**c-erbB-2 positive breast tumours behave more aggressively in the first years after diagnosis**

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**Summary**

In a retrospective study the expression of the c-erbB-2 oncogene was determined immunohistochemically in 276 breast cancer samples from 253 patients with the antibody 21N. The follow-up period was between 7 and 12 years. This study showed a trend for an inverse relationship between c-erbB-2 positive tumours and estrogen receptors (ER). A correlation was assessed between c-erbB-2 positive tumours and histological grade, liver metastases as first site of metastases, disease free survival time (DFS) in the second and third year after diagnosis and overall survival time (OST) in the third and fourth year after diagnosis. A trend was seen between c-erbB-2 positive tumours and tumour size. No correlation was found between c-erbB-2 positive tumours and age at diagnosis. The method of operation and lymph node involvement. From this study we conclude that there is a significant difference in prognosis the first years after diagnosis, but this difference seems to vanish in a longer follow-up period of 12 years. This provides us with an explanation for the discrepancies in literature concerning c-erbB-2 expression and prognosis in breast cancer. Some investigators did not show differences in prognosis between positive and negative cases after a long follow-up period whereas investigations with a short term follow-up period up to 2–3 years have indeed established a more aggressive behaviour of c-erbB-2 overexpression tumours.

The c-erbB-2 or HER-2/neu oncogene encodes a transmembrane glycoprotein with tyrosine kinase activity. The gene was first described in rat neuroglioblastoma induced by treatment with a carcinogen (Schechter et al., 1984). c-erbB-2 or neu has important sequence homology with the epidermal growth factor receptor. (Schechter et al., 1984; Bargmann et al., 1986). Amplification and overexpression were found especially in breast cancer (Yakota et al., 1986) and gastric carcinomas (Falck & Gullick, 1989).

Increased copy numbers in breast carcinomas were related to bad prognosis by some authors (Cline et al., 1987; Slamon et al., 1987; Varley et al., 1987; Slamon et al., 1989). These results were refuted by others (Ali et al., 1988; Zhou et al., 1989). Gene amplification of c-erbB-2 correlated with lymph node involvement (Slamon et al., 1987; Zhou et al., 1987; Slamon et al., 1989; Guerin et al., 1989; Tavassoli et al., 1989), histological grade (Berger et al., 1988; Tavassoli et al., 1989; Tsuda et al., 1989, Paik et al., 1990), negative ER-content (Cline et al., 1987; Guerin et al., 1989; Zeillinger et al., 1989; Heintz et al., 1990), early recurrence (Zhou et al., 1987; Cline et al., 1987; Varley et al., 1987), short overall survival time (Clime et al., 1987; Slamon et al., 1987; Varley et al., 1987; Slamon et al., 1989; Paik et al., 1990) and increased mitotic activity (Heintz et al., 1990; Ramachandra et al., 1990). All of these factors are considered to be bad prognostic indicators. According to other authors there was no correlation with tumour size (Gutman et al., 1989; Seshadri et al., 1989) and age at diagnosis (Zhou et al., 1987; Seshadri et al., 1989).

Studies were also carried out on the protein of c-erbB-2 by the immunoperoxidase method on primary cancers and metastases. Correlations were found between membrane staining tumours and patho-histological findings such as tumour size (van de Vijver et al., 1988), negative ER-content (De Potter et al., 1989a; Thor et al., 1989; Wright et al., 1989a; Kommoss et al., 1990; De Potter et al., 1990), lymph node involvement (Berger et al., 1988; Thor et al., 1989), histological grade (Berger et al., 1988; Barnes et al., 1988; Wright et al., 1989a) and survival time (Thor et al., 1989; Wright et al., 1989a).

Furthermore a trend towards worse prognosis was found by others (Barnes et al., 1988; Thor et al., 1989; Walker et al., 1989; Paik et al., 1990; De Potter et al., 1990). This immunohistochemical study with a clinical follow-up of up to 12 years was carried out to investigate a putative relationship between the c-erbB-2 oncogene and factors for prognosis. The aim of this study was to establish an explanation for the discrepancies in prognosis in the literature in a large number of patients.

**Materials and methods**

**Patients and treatment**

Tumour specimens were investigated from 251 female and two male patients with primary breast cancer from the De-Veer-Ziekenhuis in Heerlen, the Netherlands. Patients were chosen by haphazard from 1978–1982. Tumour samples were embedded in buffered formalin and could be used for the indirect immunoperoxidase method. Haematoxylin slides of the primary tumours and the metastases were reviewed. Clinical and pathohistological data as well as patient follow-ups were assessed. Table 1A shows the number of patients in each category of prognostic variables.

The patients were treated surgically depending on the clinical status at the time of diagnosis. For patients with minimal disease, T1, or tumours of <2 cm, a breast-saving quadrantectomy or radical mastectomy was performed. For patients with intermediate disease, T2, tumours of 2–5 cm, not fixed on the skin or chest wall, mastectomy and axillary clearance were carried out. In cases of breast-saving operations with negative lymph nodes the regional lymph nodes were treated with radiotherapy. In patients with positive lymph nodes adjuvant chemotherapy, six cycles with cyclophosphamide, methotrexate and fluorouracil (CMF) was administered instead of radiotherapy. After the operation, patients with T2 or T3 tumours and negative lymph nodes, but localisation of the tumour in the upper or lower medial quadrant or in the centre, received radiotherapy. If the lymph nodes were involved adjuvant chemotherapy was added.
Paget's disease of the nipple was treated with mastectomy and axillary clearance because of its central position. In patients with T4 tumours a biopsy was taken from the primary tumour to determine the ER-status; courses of chemotherapy were given immediately after diagnosis. Simple mastectomy, radiotherapy or a combination of both followed. From 1980 onward all patients with tumour stage 1–4, postmenopausal and positive ER were given tamoxifen as hormonal treatment.

The general follow-up for breast cancer patients after 1982 was based on clinical status mammography, chest X-ray and laboratory diagnosis. Bone metastases were diagnosed in the skeleton by scintigram, bronchial metastases by chest X-ray and cytology and liver metastases by ultrasound and laboratory investigations.

Local recurrences were pathohistologically confirmed and were treated surgically or with radiotherapy or both or with hormonal adjuvants regardless of the stage of the tumour.

To patients with liver metastases six cycles of CMF were administered. Bone metastases were radiated. If ER was positive, these patients were given Tamoxifen. Lung metastases were treated with six cycles of CMF and with Tamoxifen if ER was positive. Pleural metastases and pleural effusion were treated with an intra-pleural administration of neomycin. Solitary brain metastases were enucleated depending on the localisation. Table IB gives the number of patients with the method of primary and adjuvant treatment. The follow-up period varied between 7 and 12 years, depending on the age of the patients, if they were older than 80 the patients were in part reviewed by their GPs. Some of the patients were not available because of lost follow-up.

### Table 1B Primary and adjuvant treatments in invasive ductal carcinoma patients

| Method of operation                  | Total |
|--------------------------------------|-------|
| Mastectomy with ax. clearance       | 232   |
| Simple mastectomy                   | 138   |
| Quadrantectomy                       | 44    |
| Biopsy                               | 43    |
| Radiotherapy                         | 68    |
| Radio- and chemotherapy              | 7     |
| Radiotherapy and Tamoxifen           | 32    |
| Chemohormonal therapy                | 9     |
| Adjuvant chemotherapy                | 46    |
| Adjuvant Tamoxifen                   | 31    |
| Ovariectomy                          | 1     |

### Indirect immunoperoxidase method

Five micro m sections from blocks of breast cancer fixed in 4% formalin (buffered with phosphate) and embedded in paraffin were dewaxed, rehydrated and washed in phosphate buffered saline (PBS). The peroxidase-anti-peroxidase technique was applied as follows:

1. Immersion of deparaffinised sections in methanol containing 0.03% hydrogen peroxidase for 20 min to block the endogenous peroxidase activities and incubation with 5% bovine serum albumin for 30 min.
2. Incubation with rabbit polyclonal anti-c-erbB-2 antibody 21N diluted at 1/200 for 60 min (Gullick, ICRF, Hamm- mersmith Hospital, London); and rinsed three times with P.B.S. and 1% bovine serum albumin (B.S.A) for 5 min.
3. Biotinilated swine-anti-rabbit immunoglobulin diluted at 1/80 for 30 min (Dako-patts, Glostrup – Denmark); and rinsed three times with B.S.A for 5 min.
4. Avidin-biotin peroxidase complex for 30 min (Dako- patts, Glostrup – Denmark).
5. The peroxidase reaction was developed using 3–3 diaminobenzidine (Sigma, St Louis – USA) with 0.01% hydrogen peroxide for 10 min followed by washing in tap water. The nuclei were counterstained with haematoxylin. All sections were dehydrated and mounted. Control specimens were prepared by omitting the primary antibody. One slide identified as being positive for c-erbB-2 was taken as positive control.

### Antibody

21N as a polyclonal antibody was raised against a synthetic peptide derived from the c-erbB-2 oncogene product containing the amino acid residues 1243–1255 of the c-terminus of the protein of c-erbB-2 (Gullick et al., 1987).

### Oestrogen receptor content

The ER content was determined in 226 tumour specimens by the dextran coated charcoal technique. The hormonal contents were expressed as fmol mg–1 protein. Values of more than 10 fmol mg–1 protein were considered as positive, whereas values lower than 10 fmol mg–1 protein as ER negative.

### Pathological assessment

The slides were reexamined for correct grading and categorising. The size of the tumour and the number of lymph nodes were determined. The breast cancers were divided pathohisto logically into 239 invasive ductular carcinomas, 16 intra ductular carcinomas (DCIS), 21 invasive lobular carcinomas and two cases of Paget’s disease of the nipple. The invasive ductular carcinomas were again divided into stage 1, 2 and 3 according to histological grade.

All primary and secondary tumour specimens were examined by two independent observers. The immunohistochemical staining was scored as positive if there was membrane staining. Cytoplasmic staining was not considered specific for the c-erbB-2 protein since only membrane staining was considered specific as previously shown (De Potter et al., 1989b).

### Statistical analysis

Clinical and pathohistological factors in relation to c-erbB-2 over-expression were assessed by Fisher’s exact test (Hartung, 1985). Age at diagnosis was calculated with the Wilcoxon Rank Sum test (Hartung, 1985).

In a multivariate analysis, using an accelerated life model (Cox & Oakes, 1984), the relation between DFS and OST and the following prognostic factors as: c-erbB-2 over-expression, histology, lymph node status, ER, method of operation, age at diagnosis and tumour size were calculated.
The actuarial curves for DFS and OST were calculated with
the Kaplan-Meier technique. The tumour size was the
strongest prognostic factor in the accelerated life model.
Adjusting for tumour size the probability of recurrence at
fixed time periods after 6, 12, 18, . . . 36 months, respectivELY
the probability of survival at fixed time periods after 6, 12,
18, . . . 36 months was assessed under consideration of the
c-erbB-2 over-expression with the Cockran-Mantel-Haenzel
test (Agresti, 1990). Neglecting all other clinical and patho-
histological factors DFS and OST were tested for the c-erbB-
2 over-expression with the Log Rank and Wilcoxon test. All
P-values are two sided.

Results

Tumours were only scored as positive if the membrane was
stained (Figure 1). A different cytoplasmic staining pattern
was observed in some normal cells and some tumour cells,
but was not considered specific for the c-erbB-2 protein. Each
tumour was assessed according to the following criteria:

(1) Scoring of the membrane.
(2) Assessment of different components within the tumour
(e.g. invasive duct./intraductular).
(3) Comparison of the staining of primary tumours and
involved lymph nodes.

Normal breast tissue, if found, only showed granular cyto-
plasmic staining. Smooth muscle cells and the upper layers of
the epidermis tended to have cytoplasmic staining, which
again was not considered specific for the expression of the
protein of the c-erbB-2 oncogene.

For the statistical analysis only patients with invasive duct-
tacular carcinomas were used. Thirty-five of 232 (15.1%) patients
with invasive ductular carcinomas showed membrane
staining tumours. The median age of the c-erbB-2 positive
patients was 57.8 and the median age of the negative patients
was 59.3.

A trend for an inverse correlation between membrane
staining tumours and ER content was seen (P = 0.078)
(Table II). A correlation between membrane staining
tumours and the histological grade was found (P = 0.003)
(Table II). None of the twenty-one invasive lobular carci-
nomas was positive for c-erbB-2. Three of the four intra-
ductular carcinomas were positive, one of these three also
had an an intralobular component, which was negative. An
invasive carcinoma grade 3 with an in situ component showed
membrane staining in the invasive as well as in the in situ
part. The two cases of Paget’s disease of the nipple showed
membrane staining and the two underlying invasive grade 2
carcinomas. The Paget cells were of large size with large
 nuclei and prominent nucleoli.

Concerning the tumour size a trend was found between
c-erbB-2 tumours and a tumour size larger than 5 cm
(P = 0.055) (Table II). A correlation was seen between the
tumours which expressed the protein of c-erbB-2 and site of
first metastasis. The liver was the only tissue to have a
 correlation with c-erbB-2 positive tumours (P < 0.05) (Table
III). No correlation was found between the over-expression
of the c-erbB-2 oncogen and age at diagnosis (P = 0.66).
method of operation (P = 0.084) and axillary lymph-nodes
(P = 0.18) (Table II). There was some difference in the
membrane staining between primary tumours and their lymph
node metastases. In 3/7 (42.9%) of the lymph node meta-
tases the tumour cells showed a less marked membrane stain-
ing. The number of positive cells was also lower than in the
primary tumour.

There was a significant correlation between over-expression

Figure 1 Immunohistochemical staining with 21N. Invasive duct-cell carcinoma stained for the c-erbB-2 oncogene product with
21N. All tumour cells show membrane staining.
Table II c-erbB-2 membrane staining in relation to clinical and pathological findings

| Data               | c-erbB-2 pos. | c-erbB-2 neg. | Total | P value |
|--------------------|---------------|---------------|-------|---------|
| Hist. grade        |               |               |       |         |
| I                  | 0/13          | 13/13         |       |         |
| II                 | 20/164        | 144/164       |       |         |
| III                | 13/41         | 28/41         | 218   | 0.003   |
| Lymph nodes        |               |               |       |         |
| None inv. Pos.     | 12/102        | 90/102        |       | 0.185   |
|                  | 21/110        | 89/110        | 212   |         |
| ER Neg. Pos.       | 17/76         | 59/76         | 195   | 0.078   |
| Tumour size        |               |               |       |         |
| ≤5 cm              | 7/80          | 73/80         |       |         |
| >5 cm              | 28/152        | 124/152       | 232   | 0.055   |
| Method of operation|               |               |       |         |
| Mast. + ax. cl.    | 19/138        | 119/138       |       |         |
| Mast. – ax. cl.    | 9/44          | 35/44         |       |         |
| Quadrantectomy     | 5/43          | 38/43         |       |         |
| Biopsy             | 2/3           | 1/3           | 232   | 0.084   |
| Age at diag.       |               |               |       |         |
| ≤50                | 7/59          | 52/59         |       |         |
| >50                | 28/173        | 145/173       | 232   | 0.66    |

Table III Expression of c-erbB-2 in relation to site of first metastases

| Metastases       | c-erbB-2 pos. | c-erbB-2 neg. | P value |
|------------------|---------------|---------------|---------|
| Bone             | 6 (24%)       | 23 (31.1%)    | <0.05   |
| Liver            | 5 (32%)       | 9 (12.2%)     |         |
| Brain            | 0             | 2 (2.7%)      |         |
| Pleura           | 1 (4%)        | 4 (5.4%)      |         |
| Lung             | 2 (8%)        | 7 (9.5%)      |         |
| Local            | 5 (20%)       | 18 (24.3%)    |         |
| Others           | 1 (4%)        | 3 (4.0%)      |         |
| Lymph-nodes      | 2 (8%)        | 8 (10.8%)     |         |
|                  | 25 (100%)     | 78 (100%)     |         |

of c-erbB-2 and a bad prognosis. A multivariate analysis was performed to determine whether c-erbB-2 was an independent prognostic factor for DFS and OST. Clinical and pathohistological factors were tested. Tumour size was the strongest prognostic factor for both DFS (P = 0.0003) and OST (P = 0.0081). Another confounding factor for DFS was method of operation (P = 0.009). For OST age at diagnosis was a confounding factor (P = 0.011). Lymph node status showed a trend as confounding factor (P = 0.0499) (Table VA and B). The difference in DFS, neglecting all other clinical and pathohistological factors, was statistically significant with the Log Rank test (P = 0.025) and with the Wilcoxon test (P = 0.007). Most of the recurrences were seen in the first 3 years after diagnosis (Table IVA). After adjusting tumour size with the Cockran-Mantel-Haenzel test a correlation was found between c-erbB-2 positive tumours and the probability of recurrence after 18, 24 and 30 months (Table VA). A trend was found between c-erbB-2 positive patients and OST with the Wilcoxon test (P = 0.06). 61.8% of c-erbB-2 positive patients died within the first 4 years after diagnosis (Table IVB). Taking tumour size into consideration a correlation with the Cockran-Mantel-Haenzel test was found between c-erbB-2 positive tumours and OST after 36 and 42 months (Table V). Both Tables VA and V show the estimates and the standard errors of the regression-coefficients in the accelerated life model for DFS and OST. (Figures 2 and 3 show the actuarial curves for DFS and OST).

Discussion
In this retrospective study the expression of the c-erbB-2 protein was determined immunohistochemically in breast cancer patients in relation to its clinical and pathohistological features. Membrane staining with antibodies against the c-erbB-2 protein is known to be related to DNA amplification (Venter et al., 1987; Gusterson et al., 1988; Walker et al., 1989) and is considered to be the only expression of the c-erbB-2 oncogene, as cytoplasmic staining was shown not to

Table IVB c-erbB-2 positive and negative patients at 6 month periods within OST

| Months | c-erbB-2 pos. | c-erbB-2 neg. | P value |
|--------|---------------|---------------|---------|
| 0      | 34            | 185           | 0.09    |
| 6      | 27            | 171           | 0.44    |
| 12     | 24            | 155           | 0.03    |
| 18     | 18            | 144           | 0.008   |
| 24     | 15            | 136           | 0.01    |
| 30     | 13            | 124           | 0.06    |
| 36     | 13            | 116           | 0.12    |
| 42     | 13            | 111           | 0.25    |
| 48     | 13            | 103           | 0.49    |
| 54     | 13            | 95            | 0.54    |
| 60     | 12            | 88            | 0.31    |
| 66     | 10            | 83            | 0.13    |
| 72     | 10            | 74            |         |

Table VA Estimates and its standard errors of the regression coefficients in the accelerated life model for DFS and the P values for the Chi-square test

| Progn.-Factor | Value | Estimate | StdErr | P value |
|---------------|-------|----------|--------|---------|
| Intercept     | 2.938 | 0.783    | 0.0002 |
| Age           | 0.002 | 0.005    | 0.6564 |
| Tumour size   | -0.144| 0.040    | 0.0003 |
| c-erbB-2      | -0.204| 0.151    |        |
| Hist. Grade   |       |          |        | 0.1394  |
| 1              | -0.447| 0.376    |        |
| 2              | -0.570| 0.288    |        |
| 3              | -0.314| 0.288    |        |
| 5              | 1      | 0        |        |
| Lymph nodes   |       |          |        | 0.2521  |
| non. inv.     | 0.262 | 0.161    |        |
| 1 - 3         | 0.131 | 0.158    |        |
| >3             | 1      | 0        |        |
| ER            |       |          |        | 0.9696  |
| <10 fmol      | -0.005| 0.124    |        |
| >10 fmol      | 0      | 0        |        |
| Method of operation |       |          |        | 0.0009  |
| 1 mast. + ax.cl. | 1.997 | 0.705    |        |
| 2 mast. - ax.cl. | 1.542 | 0.711    |        |
| 3              | 1.687 | 0.711    |        |
| 4              | 0      | 0        |        |

Table IVA c-erbB-2 positive and negative patients at 6 months periods within DFS

| Months | c-erbB-2 pos. | c-erbB-2 neg. | P value |
|--------|---------------|---------------|---------|
| 0      | 34            | 185           | 0.09    |
| 6      | 27            | 171           | 0.44    |
| 12     | 24            | 155           | 0.03    |
| 18     | 18            | 144           | 0.008   |
| 24     | 15            | 136           | 0.01    |
| 30     | 13            | 124           | 0.06    |
| 36     | 13            | 116           | 0.12    |
| 42     | 13            | 111           | 0.25    |
| 48     | 13            | 103           | 0.49    |
| 54     | 13            | 95            | 0.54    |
| 60     | 12            | 88            | 0.31    |
| 66     | 10            | 83            | 0.13    |
| 72     | 10            | 74            |         |
be related to c-erbB-2 expression (De Potter et al., 1989b). We found positive membrane-staining in 15.1% of primary breast cancers.

Table VB Estimates and its standard errors of the regression coefficients in the accelerated life model for OST and the P values for the Chi-square test

| Progn.-Factor       | Value       | Estimate | StdErr | P value |
|---------------------|-------------|----------|--------|---------|
| Intercept           |             | 4.467    | 0.682  | 0.0001  |
| Age                 |             | -0.011   | 0.004  | 0.0110  |
| Tumour size         |             | -0.103   | 0.039  | 0.0081  |
| c-erbB-2            |             |          |        | 0.8742  |
| Hist. Grade         |             | 0.025    | 0.153  |         |
| Lymph nodes         |             | 0.052    | 0.359  |         |
| ER                  | <10 fmol    | -0.004   | 0.121  | 0.9763  |
| Method of operation |             | 0.1472   |        |         |

Method of operation: 1 mast. + ax.cl., 2 mast. - ax.cl., 3 quadrantectomy, 4 biopsy.

A trend for an inverse correlation was found between c-erbB-2 positive tumours and the ER status. This result confirms the studies done by De Potter et al., 1989a; Thor et al., 1989; Wright et al., 1989a; Kommuss et al., 1990; De Potter et al., 1990; O’Reilly et al., 1991.

It has been demonstrated that c-erbB-2 expression is under hormonal regulation (Dati et al., 1990). c-erbB-2 expression is not present in breast tissue in virgin mice and in the first 2 weeks of pregnancy when oestrogen and progesterone levels are high and maximum proliferation activity is seen. Protein expression of c-erbB-2 increases at the end of pregnancy and at the beginning of the lactation period in mice, when proliferation declines and differentiation begins. Oestrogens are a controlling factor of the protein expression of c-erbB-2. Under the influence of oestrogens, c-erbB-2 expression is inhibited. This fact agrees with our findings that c-erbB-2 expression is found more frequently in ER-negative tumours. The ER negative tumours are known to behave more aggressively and to metastasise faster than ER positive tumours (Oster, 1986). ER negative tumours, which are c-erbB-2 positive, have the tendency to respond less on hormonal therapy (Wright et al., 1989b).

The OST in c-erbB-2 negative patients after recurrence is longer, because most of these tumours are ER positive and respond much better to hormonal treatment. Metastases of ER positive tumours are found most of the time in bone, lung, pleura and are not as aggressive as metastases in liver or brain, which are often seen in ER negative and c-erbB-2 positive patients. This fact agrees with our finding, six out of eight patients who were c-erbB-2 positive and had liver metastases died within the first 2 years after diagnosis and did not respond to any hormonal treatment.

The responsiveness of c-erbB-2 positive tumours on chemo-
therapy is debated (Gullick et al., 1991). The question is raised whether these tumours are resistant on chemotherapy (O’Reilly et al., 1991), what requires further studies to investigate this hypothesis. Furthermore a trend was seen between membrane staining tumours and tumour size, confirming the findings of van de Vijver et al., 1988. Another correlation was found between over-expression of c-erbB-2 tumours and histological grade. Berger et al., 1988; Barnes et al., 1988; Wright et al., 1989a; Gullick et al., 1991; Lovekin et al., 1991 came to the same results.

From this study we conclude that c-erbB-2 membrane-staining tumours spread, especially to the liver, which confirms a previous prospective study with a short follow-up period (De Potter et al., 1989b). The particular pattern of metastasis to the liver could be explained with the production of a factor in the liver which stimulates the growth and spread of c-erbB-2 tumour cells. This factor may also be present in foetal liver tissue where c-erbB-2 is expressed (Quirke et al., 1989).

Our findings suggest that the putative ligand of c-erbB-2 is secreted into parenchymal organs in which the c-erbB-2 protein is expressed. The fact, that c-erbB-2 positive tumours show the tendency to select one parenchymal organ for metastising requires further investigation.

In conclusion our results show that c-erbB-2 positive tumours spread earlier. Most of the metastases are seen in the first three years after diagnosis. As a result of early metastases these patients have a shorter OST.

Our study is the first to provide us with an explanation for the discrepancies in literature between c-erbB-2 expression and different prognoses. Groups of authors who looked for a bad prognosis in the first years after diagnosis were able to show a difference in prognosis (Slamon et al., 1987; Varley et al., 1987; Gusterson et al., 1988; Thor et al., 1989; Tsuda et al., 1989; Wright et al., 1989a; De Potter et al., 1990). Some authors who carried out investigations in a long follow-up period of more than 5 to 10 years did not find a difference in prognosis between c-erbB-2 positive and negative patients (Gusterson et al., 1988; van de Vijver et al., 1988; Barnes et al., 1988), other authors (Lovekin et al., 1991; Wistansley et al., 1991) found a difference in prognosis in a long follow-up period of more than 5 years between c-erbB-2 positive and negative patients. Our study only showed differences within a short follow-up period of up to 3 years in DFS and up to 4 years in OST and between c-erbB-2 positive and negative tumours. These differences vanish in a longer follow-up period up to 12 years.

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