/neu oncogene amplification in early onset breast cancer. *Breast Cancer Res Treat* 1996, **39**, 203–212.
68. Chong D, Cooke TG, Reeves JR, *et al*. Quantitation of c-erbB-2 in primary breast cancer allows identification of a further poor prognostic group. *Eur J Cancer* 1999, **35**(Suppl. 4), S89 (abstract 291).
69. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. *Lancet* 1896, **2**, 162–167.
70. Allen E, Doisy EA. An ovarian hormone: preliminary reports on its localization, extraction and partial purification and action in test animals. *J Am Med Assoc* 1923, **81**, 810–821.
71. Jensen EV, Jacobson HI. Fate of steroidal estrogens in target tissues. In Pincus G, Vollmer EP, eds. *Biological Activities of Steroids in Relation to Cancer*. New York, Academic Press, 1962, 161–174.
72. Croton R, Cooke T, Holt S, George WD, Nicolson R, Griffiths K. Oestrogen receptors and survival in early breast cancer. *Br Med J* 1981, **283**, 1289–1291.
73. Thorpe SM. Estrogen and progesterone receptor determinations in breast cancer: technology, biology and clinical significance. *Acta Oncol* 1988, **27**, 1–19.
74. American Society of Clinical Oncology. 1997 update of recommendations for the use of tumor markers in breast and colorectal cancer. *J Clin Oncol* 1998, **16**, 793–795.
75. American Society of Clinical Oncology. 1997 update of recommendations for the use of tumor markers in breast and colorectal cancer. *J Clin Oncol* 1998, **16**, 793–795.
76. Carter P, Presta L, Gorman CM, *et al*. Humanization of an anti-$p185^{HER2}$ antibody for human cancer therapy. *Proc Natl Acad Sci USA* 1992, **89**, 4285–4289.
77. Norton L, Slamon D, Leyland-Jones B, *et al*. Multinational Herceptin Investigator Group Overall survival (OS) advantage to simultaneous chemotherapy (CRx) plus the humanized anti-HER2 monoclonal antibody Herceptin (H) in HER2-over-expressing (HER2+) metastatic breast cancer (MBC). *Proc Am Soc Clin Oncol* 1999, **18**, 127a (abstract 483).