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C-erbB-2 expression does not predict response to docetaxel or sequential methotrexate and 5-fluorouracil in advanced breast cancer

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Abstract

Breast cancer patients with c-erbB-2-positive tumours seem to benefit from anthracycline-based adjuvant chemotherapy. The predictive value of c-erbB-2 for taxane sensitivity is not yet clear. The purpose of this study was to assess whether c-erbB-2 expression is associated with clinical sensitivity to docetaxel (T) or sequential methotrexate and 5-fluorouracil (MF). A total of 283 patients with metastatic breast cancer were initially enrolled in a randomised multicentre trial comparing docetaxel with sequential MF in advanced breast cancer. Paraffin-embedded blocks of the primary tumour were available for 131 patients (46%). c-erbB-2 status was determined by immunohistochemistry using a polyclonal antibody to the c-erbB-2 protein. C-erbB-2 expression was scored in a semi-quantitative fashion using a 0 to 3+ scale. Staining scores 2+ or greater were considered positive. Response evaluation was performed according to World Health Organization (WHO) recommendations. Overall 54 (42%) patients had c-erbB-2-positive tumours. There was no association between treatment outcome and c-erbB-2 overexpression. The overall response rates (RR) ($n=128$) among c-erbB-2-negative and -positive patients were 35 and 44%, respectively ($P=0.359$). In the MF arm ($n=62$), the RR was somewhat higher in the c-erbB-2 overexpressors (33% versus 18%, $P=0.18$). In the docetaxel arm the RRs were very similar, regardless of the c-erbB-2 expression (53% versus 53%). While several studies have suggested a prognostic and putative predictive significance of c-erbB-2 overexpression in early breast cancer, the significance of c-erbB-2 expression as a predictive factor for response to various cytotoxic treatments in advanced breast cancer is still controversial. In this study, c-erbB-2 expression could not predict response to either MF or T. Thus, tumours over-expressing c-erbB-2 are not uniformly more sensitive to taxanes and c-erbB-2 expression cannot yet be applied clinically as a predictive factor for response in advanced breast cancer.

Keywords: Breast cancer; c-erbB-2; Chemotherapy; Docetaxel; Predictive factor

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1. Introduction

Factors predicting response to chemotherapy can assist the clinician in selecting the right patients for chemotherapy and save the remaining patients from experiencing unnecessary toxicity. Additionally, such factors might also help in selecting the best possible regimen among the various cytotoxic agents for individual patients.

One of the most extensively studied prognostic factors in breast cancer is the *c-erbB-2* oncogene, also known as *HER-2/neu*. The protein product of the corresponding gene is a 185-kDa transmembrane receptor with tyrosine kinase activity [1]. Overexpression or amplification of this oncogene has been demonstrated in 15–30% of patients with breast cancer, and this has been associated with poor survival, mainly in node-positive patients [2].

While several studies have enlightened the prognostic and putative predictive significance of c-erbB-2 in early breast cancer, the significance of c-erbB-2 expression as a predictive factor for response to various cytotoxic treatments in advanced breast cancer is still a controversial issue. So far, only one trial addressing the role of c-erbB-2 expression and clinical taxane sensitivity has been published in short form [3]. In this study, c-erbB-2 overexpression seemed to confer increased taxane sensitivity. After adjusting for prognostic factors, c-erbB-2-positive patients were 3 times more likely to respond clinically to taxane therapy.

In the current study, pre-treatment expression of c-erbB-2 has been correlated with response to therapy, time to progression and overall survival in a series of patients enrolled in an international randomised trial comparing docetaxel (T) with sequential methotrexate and 5-fluorouracil (MF) regimen after failure on anthracycline-based chemotherapy in advanced breast cancer [4].

The main purpose of this study was the need to confirm the clinical sensitivity to taxanes observed previously [3] and assess the generalisation of these results in a more uniform patient population.

2. Patients and methods

2.1. Patients

A total of 283 patients with metastatic breast cancer were initially randomised in a multicentre trial comparing T with sequential MF in advanced breast cancer (Fig. 1). Paraffin-embedded blocks of the primary tumour were available for 131 patients (46%). The blocks were obtained from seven participating centres; Helsinki ($n=71$), Umeå ($n=13$), Rogaland ($n=12$), Lund ($n=10$), Ullevål ($n=8$), Tromsoe ($n=6$), Estonia ($n=6$), Uppsala ($n=4$) and Danderyd ($n=1$). To enter the trial, patients were required to have histologically-proven primary breast cancer that had progressed during or after first-line anthracycline treatment for advanced disease or relapsed within 12 months after discontinuation of adjuvant anthracycline therapy. The patients were required to be 18–70 years old with a performance status $\leqslant 2$ and have no more than one previous chemotherapy regimen for advanced disease (multiple endocrine treatments and radiotherapy were allowed). Patients with measurable lesions or evaluable lesions were eligible. Response evaluation was done every third course, at treatment discontinuation and every 3 months during follow-up. Response evaluation was performed according to World Health Organization (WHO) recommendations [5]. The main patient and tumour characteristics of the 131 analysed patients are shown in Table 1.

2.2. Immunohistochemical assays

All tissues had been fixed in 4% buffered formalin, processed and embedded in paraffin. From each block, 4 μm thick sections were cut on coated slides (Superfrost plus, Menzel-Gläser) and dried for 12 h at 37 °C. The sections were deparaffinised in xylene and rehydrated through graded concentrations of ethanol to distilled water. Subsequently, endogenous peroxidase was quenched by incubating the sections for 30 min in methanol and hydrogen peroxide. Immunohistochemical stainings

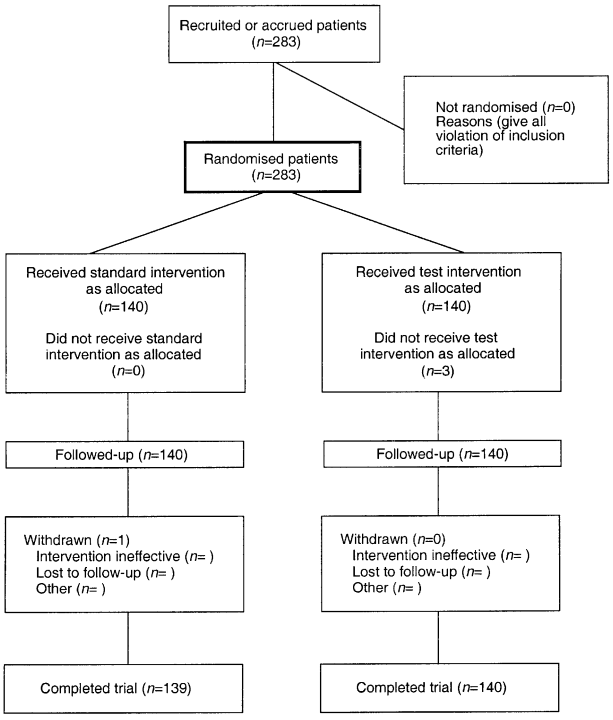

Fig. 1. Flow chart of the progress of patients through the trial (adapted from Ref. [21]).

were performed by using an Elite ABC Kit (Vectastain, Vector Laboratories, Burlingame, CA, USA). Blocking serum was applied for 15 min and was followed by an overnight incubation with rabbit anti-human c-erbB-2 oncoprotein (Dako, Glostrup, Denmark) diluted 1:500. Antigen retrieval techniques were not used. On the following day, sections were incubated with the biotinylated secondary antibody and the peroxidase-labelled advanced breast cancer (ABC) for 30 min each. Bound peroxidase was visualised by using 3-amino-9-ethyl-carbazole (AEC) (Sigma A-5754) as a chromogen. Between each step in the staining procedure (except before the incubation with the primary antibody), the slides were rinsed three times in phosphate-buffered saline. Finally, the sections were counterstained with Mayer's haematoxylin and mounted with Aquamount (BDH Ltd, Poole, UK). C-erbB-2 overexpression was scored in a semi-quantitave fashion using a 0 to 3+ scale (0 completely negative, 1+ faint perceptible staining of the membrane, 2+ moderate staining of the entire membrane observed in more than 10% of the tumour cells, 3+ strong circumferential staining of the entire membrane creating a fishnet pattern). Cytoplasmic staining was considered non-specific and was not included in the final scoring.

Table 1
Tumour and patient characteristics ($n=131$)

Characteristics	Patients n (%)
Histology	
Ductal	119 (91)
Lobular	11 (8)
Medullary	1 (1)
Histological grade	
Gr I	16 (12)
Gr II	80 (61)
Gr III	35 (27)
ER status	
Positive	60 (46)
Negative	58 (44)
Unknown	13 (10)
Treatment arm	
Docetaxel	69 (53)
Methotrexate and 5-fluorouracil	62 (47)
Median age (years)	50.8
Menopausal status	
Pre-menopausal	14 (11)
Post-menopausal	115 (88)
Unknown	2 (2)
Median disease-free interval (years)	1.6
Previous chemotherapy for metastatic disease	118 (90)
Previous adjuvant chemotherapy only	13 (10)
Visceral disease	102 (78)
WHO performance status	
WHO 0	33 (25)
WHO 1	75 (57)
WHO 2	23 (18)

Gr, grade; WHO, World Health Organization; ER, oestrogen receptor.

2.3. Statistical methods

Statistical analyses were carried out using a personal computer and the Statistical Package for the Social Sciences (SPSS). Overall survival (OS) and time to progression (TTP) curves were prepared by the Kaplan–Meier method and survival analysis was performed using the Mantel–Cox logrank test. The association between treatment response and c-erbB-2 overexpression was evaluated using the Mann–Whitney U test with Complete Response (CR) classified as 4, Partial Response (PR) as 3, No Change (NC) as 2 and Progressive Disease (PD) as 1. The association between response rate and other clinicopathological factors was tested either by Chi-squared test or by Chi-squared test for trends when the clinicopathological factor was divided in three groups (WHO-status, histological grade). In these Chi-squared tests, patients with CR and PR were regarded as one group and those patients with NC and PD response as the other. Variables that were statistically significantly associated with response in the univariate analysis were further evaluated in a multivariate analysis using a stepwise logistic regression to evaluate the independent predictive value of each variable. The Spearman correlation co-efficient factors were calculated for c-erbB-2 and various tumour biological factors.

3. Results

3.1. All patients

Out of 131 patients, 128 were evaluable for response. The overall response rate (CR + PR) was 53% in the T and 24% in the MF arm, respectively ($P<0.01$) (Table 2). In the parent study ($n=283$), the corresponding response rates were 42 and 21% [4].

Of various clinicopathological variables, only pretreatment WHO performance status and treatment group (T versus MF) were significantly associated with treatment response ($P<0.05$) according to the univariate analysis (Table 2). None of the other clinicopathological parameters (menopausal status, presence of visceral disease, previous chemotherapy for metastatic disease, tumour grade) was associated with overall response rate (data not shown). In the multivariate analysis (stepwise logistic regression), only the treatment group remained an independent predictor for the response rate ($P=0.01$).

Overall, 54 patients (42%) patients were c-erbB-2-positive (c-erbB-2 score 2+ or 3+). There was no association between treatment outcome and c-erbB-2

Table 2
The predictive value of various patient and tumour-related factors ($n=128$)

	CR + PR	NC + PD	
All patients	39% (50/128)	61% (78/128)	
Treatment arms			
Docetaxel (T)	53% (35/66)	47% (31/66)	
Methotrexate–5-FU (MF)	24% (15/62)	76% (47/62)	$P<0.01$
ER status[a]			
Positive	46% (26/57)	54% (31/57)	
Negative	37% (22/59)	63% (37/59)	NS
Histological grade			
Grade I	40% (6/15)	60% (9/15)	
Grade II	39% (31/79)	61% (48/79)	
Grade III	38% (13/34)	62% (21/34)	NS
Visceral metastasis			
No	34% (10/29)	66% (19/29)	
Yes	40% (40/99)	60% (59/99)	NS
WHO performance status			
WHO 0	38% (12/32)	63% (20/32)	
WHO 1	47% (34/73)	53% (39/73)	
WHO 2	17% (4/23)	83% (19/23)	$P<0.05$
c-erbB-2 overexpression			
Negative	35% (26/74)	65% (48/74)	
Positive	44% (24/54)	56% (30/54)	NS

CR, complete response; PR, partial response; NC, no change; PD, progressive disease; 5-FU, 5-fluorouracil; NS, non-significant; WHO, World Health Organization; ER, oestrogen receptor.
[a] Data is missing for some patients.

overexpression (Table 3). The overall response rates among c-erbB-2-negative and -positive patients were 35 and 44%, respectively ($P=0.359$).

Time to progression did not differ significantly between the c-erbB-2 overexpressors and non-overexpressors (Fig. 2). There was a trend towards a shorter median overall survival in patients with c-erbB-2-positive tumours than in patients with c-erbB-2-negative tumours (12.5 months versus 9.5 months; $P=0.08$). None of the other clinicopathological parameters was associated with either time to progression or with survival in the whole patient population.

We have previously assessed by immunohistochemistry the expression of p21, p53, mdm-2, mib-1, bcl-2, bax, bcl-xL, bag-1, fas receptor and fas ligand from the present tumour material. Of these factors, c-erbB-2 expression was positively correlated with fas ligand expression ($r=0.30$; $P=0.001$) and negatively correlated with bcl-2 expression ($r=-0.26$; $P=0.004$).

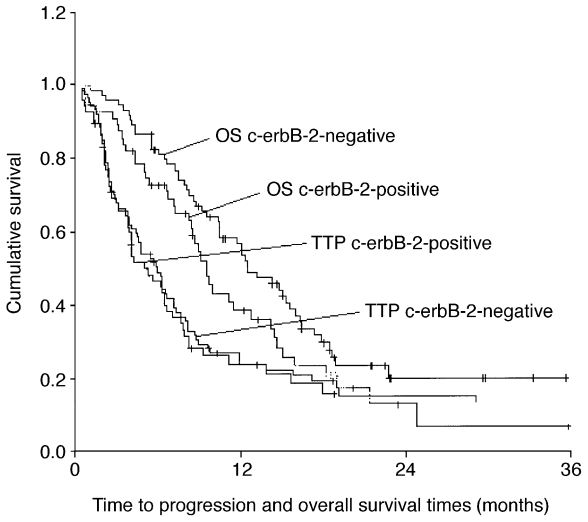

Fig. 2. Overall survival (OS) and time to progression (TTP) in patients with c-erbB-2-positive and -negative tumours.

3.2. MF arm

Among the 62 patients in the MF arm, 24 were c-erbB-2-positive (39%). The overall response rate was higher in the c-erbB-2 overexpressors than in c-erbB-2-negative patients (33% versus 18%), but the difference did not reach statistical significance ($P=0.18$). Survival from the start of treatment and time to progression were similar irrespective of the c-erbB-2 status (data not shown). Of all the clinicopathological variables analysed, none was associated with time to progression or overall survival (data not shown).

3.3. T arm

In the T arm, 45% ($n=30$) of the patients overexpressed c-erbB-2. The response rate was similar

Table 3
The association between c-erbB-2 expression and response to treatment

			PD (%)	NC (%)	PR (%)	CR (%)	P value
All patients $n=128$	c-erbB-2	Positive $n=54$	20 (37)	10 (19)	20 (37)	4 (7)	0.94
		Negative $n=74$	21 (28)	27 (36)	21 (28)	5 (7)	
Methotrexate–5-FU (MF) arm $n=62$	c-erbB-2	Positive $n=24$	11 (46)	5 (21)	7 (29)	1 (4)	0.60
		Negative $n=38$	17 (45)	14 (37)	6 (16)	1 (3)	
Docetaxel (T) arm $n=66$	c-erbB-2	Positive $n=30$	9 (30)	5 (17)	13 (43)	3 (10)	0.50
		Negative $n=36$	4 (11)	13 (36)	15 (42)	4 (11)	

5-FU, 5-fluorouracil; CR, complete response; PR, partial response; NC, no change; PD, progressive disease

regardless of the c-erB-2 expression (53% in both groups). Time to progression and overall survival did not differ between patients with c-erbB-2-positive or -negative tumours in the T arm (data not shown). Of all the prognostic variables analysed, only worse WHO performance status ($P = 0.04$) and presence of visceral disease ($P = 0.04$) were associated with a shorter time to progression (data not shown).

4. Discussion

Recent interest in c-erbB-2 as a possible predictive factor for chemotherapy response is derived largely from studies done in the adjuvant setting. In the early 1990s, two studies were published suggesting that c-erbB-2 overexpression determined by immunohistochemistry could predict a relative resistance to CMF (cyclophosphamide/methotrexate/5-fluorouracil)-based adjuvant therapy. In the Intergroup study, high-risk node-negative patients were randomised to CMFp (cyclophosphamide/methotrexate/5-fluorouracil/prednisone) or observation [6]. In a retrospective analysis of tumour material from this trial, c-erbB-2 overexpressing patients did not appear to derive significant benefit in disease-free survival, while among non-overexpressors benefit was observed. In the other retrospective study, c-erbB-2 expression was determined by immunohistochemistry from patients initially entered in the International (Ludwig) Breast Cancer Study Group trial where patients were randomised to single perioperative treatment or prolonged CMF-based therapy [7]. In this trial, significant benefit in terms of disease-free survival and overall survival was seen for prolonged chemotherapy only in the c-erbB-2-negative patients. These results were interpreted to indicate that tumours overexpressing the *c-erbB-2* oncogene are less responsive to CMF-containing adjuvant therapy.

The potential relationship between c-erbB-2 status and response to anthracycline-based chemotherapeutic regimens has also been examined in the adjuvant setting. The study by Muss and colleagues was the first one to suggest that c-erbB-2-positive patients benefit more from higher doses of doxorubicin in terms of a better disease-free and overall survival [8]. These results have recently been updated and continue to show an association between the anthracycline dose and c-erbB-2 overexpression, although definitive conclusions cannot be made [9]. In another study, axillary node-positive patients with c-erbB-2-positive tumours seemed to benefit from doxorubicin [10]. Interaction between doxorubicin treatment and c-erbB-2 overexpression was statistically significant for disease-free survival, but not for overall survival. The results of these two studies indicate that as a group patients with c-erbB-2-positive tumours benefit from anthracycline-based adjuvant therapy. However, these results are not conclusive for c-erbB-2 being a predictive factor for absolute sensitivity to anthracyclines.

The results from studies addressing the predictive value of c-erbB-2 overexpression in locally advanced or metastatic breast cancer have been conflicting (Table 4). Thus far, the only published studies which indicate that c-erbB-2-positive patients respond better to chemotherapy have been with patients receiving either the latest vinca-alkaloid vinorelbine or taxanes. In the remaining studies where patients have been treated with either an anthracycline- or anthracendione-based chemotherapy regimen, a positive c-erbB-2 status was indicative of a worse or equal response compared with c-erbB-2-negative tumours. This is contrary to the adjuvant setting where patients with c-erbB-2-positive tumours benefit from anthracyclines. Indeed, c-erbB-2 overexpression indicated resistance to epirubicin in a trial of 55 patients with metastatic breast cancer receiving weekly epirubicin [11]. However, one might speculate that this result was partly explained by the low dose of anthracycline used (30 mg per square metre of body surface area weekly) which might be suboptimal considering the possible interaction of dose and c-erbB-2 overexpression observed previously [8]. However, in two separate studies no association between treatment response and c-erbB-2 expression was seen in patients receiving either the 5-fluorouracil/doxorubicin/cyclophosphamide (FUC) regimen as neoadjuvant treatment [12] or the 5-fluorouracil/epirubicin/cyclophosphamide (FEC) regimen for metastatic disease [13]. As for the anthracyclines, some interesting recent data indicate that topoisomerase IIalpha may be a better indicator of anthracycline sensitivity than c-erbB-2 [14]. Theoretically, this seems logical since topoisomerase IIalpha is also the target for anthracycline therapy.

So far, only one trial addressing the role of c-erbB-2 expression and clinical taxane sensitivity has been published and even these results are only available in truncated form [3]. In this study, archived paraffin-embedded tumour tissue from 122 patients who were treated with single agent paclitaxel or docetaxel was obtained for immunohistochemistry. Tumours were considered to overexpress c-erbB-2 if at least 10% of tumour cells exhibited characteristic membrane staining. Eighty-four percent of the patients received paclitaxel treatment and 16% docetaxel. Significantly more patients with c-erbB-2-positive tumours than patients with c-erbB-2-negative tumours responded to treatment (64% versus 35%; $P = 0.02$). After adjusting for prognostic factors, it was concluded that the odds for c-erbB-2-positive patients responding clinically were greater than 3 times those the of c-erB-2-negative patients.

In this study, the response in the T group was very similar, irrespective of the c-erbB-2 status. The reason

Table 4
Studies on the predictive value of c-erbB-2 for response to chemotherapy in locally advanced or metastatic breast cancer

Investigator and year [Ref.]	*n*	Chemotherapy	Method and antibody	Cut point	Positive (%)	RR% low	RR% high	Association with RR	*P*-value
MacGrogan *et al.* (1996) [16]	128	Neoadjuvant EVM, MTV	IHC Rabbit poly- clonal, Dako	1	22	NA	NA	Not predictive	NS
Rozan *et al.* (1998) [12]	167	Neoadjuvant FAC	IHC NCL-CB11, Novocastra	No/weak/ strong	19	CR score 0, 20; score 1, 24	CR score 2, 31%	Not predictive for CR	NS
Willsher *et al.* (1998) [17]	50	Neoadjuvant MMM	IHC Rabbit poly- clonal, Dako	5	40	73	30	High worse	0.0025
Colleoni *et al.* (1999) [18]	73	Neoadjuvant FUFAvino	IHC TAB250, Triton	10	10	56	86	High better	0.03
Wright *et al.* (1992) [19]	68	First-line mitoxantrone	IHC NCL-CB11, Novocastra	50	23	40	31	Not predictive	NS
Niskanen *et al.* (1997) [13]	103	First-line FEC	IHC NCL-CB11, Novocastra	50	14	Score 0, 34; score 1, 22	Score 2, 50	Not predictive	NS
Baselga *et al.* (1997) [3]	122	Taxane metastatic	IHC Mouse mono- clonal 4D5	10	38	36	65	High better	0.002
Järvinen *et al.* (1998) [17]	55	First-line E weekly	IHC TAB250, Triton	NA	35	64	32	High worse	0.006
Jukkola *et al.* (2001) [20]	72	Anthracyclines metastatic	IHC NCL-CB11, Novocastra	NA	32	35	4	High worse	NA
Jukkola *et al.* (2001) [20]	52	Non-anthracyclines metastatic	IHC NCL-CB11, Novocastra	NA	29	27	7	High worse	NA

EVM, epirubicin, vincristine, methotrexate; MTV, methotrexate, thiotepa, vincristine; FAC, 5-fluorouracil, doxorubicin, cyclophosphamide; MMM, mitoxantrone, mitomycinc, methotrexate; FEC, 5-fluorouracil, epirubicin; cyclophosphamide; IHC, immunohistochemistry; NA, not available; RR, response rate; CR, complete response; NS, non-significant; E, epirubicin.

for these different results can probably be explained to some extent by patient selection and the different taxane used. Additionally, the primary detecting antibodies used in the two studies were also different. Baselga and colleagues performed immunohistochemical stainings by using murine MoAb4D5 directed at the extracellular domain of $p185^{HER}$, while in this study a polyclonal antibody directed at the intracellular domain of $p185^{HER}$ was used. The US Food and Drug administration-approved HercepTest (DAKO), a commercially available immunohistochemical test kit to determine Her2/neu or c-erbB-2 status, is based on the same rabbit polyclonal antibody as the antibody used in this study. Moreover, a high level of correlation has been reported between fluoresence *in-situ* hybridisation and immunohistochemistry with this antibody in assessing c-erbB-2 status [15].

In this study, 42% of tumours were c-erbB-2-positive. According to the literature the frequency of c-erbB-2 overexpression determined by immunohistochemistry has varied between 14 and 38% in patients with metastatic disease (Table 4). Besides the immunohistochemical procedure and the cut-off point used, the percentage of positive tumours depends also on the patient population. The high incidence of c-erbB-2 overexpression in this study is comparable to the 38% obtained in the study by Baselga and colleagues, where the patients were also heavily pretreated. A cut-off point of 10% between positive and negative tumours was used in both studies and may, in part, account for the high incidence of positive tumours.

The controversial results in trials set in the advanced disease setting makes it difficult to make explicit statements about the definitive value of c-erbB-2 as a predictor of response to chemotherapy. Furthermore, the lack of both a uniformly approved scoring system and method for detecting c-erbB-2 expression emphasises that any conclusions must still be regarded as preliminary. However, our retrospective analysis on the predictive value of c-erbB-2 expression in archived tissues samples of well-characterised patients suggests that tumours overexpressing c-erbB-2 are not uniformly sensitive to taxanes. Taken together with results from other studies, it seems apparent that c-erbB-2 overexpression cannot yet be readily applied as a predictive factor for response to chemotherapy in advanced breast cancer.

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