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Report

The prognostic value of c-erbB2 in primary breast carcinomas: A study on 942 cases

Nathalie Quénel[1], Jean Wafflart[1], Françoise Bonichon[1], Isabelle de Mascarel[1], Monique Trojani[1], Michel Durand[1], Antoine Avril[1] and Jean-Michel Coindre[1,2]
[1] *Fondation Bergonié, Comprehensive Cancer Center, Bordeaux, France;* [2] *Laboratory of Pathology, UFR II, University of Bordeaux II, Bordeaux, France*

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Summary

To assess the practical prognostic value of c-erbB2, we performed a study on 942 invasive ductal carcinomas treated with primary surgery between 1980 and 1986 in our center. We evaluated its expression by immunohistochemistry in paraffin-embedded tissue using a polyclonal antipeptide antibody. Of 942 tumors, 229 (24%) showed a positive membrane staining. We observed a significant association between c-erbB2 and Scarff-Bloom-Richardson grading ($p < 0.0001$) and a negative correlation between c-erbB2 and both estrogen and progesterone receptors ($p < 0.0001$). In our analysis, with respect to overall survival (OS), relapse-free survival (RFS), and metastasis-free survival (MFS), c-erbB2 was statistically significant ($p \leq 0.0001$) for the whole group and the node-positive subgroup. In multivariate analysis, c-erbB2 appeared to be an independant variable for RFS and MFS in the node-negative group. However, in our hands, c-erbB2 had a poor prognostic value in comparison with the classical prognostic variables such as histological grade, nodal status (N), hormonal receptor status (estrogen and progesterone receptors), and tumor size, and it did not supersede the classical parameters.

Introduction

The number of newly proposed prognostic factors in breast carcinoma has grown during the last 10 years at an increasing rate. Much attention has been focused recently on the amplification of the c-erbB2 oncogene associated with increased c-erbB2 oncoprotein expression [1]. This gene, which belongs to the tyrosine kinase oncogene family, is located on chromosome 17 at q21 and encodes for a transmembrane receptor-like phosphoglycoprotein that is closely related in structure but is biologically distinct from the epidermal growth factor receptor (EGFR) [1–6].

The c-erbB2 proto-oncogene is found to be overexpressed in approximately 20 to 30% of primary human breast carcinomas and has been reported to correlate with poor clinical prognosis [7–9].

To assess the practical prognostic value of c-erbB2, we performed two studies: first, we compared levels of c-erbB2 mRNA by dot blot and presence of c-erbB2 oncoprotein by immunohistochemistry (IHC) on 83 breast carcinomas. Then, we analyzed 942 operable infiltrating ductal carcinomas (IDC) treated in our center between 1980 and

Address for offprints: N. Quénel, Department of Medical Oncology, Fondation Bergonié, 180, rue de Saint-Genès, 33076 Bordeaux Cedex, France

Table 1. Characteristics of patients and tumors

Mean age	56 years (23 to 86 years)
Tumor size	21 ± 9 mm (1 to 88 mm)
*Nodal status**	
Node-negative (N–)	398 (42%)
Node-positive (N+)	544 (58%)
Scarff-Bloom and Richardson (SBR) grade	
I	196 (21%)
II	405 (43%)
III	341 (36%)
*Hormonal receptor status (HR)***	
ER+	688 (73%)
PR+	526 (56%)
ER–	254 (27%)
PR–	416 (44%)

* The mean number of lymph nodes histologically examined per case was 15.

** By the dextran-coated charcoal method with a cut-off level of 10 and 15 fmol/mg of protein for ER and PR status respectively.

1986 by IHC using a polyclonal antipeptide antibody (Dako).

Material and methods

Patients and tumors

The study was carried out in a group of 942 consecutive distant metastasis-free primary IDC (M_0) of the breast initially resected in our center between 1980 and 1986. Characteristics of patients and tumors, initial treatment, and follow-up were previously described [10]. Mean age was 56 years, and mean tumor size 21 mm. Data on nodal status, Scarff-Bloom-Richardson (SBR) grading [11], and hormonal receptor status are shown in Table 1. Adjuvant treatments are shown in Table 2.

c-erbB2 analysis

To assess the validity of immunohistochemistry (IHC), 83 recent cases were studied concomitantly by RNA dot blot and IHC.

The quantification of c-erbB2 RNA levels by densitometry was performed using a c-erbB2 probe kindly provided by T. Yamamoto. Relative RNA levels were quantified by comparison with a MCF7 WT cell line used as reference. An actin probe from D. Wallitz was used as standard.

IHC was performed on Bouin-fixed paraffin-embedded sections on the 83 tumors with a previously described technique [10]. A polyclonal antipeptide antibody (Dako) was used at a 1:600 dilution. The streptavidin biotin peroxidase method was performed with the LSAB kit from Dako.

Then, the 942 tumors were tested by IHC using the same method. All the slides were read by the same pathologist. Only positive invasive tumor cells were evaluated. Tumor cells were considered as positive when they showed a positive membrane staining. The proportion of c-erbB2 positive tumor cells was determined as a percentage of invasive tumor cells, ranging from 0 to 100%, from the entire stained tissue section. For each tumor, both % of stained cells and the intensity of positivity evaluated on a 3-point scale (1+, 2+, 3+) were analysed, but for the statistical evaluation, we used only % of stained cells.

Table 2. Adjuvant treatment decided following nodal status and hormonal receptor status of tumors and the current protocols at the time of surgery

N– H+ (n = 288)	→	No adjuvant treatment		
N– HR– (n = 110)	→	Chemotherapy*	68	(62%)
N+ HR+ (n = 435)	→	Chemotherapy*	252	(58%)
		Chemotherapy + hormonotherapy**	113	(26%)
N+ HR– (n = 109)	→	Chemotherapy	98	(90%)

* CMF: cyclophosphamide, methotrexate, 5-fluorouracil.
or 1041: farmorubicin, oncovin, methotrexate.
or 1043: mitomycin C, thiotepa, eldisine.
** Tamoxifen.
N–: node-negative, N+: node-positive, HR–: hormonal receptor status –, HR+: hormonal receptor status +.

Table 3. Study of c-erbB2 on 83 recent tumors concomitantly by dot blot and IHC

		IHC		Total
		–	+	
RNA dot blot	–	63	2	65
	+	6	12	18
Total		69	14	83

Statistical analysis

Association between c-erbB2 expression and histological grade was assessed by the Chi-square-test while the Spearman rank correlation test was used to study the association between c-erbB2 protein and other parameters such as age, nodal status, hormonal receptor status, and tumor size.

All patients were followed quarterly for two years, twice a year for the next year, and then yearly. The median follow-up was 83.5 months, ranging from 33 to 140 months. The cut-off date for the current analysis was 31 July 1992. Survival curves were established by the Kaplan-Meier method. For overall survival (OS), survival duration was calculated from the date of surgery to death or the date they were last known alive. All causes of deaths were considered as events. For metastasis-free survival (MFS) and for relapse-free survival (RFS), time-to-failure was computed from the date of surgery until metastasis or relapse or the date they were last known to be disease-free, respectively. Patients who died from unrelated causes were considered as censored by the time of their death. For RFS, the event was either local failure and/or metastasis. For comparison between survival curves, logrank tests were used. Univariate analyses were performed using BMDP software (Program 1L). Multivariate analyses using the stepwise Cox-model were performed with BMDP software (Program 2L).

Results

Validity of technique

We found 18 tumors (21%) with c-erbB2 gene overexpression by dot blot, and 14 tumors (17%) were positive by IHC (Table 3).

Six tumors were considered as positive with dot

Table 4. Relationship between c-erbB2 expression and classical prognostic parameters

	c-erbB2 +	c-erbB2 –	p value
Age			
< 50	81 (27.8%)	210 (72.2%)	0.10
≥ 50	148 (22.7%)	503 (77.3%)	
Tumor size			
≤ 20 mm	124 (23.6%)	402 (76.4%)	0.70
> 20 mm	97 (24.9%)	293 (75.1%)	
Nodal status			
N–	95 (23.9%)	303 (76.1%)	0.84
N+	134 (24.6%)	410 (75.4%)	
SBR grade			
I	22 (11.2%)	174 (88.8%)	
II	87 (21.5%)	318 (78.5%)	< 0.0001
III	120 (35.2%)	221 (64.8%)	
Estrogen receptor status			
ER+	143 (20.8%)	545 (79.2%)	< 0.0001
ER–	86 (33.9%)	168 (66.1%)	
Progesterone receptor status			
PR+	91 (17.3%)	435 (82.7%)	< 0.0001
PR–	138 (33.2%)	278 (66.8%)	

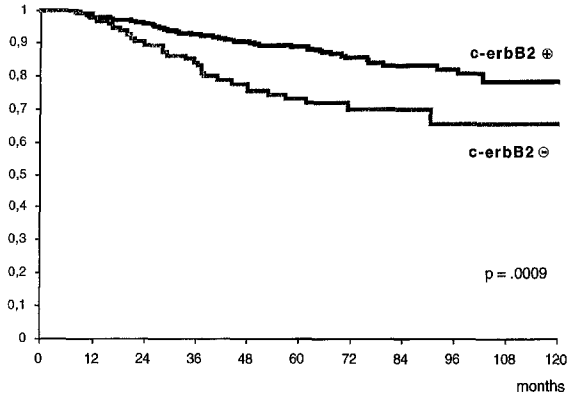

Fig. 1. RFS in node-negative group of patients with c-erbB2– (303 patients) and with c-erbB2+ (95 patients).

blot analysis and negative with IHC. Only one out of these dot-blot-positive/IHC-negative tumors showed high levels of c-erbB2 mRNA. In contrast, two tumors were negative with dot blot analysis and positive with IHC but with low values (1% and 2% of stained tumor cells respectively).

Results were concordant in 75 cases (90%).

c-erbB2 assay in the IDC series

Of 942 tumors, 229 cases (24%) showed a positive membrane staining (> 0%): 6% with 1 to 10% of positive tumor cells, 8% between 10 and 50%, and 10% with more than 50% positivity.

Among the 229 tumors with positive stained cells, we evaluated the intensity of membrane staining: we observed 89 cases with intensity evaluated at 1+, 82 tumors with intensity evaluated at 2+, and 58 tumors with intensity at 3+.

c-erbB2 expression and classical prognostic parameters

Results are shown in Table 4: there was a significant association between c-erbB2 and grade ($p < 0.0001$) and a negative correlation with both estrogen receptors (ER) ($p < 0.0001$) and progesterone receptors (PR) ($p < 0.0001$), but no association with tumor size ($p = 0.7$), nodal status ($p = 0.8$), or age ($p = 0.1$).

Grading was performed according to the criteria of Scarff-Bloom-Richardson. This technique assesses nuclear pleomorphism, tubule formation, and mitotic rate estimated after analysis of at least 10 high power fields at a 400 times magnification.

When considering SBR grade parameters, we observed that c-erbB2 positivity correlated with the least differentiated tumors (i.e. with no tubule formation) ($p = 0.01$), with higher mitotic rate (≥ 15 mitotic figures for 10 HPF) ($p < 0.0001$), and with the most marked polymorphism ($p < 0.0001$).

Univariate analysis

In the univariate analysis, overall survival, relapse-free survival, and metastasis-free survival curves were calculated for age, tumor size, nodal status, SBR grade, ER, PR, and c-erbB2 status.

All variables other than age were statistically significant with respect to overall survival (OS), relapse-free survival (RFS), and metastasis-free survival (MFS) (Tables 5, 6, 7) in the whole group.

In the N– patients (n = 398), c-erbB2 was significant for RFS ($p = 0.0009$) and MFS ($p = 0.002$) but not for OS (Table 6) (Fig. 1).

Table 5. Invasive ductal carcinomas for the whole group (n = 942). Univariate analysis

	Overall survival	Relapse-free survival	Metastasis-free survival
Age	NS	NS	NS
Tumor size	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Nodal status	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
SBR grade	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
ER	$p < 0.0001$	$p = 0.0001$	$p = 0.0008$
PR	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
c-erbB2	$p = 0.0001$	$p < 0.0001$	$p = 0.0001$

Table 6. Invasive ductal carcinomas for the node-negative group (n = 398). Univariate analysis

	Overall survival	Relapse-free survival	Metastasis-free survival
Age	NS	NS	NS
Tumor size	p = 0.10	p = 0.0098	p = 0.02
SBR grade	p = 0.012	p = 0.026	p = 0.008
ER	p = 0.003	NS	NS
PR	p < 0.0001	p = 0.006	p = 0.001
c-erbB2	NS	p = 0.0004	p = 0.002

In the N+ patients (n = 544), c-erbB2 was significant for OS (p = 0.0002), RFS (p = 0.008), and MFS (p = 0.008) (Table 7).

Multivariate analysis

For Cox multivariate analysis, 8 factors were tested: tumor size, nodal status, histological grade, ER status, PR status, chemotherapy, hormonal treatment, and c-erbB2.

Results are listed in Tables 8, 9, and 10.

In our study, c-erbB2 is an independent prognostic factor regarding RFS and MFS only in the node-negative group of patients: grade is one of the most important predictive factors with respect to OS, RFS, and MFS for the three groups (whole group, N–, N+). Nodal status is the second with respect to OS, and the first with respect to RFS and MFS for the whole group. Tumor size and PR status are significant in terms of OS, RFS, and MFS for the whole group and N+ patients. In the N– patients, tumor size is significant in terms of RFS, and PR status is significant regarding OS and MFS. For N+ patients and the whole group, hormonotherapy is of lesser importance in terms of OS, RFS, and MFS, while chemotherapy is of lesser importance in terms of OS.

Discussion

In this retrospective study, analysis of protein c-erbB2 by IHC in 942 infiltrating ductal carcinomas surgically removed as first-line treatment, revealed 24% of positive tumors. This is in perfect agreement with the 20 to 30% positivity usually found in the literature (1, 7, 8, 12–22].

This staining represents an overexpression of the protein associated with amplification of the gene coding for this protein. As in other studies, a dot blot analysis of c-erbB2 mRNA was performed and a 90% concordance was found between IHC and dot blot analysis [9, 23–26]. However, this molecular biological method is time-consuming and consequently difficult to apply routinely to a large number of patients. It is easier to use the IHC method, which is what the various authors usually do to study large groups [27].

When we looked for a link between c-erbB2 overexpression and the other prognostic factors usually assessed, we found that c-erbB2 was associated with grade III tumors [7, 14, 23, 24, 28–31], e.g.

Table 7. Invasive ductal carcinomas for the node-positive group (n = 544). Univariate analysis

	Overall survival	Relapse-free survival	Metastasis-free survival
Age	NS	NS	NS
Tumor size	p < 0.0001	p < 0.0001	p < 0.0001
SBR grade	p < 0.0001	p < 0.0001	p < 0.0001
ER	p < 0.0001	p < 0.0001	p < 0.0001
PR	p < 0.0001	p < 0.0001	p < 0.001
c-erbB2	p = 0.0002	p = 0.008	p = 0.008

Table 8. Invasive ductal carcinomas. Multivariate analysis. Overall survival

		Improvement chi square	p value	RR*
Whole group	1. SBR grade III	43.4	< 0.0001	2.3
(n = 942)	2. N+	32	< 0.0001	3.6
	3. PR–	29.6	< 0.0001	2.0
	4. Size	14.3	< 0.0001	1.8
	5. Chemotherapy	10.3	= 0.001	0.5
	6. ER–	4.1	= 0.042	1.4
N– group	1. PR–	16.4	< 0.0001	2.8
(n = 398)	2. SBR grade III	5	= 0.0025	1.9
N+ group	1. SBR grade III	36.6	< 0.0001	2.5
(n = 544)	2. PR–	19.7	< 0.0001	2.0
	3. Size	15.2	< 0.0001	2.0
	4. Chemotherapy	9	= 0.003	0.5
	5. Hormonotherapy	3	= 0.008	0.6

* RR = Relative Risk in final model.

undifferentiated tumors, and was inversely correlated with tumors rich in hormonal receptors [3, 13, 14, 26, 27, 32–36]. These findings have already been reported in previous studies [9, 12, 18, 24, 27, 32]. We have found no other correlation with either age, tumor burden, or nodal involvement, which contrasts with the results of other authors [3, 14, 15, 23, 26, 28, 37].

The multivariate analysis showed that c-erbB2 was an independent prognostic factor, associated with earlier relapses or metastasis in the group of node-negative patients; therefore our results indicate that overexpression of c-erbB2 is associated with poor prognosis in patients with node-negative tumors. Previous studies have shown that c-erbB2 was an important prognostic factor for patients with node-negative and EGFR-negative tumors [27]. However, conflicting results have been observed by other authors [7, 14, 18, 24, 26, 28, 30, 33, 36, 38–40]. This may be due to the small number of patients included in these series or to the heterogeneity of the groups studied [38].

The interest of our study lies in the fact that it concerns a large consecutive series of infiltrating

Table 9. Invasive ductal carcinomas. Multivariate analysis. Relapse-free survival

		Improvement chi square	p value	RR*
Whole group	1. N+	52.6	< 0.0001	2.9
(n = 942)	2. SBR grade III	49	< 0.0001	2.0
	3. Size	26	< 0.0001	1.8
	4. PR–	13.5	< 0.0001	1.5
	5. Hormonotherapy	5.6	= 0.02	0.6
N– group	1. SBR grade III	8.3	= 0.004	1.7
(n = 398)	2. c-erbB2	5.6	= 0.02	1.8
	3. Size	4.1	= 0.04	1.6
N+ group	1. SBR grade III	41.3	< 0.0001	2.2
(n = 544)	2. Size	22.2	< 0.0001	1.9
	3. PR–	11.5	= 0.001	1.5
	4. Hormonotherapy	5	= 0.025	0.7

* RR = Relative Risk in final model.

Table 10. Invasive ductal carcinomas. Multivariate analysis. Metastasis-free survival

		Improvement chi square	p value	RR*
Whole group	1. N+	47	< 0.0001	2.8
(n = 942)	2. SBR grade III	48.4	< 0.0001	2.1
	3. Size	24.7	< 0.0001	1.8
	4. PR–	12	= 0.001	1.5
	5. Hormonotherapy	4	= 0.04	0.7
N– group	1. SBR grade III	12.4	< 0.0001	1.9
(n = 398)	2. PR–	4.5	= 0.03	1.7
	3. c-erbB2	4	= 0.04	1.7
N+ group	1. SBR grade III	36.1	< 0.0001	2.2
(n = 544)	2. Size	22.4	< 0.0001	1.9
	3. PR–	8.3	= 0.0004	1.4
	4. Hormonotherapy	4.1	= 0.043	0.7

* RR = Relative Risk in final model.

ductal carcinomas. However, the tumors of our study may be at different stages but all were surgically removed as first-line treatment in the same center. Adjuvant treatments (chemotherapy, hormonal therapy) were given in relation to prognostic factors and were included in the multivariate analysis, which can of course modify results especially if there are small numbers of patients in the subgroups. However despite this fact, the value of classical prognostic factors such as histologic nodal involvement, SBR grade, tumor burden, and hormonal receptors is considerable and is not significantly different when treatment is added or excluded from the multivariate analysis.

In conclusion, c-erbB2 seems to be an adverse prognostic factor indicating poor prognosis in node-negative patients [13, 14, 15, 16, 41, 42]. Its value seems minimal in relation to the classical prognostic factors [27, 29, 31, 42]. However, it is important to find new prognostic factors for node-negative patients which will make it possible to better distinguish those requiring intensified treatment, and to adjust their management in order to improve their relapse-free and metastasis-free survival.

References

1. 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 235: 177–182, 1987
2. Guerin M, Galillot M, Mathieu MC, Travagli JP, Spielmann M, Andrieu N, Riou G: Structure and expression of c-erbB2 and EGF receptor genes in inflammatory and non inflammatory breast cancer: Prognostic significance. Int J Cancer 43: 201–208, 1989
3. May E, Mouriesse H, May-Levin F, Qian JF, May P, Delarue JC: Human breast cancer: identification of populations with a high risk of early relapse in relation to both oestrogen receptor status and c-erbB-2 overexpression. Br J Cancer 62: 430–435, 1990
4. Wolber RA, Dupuis BA, Wick MR: Expression of c-erbB2 oncoprotein in mammary and extramammary Paget's disease. Am J Clin Pathol 96: 243–247, 1991
5. Gullick WJ, Tuzi NL, Kumar S, Paterson H, Quirke P, Venter DJ: c-erbB2 and c-myc genes and their expression in normal tissues and in human breast cancer. Cancer Cells 7: 393–398, 1989
6. Venter DJ, Tuzi NL, Kumar S, Gullick WJ: Overexpression of the c-erbB2 oncoprotein in human breast carcinomas: Immunohistological assessment correlates with gene amplification. Lancet ii: 69–71, 1987
7. Parkes HC, Lillycrop K, Howell A, Craig RK: c-erbB2 mRNA expression in human breast tumours: comparison with c-erbB2 DNA amplification and correlation with prognosis. Br J Cancer 61: 39–45, 1990
8. Tsutsumi Y, Naber SP, DeLellis RA, Wolfe HJ, Marks PJ, McKenzie SJ, Yin S: Neu oncogene protein and epidermal growth factor receptor are independently expressed in benign and malignant breast tissues. Hum Pathol 21: 750–758, 1990
9. Dati C, Muraca R, Tazartes O, Antoniotti S, Perroteau I, Giai M, Cortese P, Sismondi P, Saglio G, de Bortoli M: c-erbB-2 and ras expression levels in breast cancer are corre-

lated and show a co-operative association with unfavorable clinical outcome. Int J Cancer 47: 833–838, 1991
10. Soubeyran I, Coindre JM, Wafflart J, Bonichon F, de Mascarel I, Trojani M, Durand M, Avril A: Immunohistochemical determination of PS2 in invasive breast carcinomas: a study on 942 cases. Breast Cancer Res Treat 34: 119–128, 1995
11. Bloom HJG, Richardson WW: Histological grading and prognosis in breast cancer. Br J Cancer 11: 359, 1957
12. Børresen AL, Ottestad L, Gaustad A, Andersen TI, Heikkilä R, Jahnsen T, Tveit KM, Nesland JM: Amplification and protein over-expression of the neu/HER-2/c-erbB-2 protooncogene in human breast carcinomas: relationship to loss of gene sequences on chromosome 17, family history and prognosis. Br J Cancer 62: 585–590, 1990
13. Kallioniemi OP, Holli K, Visakorpi T, Koivula T, Helin HH, Isola JJ: Association of c-erbB-2 protein over-expression with high rate of cell proliferation, increased risk of visceral metastasis and poor long-term survival in breast cancer. Int J Cancer 49: 650–655, 1991
14. Press MF, Schwartz AM: Her-2/neu and breast cancer. In: Prognostic markers in breast cancer: laboratory analysis of breast biopsies. USCAP 83rd Annual Meeting San Francisco 1994 (Abstract)
15. Rilke F, Colnaghi MI, Cascinelli N, Andreola S, Baldini MT, Bufalino R, Della Porta G, Ménard S, Pierotti MA, Testori A: Prognostic significance of HER-2/neu expression in breast cancer and its relationship to other prognostic factors. Int J Cancer 49: 44–49, 1991
16. Ro J, El-Naggar A, Ro JY, Blick M, Frye D, Fraschini G, Fritsche H, Hortobagyi G: c-erbB-2 amplification in node-negative human breast cancer. Cancer Res 49: 6941–6944, 1989
17. Seshadri R, Matthews C, Dobrovic A, Horsfall DJ: The significance of oncogene amplification in primary breast cancer. Int J Cancer 43: 270–272, 1989
18. Toikkhanen 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 10: 1044–1048, 1992
19. Van de Vijver MJ, Mooi WJ, Peterse JL, Nusse R: Amplification and over-expression of the neu oncogene in human breast carcinomas. Eur J Surg Oncol 14: 111–114, 1988
20. Varley JM, Swallow JE, Brammar WJ, Whittaker JL, Walker RA: Alterations to either c-erbB-2 (neu) or c-myc protooncogenes in breast carcinomas correlate with poor short-term prognosis. Oncogene 1: 423–430, 1987
21. Winstanley J, Cooke T, Murray GD, Platt-Higgins A, George WD, Holt S, Myskov M, Spedding A, Barraclough BR, Rudland PS: The long term prognostic significance of c-erbB-2 in primary breast cancer. Br J Cancer 63: 447–450, 1991
22. Zhou D, Battifora H, Yokota J, Yamamoto T, Cline MJ: Association of multiple copies of the c-erbB-2 oncogene with spread of breast cancer. Cancer Res 47: 6123–6125, 1987
23. Berger MS, Locher GW, Saurer S, Gullick WJ, Waterfield MD, Groner B, Hynes NE: Correlation of c-erbB-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. Cancer Res 48: 1238–1243, 1988
24. Gullick WJ, Love SB, Wright C, Barnes DM, Gusterson B, Harris AL, Altman DG: c-erbB-2 protein overexpression in breast cancer is a risk factor in patients with involved and uninvolved lymph nodes. Br J Cancer 63: 434–438, 1991
25. Iglehart JD, Kraus MH, Langton BC, Huper G, Kerns BJ, Marks JR: Increased erbB-2 gene copies and expression in multiple stages of breast cancer. Cancer Res 50: 6701–6707, 1990
26. Tandon AK, Clark GM, Chamness GC, Ullrich A, McGuire WL: HER-2/neu oncogene protein and prognosis in breast cancer. J Clin Oncol 7: 1120–1128, 1989
27. Wright C, Angus B, Nicholson S, Sainsbury JRC, Cairns J, Gullick WJ, Kelly P, Harris AL, Horne CHW: Expression of c-erbB-2 oncoprotein: a prognostic indicator in human breast cancer. Cancer Res 49: 2087–2090, 1989
28. Barnes DM: Breast cancer and a proto-oncogene. c-erB-2 is a reliable prognostic marker. Br Med J 299: 1061, 1989
29. Lovekin C, Ellis IO, Locker A, Robertson JFR, Bell J, Nicholson R, Gullick WJ, Elston CW, Blamey RW: c-erbB-2 oncoprotein expression in primary and advanced breast cancer. Br J Cancer 63: 439–443, 1991
30. McCann AH, Dervan PA, O'Regan M, Codd MB, Gullick WJ, Tobin BMJ, Carney DN: Prognostic significance of c-erbB-2 and estrogen receptor status in human breast cancer. Cancer Res 51: 3296–3303, 1991
31. Tsuda H, Hirohashi S, Shimosato Y, Hirota T, Tsugane S, Watanabe S, Terada M, Yamamoto H: Correlation between histologic grade of malignancy and copy number of c-erbB-2 gene in breast carcinoma. Cancer 65: 1794–1800, 1990
32. De Potter CR, Beghin C, Makar AP, Vandekerckhove D, Roels HJ: The neu-oncogene protein as a predictive factor for haematogenous metastases in breast cancer patients. Int J Cancer 45: 55–58, 1990
33. Gusterson BA, Gelber RD, Goldhirsch A, Price KN, Sâve-Söderborgh J, Anbazhagan R, Styles J, Rudenstam CM, Golough R, Reed R, Martinez-Tello F, Tiltman A, Torhorst J, Grigolato P, Bettelheim R, Neville AM, Bürki K, Castiglione M, Collins J, Lindtner J, Senn HJ, for the International (Ludwig) Breast Cancer Study Group: Prognostic importance of c-erbB-2 expression in breast cancer. J Clin Oncol 10: 1049–1056, 1992
34. Heintz NH, Leslie KO, Rogers LA, Howard PL: Amplification of the c-erbB-2 oncogene and prognosis of breast adenocarcinoma. Arch Pathol Lab Med 114: 160–163, 1990
35. Le Roy X, Escot C, Brouillet JP, Theillet C, Maudelonde T, Simony-Lafontaine J, Pujol H, Rochefort H: Decrease of c-erbB-2 and c-myc RNA levels in tamoxifen-treated breast cancer. Oncogene 6: 431–437, 1991
36. O'Reilly SM, Barnes DM, Camplejohn RS, Bartkova J, Gregory WM, Richards MA: The relationship between c-erbB-2 expression, S-phase fraction and prognosis in breast cancer. Br J Cancer 63: 444–446, 1991
37. Querzoli P, Marchetti E, Fabris G, Marzola A, Ferretti S, Iacobelli S, Hazan R, King CR, Nenci I: Immunohistochem-

ical expression of c-erbB-2 in human breast cancer by monoclonal antibody: correlation with lymph node and ER status. Tumori 76: 461–464, 1990

38. Perren TJ: c-erbB-2 oncogene as a prognostic marker in breast cancer. Br J Cancer 63: 328–332, 1991
39. Thor AD, Schwartz LH, Koerner FC, Edgerton SM, Skates SJ, Yin S, McKenzie SJ, Panicali DL, Marks PJ, Fingert HJ, Wood WC: Analysis of c-erbB-2 expression in breast carcinomas with clinical follow-up. Cancer Res 49: 7147–7152, 1989
40. Tsuda H, Hirohashi S, Shimosato Y, Hirota T, Tsugane S, Yamamoto H, Miyajima N, Toyoshima K, Yamamoto T, Yokota J, Yoshida T, Sakamoto H, Terada M, Sugimura T: Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units: hst-1/int-2 and c-erbB-2/ear-1. Cancer Res 49: 3104–3108, 1989
41. Dykins R, Corbett IP, Henry JA, Wright C, Yuan J, Hennessy C, Lennard TJW, Angus B, Horne CHW: 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 163: 105–110, 1991
42. Richner J, Gerber HA, Locher GW, Goldhirsch A, Gelber RD, Gullick WJ, Berger MS, Groner B, Hynes NE: c-erbB-2 protein expression in node negative breast cancer. Ann Oncol 1: 263–268, 1990