J Cancer Res Clin Oncol (2007) 133:423–429
DOI 10.1007/s00432-006-0176-7

ORIGINAL PAPER

HER-2/*neu* status and response to CMF: retrospective study in a series of operable breast cancer treated with primary CMF chemotherapy

Catalina Falo · Abelardo Moreno · Mar Varela · Belen Lloveras · Agnès Figueras · Agustín Escobedo

Received: 3 July 2006 / Accepted: 23 November 2006 / Published online: 24 January 2007

Abstract
Purpose Primary chemotherapy brings the opportunity for an early and accurate assessment of response and offers an ideal model to search for new predictors of response. HER-2/*neu* is one of the most studied genes for this purpose.
Patients and methods Her-2/*neu* was tested in a non-randomized series of 300 patients with operable breast carcinomas treated with primary CMF. Response was assessed by mammography. Disease-free survival (DFS) and overall survival (OS) were calculated after a mean follow-up of 116 months. Statistical analysis was performed to study the association between HER-2/*neu* status and response to CMF.
Results Overexpression/amplification was found in 23.66% cases. Univariate analysis showed that response was similar in HER-2/*neu* positive and negative tumors (51.38 vs. 47.36%, $P = 0.6$). Triple negative tumors (ER, PR and HER-2/*neu* negative) presented the highest response rate (64.9%). By multivariate analysis, response was significantly correlated to higher nuclear grade and negative estrogen receptor status ($P = 0.02$ and 0.007, respectively). Patients with HER-2/*neu* positive tumors presented shorter survival rates ($P = 0.06$). Patients with response to CMF showed a better survival over non-responders independent of Her-2/*neu* status. Patients with the combination of response to CMF and Her-2/*neu* negative tumors presented the best outcome. On the other hand, the association of no response to CMF and positive Her-2/*neu* score was statistically related to poor DFS and OS.
Conclusions CMF indication is independent of Her-2/*neu* status.

We have not received any financial support for the development of this study. The authors thank Edurne Arriola for the assistance in the correction of the paper.

C. Falo · A. Moreno · M. Varela · B. Lloveras · A. Figueras · A. Escobedo
Breast Cancer Unit, Institut Català d'Oncologia, Hospital Duran i Reynals, Hospital Universitari de Bellvitge, Barcelona, Spain

C. Falo
Department of Medical Oncology, Breast Cancer Unit, Institut Català d'Oncologia, Hospital Duran i Reynals, L'Hospitalet, Spain

A. Moreno
Department of Pathology, Breast Cancer Unit, Hospital Universitari de Bellvitge, L'Hospitalet, Spain

M. Varela
Tumour Bank, Institut Català d'Oncologia, Hospital Duran i Reynals, L'Hospitalet, Spain

B. Lloveras
Department of Pathology, Institut Català d'Oncologia, Hospital Duran i Reynals, L'Hospitalet, Spain

A. Figueras
Translational Research Laboratory, Institut Català d'Oncologia, Hospital Duran i Reynals, L'Hospitalet, Spain

A. Escobedo
Breast Cancer Unit, Institut Català d'Oncologia, Hospital Duran i Reynals, L'Hospitalet, Spain

C. Falo (✉)
Unitat Funcional de Mama, Institut Català d'Oncologia, Avda Gran Via s/n, Km 2.7, 08907 L'Hospitalet, Barcelona, Spain
e-mail: cfalo@iconcologia.net

Keywords Breast carcinoma · Her-2/*neu* · Primary chemotherapy · Response · Survival

Introduction

Systemic treatment has improved survival in breast cancer. However, prediction of response in a particular patient is not possible because of the absence of reliable predictive markers. Primary chemotherapy brings the opportunity for an early and accurate assessment of response. It offers an ideal model to test chemo-sensitivity in vivo and also to find predictive markers (Bonadonna et al. 1998; Fisher et al. 1998). Until recently, CMF has been the treatment of choice for breast cancer and it is still recommended by St Gallen expert consensus (Goldhirsch et al. 2005).

Amplification/overexpression of Her-2/*neu* is present in about 20–25% of invasive breast carcinomas. Several preclinical studies suggested that its overexpression played a direct role in pathogenesis and aggressiveness of tumors (Bargmann et al. 1986). Her-2/*neu* status has been studied as a predictor of poor prognosis in both lymph node positive and negative cases (Paik et al. 1990; Van de Vijver et al. 1988; Moreno et al. 1997; Reed et al. 2000). Several studies have also reported the relationship between Her-2/*neu* status and response to chemotherapy or hormonotherapy (Allred et al. 1992; Wright et al. 1992).

Herein we report our results about the relationship between Her-2/*neu* and CMF in a series of 300 patients with operable breast carcinomas treated with primary CMF in a single institution, after a mean follow-up of 116 months.

Patients and methods

HER-2/*neu* status was assessed in sections of paraffin-embedded surgical specimens of a series of operable breast cancers. Patients had been included in a protocol of primary chemotherapy approved by our local Research Ethics Committee (number 3490) from January 8, 1990 to December 31, 1999. The study enrolled 305 women with operable breast cancer $T > 3$ cm classified T2-3/N0-1M0. Palpable tumors were required to be assessable by mammography in two dimensions. The criteria for exclusion were: locally advanced or metastatic breast cancer, multicentricity, age under 18 years or over 65 years, pregnancy, history of prior malignancies or severe concomitant systemic disease.

The general characteristics of the series, response and survival results have been previously published (Falo et al. 2005).

Primary CMF protocol

Chemotherapy

Patients were treated with CMF. The CMF consisted of cyclophosphamide, methotrexate and fluorouracil at doses of 600/40/600 mg/m^2 intravenously on days 1 and 8 of each treatment cycle, every 28 days for three courses. In the presence of tumor progression primary chemotherapy was discontinued.

Assessment of response

The size of primary tumor was measured by palpation on the first day of each treatment cycle and before surgery. Response was assessed by mammography by measuring the product of the two largest tumor diameters. The response was classified as complete (cCR) in the absence of evidence of tumor in the breast and the axilla. Partial response (cPR) was defined as a reduction $\geq$50%. Tumor progression was defined as an increase by at least 25%. Cases with complete clinical response that presented breast cancer cells on the surgical specimen, were considered as partial response for statistical purposes (Moreno et al. 2002).

Local treatment

Surgery was planned after three to 4 weeks after the third course of CMF. Conservative surgery with wide tumorectomy was performed when radical and aesthetic criteria allowed it. Selected cases with great response required radiological guided surgery. A few cases were submitted to conservative surgery with the aid of plastic surgery. The remaining patients underwent modified radical mastectomy. In all patients, three level axillary dissection was performed.

Radiotherapy was delivered to all patients subjected to breast-conservative surgery, to mastectomy patients classified as T3 or T4, and to those cases with more than four metastasic axillary lymph nodes. Patients with surgical margins less than 10 mm had a boost of 20 Gy instead of 10 Gy on the tumor bed. Radiotherapy started 3–4 weeks after adjuvant chemotherapy was completed.

Adjuvant treatment

Adjuvant treatment started 15 days after surgery. Cases with response (complete or partial) received three more courses of CMF. Patients without response were treated with doxorubicin at doses of 75 mg/m2 in four courses. Tamoxifen was not the standard of care at that time.

Follow-up

After the completion of the treatment program, physical examination, and hematological tests and blood biochemistry were performed every 3 months for the first 3 years, every 4 months in the 4th and 5th year, and every 6 months thereafter. Mammography was performed once a year starting 6 months from the end of breast irradiation therapy. Other studies were performed annually and included chest X-ray and bone X-ray or bone scans. After the 10th year the follow-up became annual.

Her-2/neu assessment

Her-2/*neu* was detected on formalin-fixed, paraffin-embedded surgical specimens by immunohistochemistry (IHC) and by fluorescence in situ hybridization (FISH), according to the algorithm of our institution (Falo et al. 2003).

The algorithm consisted on testing all the cases by IHC with the monoclonal antibody CB-11 from Biogenex™ (mab CB-11). The negative cases were submitted to a second immunohistochemical run with the HercepTest kit (DAKO™). Finally, those cases considered positive with the HercepTest (score 2+ and score 3+) that had been negative with the monoclonal antibody CB-11 were selected to quantify amplification by FISH with the Oncor Ventana Inform Her-2/*neu* gene detection system™.

Positive cases were defined as a positive immunostaining with the mab CB-11 or as amplified by FISH.

Tumour subclassification

Genomic expression profiling studies on breast tumors have identified distinct subtypes of breast carcinomas that are associated with different responses to chemotherapy and to different clinical outcomes (Van't veer et al. 2002; Sorlie et al. 2001).

Taking into account the recent subclassification of breast carcinomas according to gene expression profiling, we have subdivided our series into the three main groups, i.e., triple negative [estrogen receptor (ER), progesterone receptor (PR) and HER-2/*neu*], luminal tumors (ER or PR positive, HER-2/*neu* negative) and HER-2/*neu* positive tumors.

Statistical analyses

Disease characteristics according to Her-2/*neu* status were compared using chi square tests.

In the univariate analysis, Her-2/*neu* amplification/overexpression has been related to response using chi square tests. Corrections for hormonal receptor expression have been done.

In the multivariate analysis, interrelationship between the different predictive factors (including nuclear grade, hormonal receptor and HER-2/*neu* status) was determined using a logistic regression test. The variable to predict was tumor reduction $\geq$50%, including complete tumor remission.

Disease-free survival (DFS) and overall survival (OS) have been calculated according to the method of Kaplan-Meier. Her-2/*neu* amplification/overexpression has been related to survival (DFS and OS) using the Cox-regression analysis. Survival curves were performed according to triple negative, luminal and HER-2/*neu* positive tumors using the cox-regression model as a survival function for patterns 1–3. Finally, survival was related both to HER-2/*neu* status and to response using the Log-rank test between groups.

All P values were two-sided and a value of $P < 0.05$ was considered statistically significant. Data were analyzed with SPSS™ (version 13.0 Chicago, IL).

Results

Her-2/*neu* status

Three hundred tumors were available for determination. After the application of the mentioned algorithm, 71 cases (23.66%) were considered positive. Disease characteristics according to Her-2/*neu* status are pictured in Table 1. HER-2/*neu* positive tumors were significantly associated with negative hormonal receptors ($P = 0.02$) and with the mean value of pathological involved lymph nodes ($P = 0.05$).

Tumour subclassification

Of the 284 breast carcinomas available for the subclassification, 57 (20%) corresponded to the triple negative subtype, 156 (55%) were luminal and 71 (25%) were HER-2/*neu* positive tumors. These figures are in

Table 1 Tumor characteristics according to HER-2/*neu* status

	HER-2 negative ($n = 229$)		HER-2 positive ($n = 71$)		P (chi square)
	N	%	N	%	
Estrogen R					
Positive	146	65.5	35	51.5	0.027
Negative	77	34.5	33	48.5	
Progesterone R					
Positive	118	55.1	31	47.7	0.153
Negative	96	44.9	35	53.0	
Nuclear grade					
I	19	9	2	2.9	0.253
II	149	70.3	50	73.5	
III	44	20.5	16	23.5	
Tumor size (mm)[a]	37.16		39.31		0.08
Involved nodes[b]	1.88		2.96		0.05

[a] Mean value of clinical tumor size

[b] Mean value of pathological involved lymph nodes

agreement of those published in the literature (Sorlie et al. 2001).

Response

The overall response rate for the whole series was 48.3%.

Univariate analysis

No statistical differences in terms of response rate, were found according to the HER-2/*neu* status. Response was similar for HER-2/*neu* negative and positive tumors (47.59 vs. 50.70% respectively, $P = 0.06$).

The relation between HER-2/*neu* and response was corrected by hormonal receptor status (Fig. 1). The highest response rates were seen in the triple negative tumors (64.9%), compared to HER-2/*neu* positive tumors (50.7%) and luminal tumors (39.7%).

Multivariate analysis

By multivariate analysis, no association was observed between HER-2/*neu* status and tumor response ($P = 0.94$). The only independent predictors of response were estrogen receptor status and nuclear grade ($P = 0.007$ and 0.02, respectively).

Survival

Events

After a mean follow-up of 116 months (range 63+ to 173+), there have been 117 relapses (21 local, 78 systemic, and 18 local and systemic) and 75 deaths. Events according to Her-2/*neu* status are shown in Table 2.

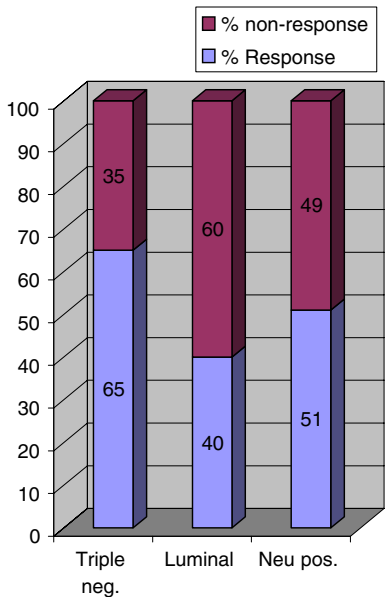


Fig. 1 Response rate according to hormonal receptor and Her-2/*neu* status

Table 2 Events according to HER-2/*neu* status

	N	HER-2 negative	HER-2 positive	P (chi square)
Recurrences	117	87	30	0.29
Local recurrence	21	14	7	0.22
Local failure	39	25	14	0.04
Deaths	75	52	23	0.06

Local recurrence and failure

Local recurrence is defined as recurrence on the ipsilateral breast, or on the ipsilateral chest wall, or in the ipsilateral draining lymph nodes in the absence of systemic disease. Local failure is defined as local recurrence independent of systemic disease.

At the time of the present analysis, there have been 21 local recurrences and 39 local failures.

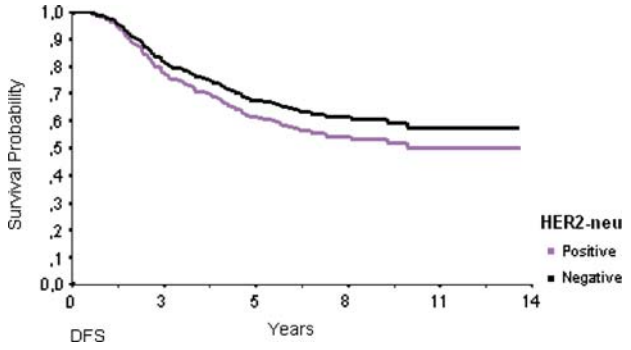


Fig. 2 Disease-free survival (*DFS*) according to HER-2/*neu* status. The *P* values for these two curves are 0.29

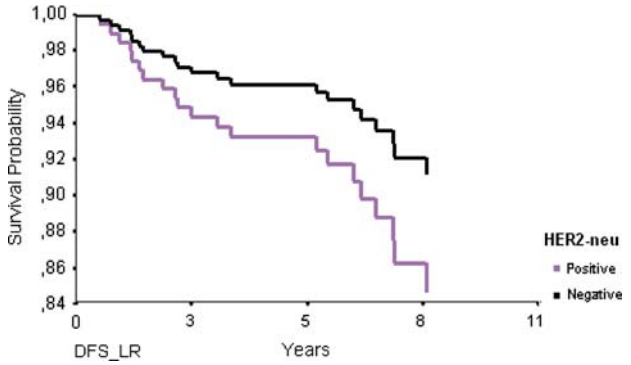


Fig. 3 Disease-free of local recurrence (*DFS LR*) according to HER-2/*neu* status. The *P* values for these two curves are 0.22

Patients with Her-2/*neu* positive tumors presented an increased rate of local failure compared with patients with Her-2/*neu* negative tumors: 19.7 versus 10.9%, respectively. This difference reached standard levels of significance ($P = 0.04$).

Disease free survival, disease free of local recurrence, disease free of local failure and overall survival

The DFS, DFS of local recurrence, DFS of local failure and OS curves are provided in Figs. 2, 3, 4 and 5, respectively.

Patients with Her-2/*neu* positive tumors presented a poorer outcome compared to patients with negative tumors. DFS at 8 years was 53.3 versus 61.5% ($P = 0.29$); disease free of local recurrence was 86.6 versus 92.6% ($P = 0.22$); disease free of local failure was 77.6 versus 88.2% ($P = 0.04$): and OS was 63.3 versus 75.8% ($P = 0.06$).

Survival rates were analyzed taking into account the three subtypes of breast cancer, i.e., basal, luminal and HER-2/*neu* positive tumors. No significant statistical differences were seen between the three groups ($P = 0.6$).

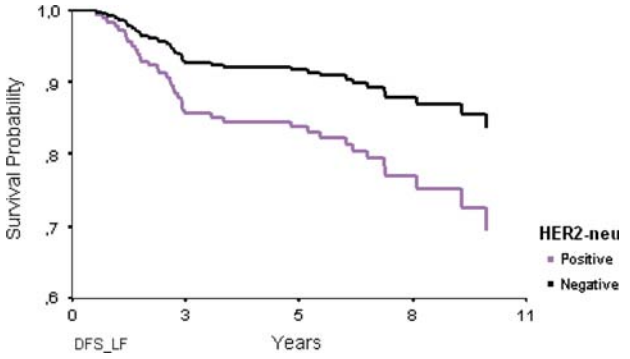


Fig. 4 Disease-free of local failure (*DFS LF*) according to HER-2/*neu* status. The *P* values for these two curves are 0.04

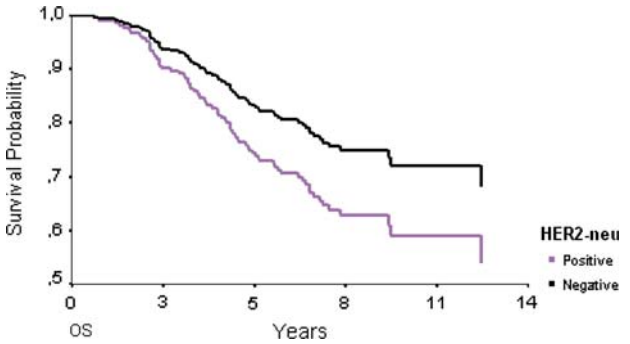


Fig. 5 Overall survival according to HER-2/*neu* status. The *P* values for these two curves are 0.06

Survival related to combined Her-2/*neu* status and response to chemotherapy

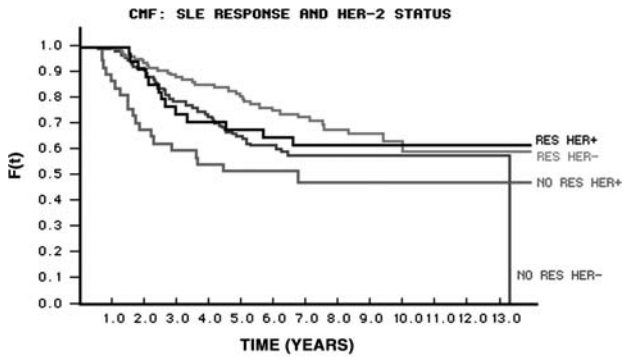
To evaluate survival according to Her-2/*neu* status and response to chemotherapy, four groups were defined as follows: group 1 ($n = 120$): non-response and Her-2/*neu* negative; group 2 ($n = 35$): non-response and Her-2/*neu* positive; group 3 ($n = 109$): response and Her-2/*neu* negative; group 4 ($n = 36$): response and Her-2/*neu* positive.

Events

The number of recurrences by subgroups was the following: group 1: 50 (41.6%); group 2: 19 (54.2%); group 3: 35 (32.1%); group 4: 13 (36.1%). The number of deaths by subgroups was distributed as follows: group 1: 34 (28.3%); group 2: 15 (42.8%); group 3: 16 (14.6%); group 4: 10 (27.7%).

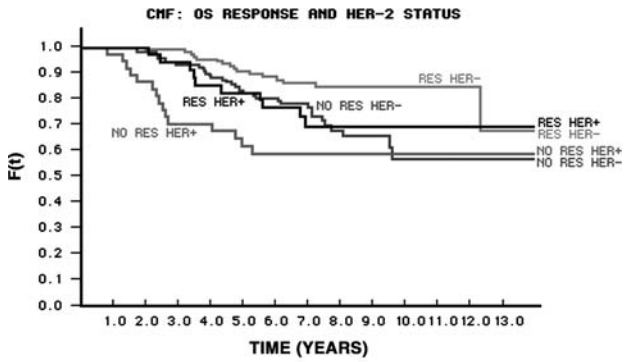
Disease free and overall survival, according to response and HER-2/neu status

The DFS and OS curves according to Her-2/*neu* status and response are provided in Figs. 6 and 7, respectively. Patients with response presented better



Log rank test between groups:	
Groups (1-2): 2.45	p= 0.1171
Groups (1-3): 3.94	p= 0.0471
Groups (1-4): 0.31	p= 0.5751
Groups (2-3): 9.22	p= 0.0023
Groups (2-4): 2.25	p= 0.1333
Groups (3-4): 0.48	p= 0.4903

Fig. 6 Patients with response to *CMF* presented a better DFS compared to non-responders regardless of HER-2/*neu* status



Log rank test between groups:	
Groups (1-2): 4.11	p= 0.0425
Groups (1-3): 7.43	p= 0.0064
Groups (1-4): 0.02	p= 0.8858
Groups (2-3): 15.14	p= 0.0001
Groups (2-4): 1.92	p= 0.1656
Groups (3-4): 2.84	p= 0.0919

Fig. 7 Patients with response to *CMF* presented a better *OS* compared to non-responders regardless of HER-2/*neu* status

survival rates than non-responders, regardless of HER-2/*neu* status.

The best outcome (DFS and OS) was achieved in patients with both response and Her-2/*neu* negative tumors (group 3). On the opposite, non-responders with Her-2/*neu* positive tumors (group 4) presented the worst survival rates.

Discussion

Several studies suggested that Her-2/*neu* was a factor of resistance to tamoxifen (Wright et al. 1992) and to CMF-like regimens (Allred et al. 1992; Gusterson et al. 1992) whereas its overexpression benefited from optimal doses of antracyclines (Muss et al. 1994). Those studies, however, lacked from an optimal methodology for Her-2/*neu* assessment and were not directly designed to answer that question. Other works do not support chemoresistance linked to CMF (Ménard et al. 2001; Miles et al. 1999) and indicate that the benefit of antracyclines is only marginal (Paik et al. 2000), although some recent papers still support the idea of CMF resistance related to overexpression of Her-2/*neu* and $p21^{cip1}$ (Yang et al. 2003).

Information on the association between Her-2/*neu* and response to taxane-based regimens is increasing. Preliminary results from small phase II studies in metastatic breast cancer, showed better response rates in patients with positive tumors (Baselga et al. 1997). These results are being consolidated in further studies (Konecny et al. 2004; Martin et al. 2005).

In the current series, tumor response was measured after primary CMF, which brings the advantage of a direct and earlier assessment of response. By univariate analysis we found no statistical significant differences in the rate of response between Her-2/*neu* negative and positive tumors (47.59 vs. 50.70%, $P = 0.6$). Correction of Her-2/*neu* status for hormone receptor expression was done. Our analysis confirmed that triple negative tumors present the highest response rate to CMF (64.9%) In the multivariate analysis, response to CMF was confirmed to be independent of HER-2/*neu* status ($P = 0.94$), whereas it was significantly related to estrogen receptor status and nuclear grade ($P = 0.007$ and 0.02, respectively).

Patients with response to primary CMF improved survival over non-responders both in Her-2/*neu* positive and negative tumors. These results are in accordance with those of other studies on adjuvant chemotherapy as those of Ménard and Miles but contradict those initial studies of Gusterson and Muss.

As in other studies from the literature (Slamon et al. 1987), in our work, Her-2/neu status is an indicator of poor outcome. Patients with HER-2/*neu* positive tumors presented a shorter DFS and OS, even if the differences did not reach stastistical levels of significance ($P = 0.29$ and 0.06, respectively). In terms of local failure, patients with HER-2/*neu* positive tumors presented a double rate of events (19.7 vs. 10.9, $P = 0.04$). The higher local failure rate has been previously related to resistance to radiotherapy (Haffty et al. 1996), however it has not been confirmed in recent studies (Buchholz et al. 2004).

An interesting point of our study is the analysis of survival combining Her-2/*neu* status and tumor

response. Patients with response to CMF and Her-2/*neu* negative tumors presented the best survival rates. On the opposite, patients without response and Her-2/*neu* positive tumors presented the worst for both DFS and OS. This combination seems more valuable for prediction of prognosis than Her-2/*neu* status or response alone, although a larger series is necessary to support this hypothesis.

In summary, the present study is a retrospective study developed during the 1990's, when CMF was the treatment of choice in breast cancer, which cannot be considered a standard of primary chemotherapy nowadays. However this study gives one of the most solid data sets addressing the question as to whether HER-2/*neu* positive breast cancers are resistant to CMF. By univariate and multivariate analysis, we report no statistical differences in the overall response rate to primary CMF between HER-2/*neu* positive and negative tumors. Accordingly, the indication of CMF in breast cancer must be independent of Her-2/*neu* status.

References

Allred DC, Clark GM, Tandon AK et al (1992) HER-2/*neu* in node-negative breast cancer: prognostic significance of overexpression influenced by the presence of in situ carcinoma. J Clin Oncol 10:599–605

Bargmann CI, Hung MC, Weinberg (1986) The *neu* oncogene encodes an epidermal growth factor receptor-related protein. Nature 319:226–230

Baselga J, Seidman AD, Rosen PP et al (1997) HER2 overexpression and paclitaxel sensitivity in breast cancer: therapeutic implications. Oncology 11:43–48

Bonadonna G, Valagussa P, Brambilla C et al (1998) Primary chemotherapy in operable breast cancer: eight-year experience at the Milan Cancer Institute. J Clin Oncol 16:93–100

Buchholz TA, Huang EH, Berry D et al (2004) HER2/*neu* positive disease does not increase risk of locorregional recurrence for patients treated with neoadjuvant doxorrubicin-based chemotherapy, mastectomy, and radiotherapy. Int J Radiat Oncol Biol Phys 59:1337–1342

Falo C, Moreno A, Lloveras B et al (2003) Algorithm for the diagnosis of HER-2/*neu* status in breast infiltrating carcinomas. Am J Clinical Oncol 26:465–470

Falo C, Moreno A, Benito E et al (2005) Primary chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil in operable breast carcinoma. Cancer 12:657–663

Fisher B, Bryant J, Wolmark N et al (1998) Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 16:2672–2685

Goldhirsch A, Glick JH, Gelber RD et al (2005) Meeting Highlights: International Expert consensus on the primary therapy of early breast cancer 2005. Ann of Oncol 16:1569–1583

Gusterson BA, Gelber RD, Goldhirsch A et al (1992) Prognostic importance of c-erbB-2 expression in breast cancer. J Clin Oncol 10:1049–1056

Haffty BG, Brown F, Carter D et al (1996) 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 Radiat Oncol Biol Phys 35:751–757

Konecny GE, Thomssen Ch, Lück HJ et al (2004) HER-2/*neu* gene amplification and response to paclitaxel in patients with metastatic breast cancer. J Natl Cancer Inst 96:1141–1151

Martin M, Pienkowski T, Mackey J et al (2005) Adjuvant docetaxel for node-positive breast cancer. N Engl J Med 352:2302–2313

Ménard S, Valagussa P, Pilotti S et al (2001) Response to cyclophosphamide, methrotrexate and fluorouracil in lymph node-positive breast cancer according to HER-2 overexpression and other tumor biologic variables. J Clin Oncol 19:329–335

Miles DW, Harris WH, Gillett CE et al (1999) Effect of c-erbB(2) and estrogen receptor status on survival of women with primary breast cancer treated with adjuvant cyclophosphamide/methotrexate/fluorouracil. Int J Cancer 84:354–359

Moreno A, Lloveras B, Figueras A et al (1997) Ductal carcinoma in situ of the breast: correlation between histologic classifications and biologic markers. Mod Pathol 10(11):1088–1092

Moreno A, Escobedo A, Benito E, Serra JM, Guma A, Riu F (2002) Pathologic changes related to CMF primary chemotherapy in breast cancer. Pathological evaluation of response predicts clinical outcome. Breast Cancer Res Treat 75(2):119–125

Muss HB, Thor AD, Berry DA et al (1994) C-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. N Engl J Med 330:1260–1266

Paik S, Hazan R, Fisher ER et al (1990) Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: prognostic significance of c-erbB-2 protein overexpression in primary breast cancer. J Clin Oncol 8:103–112

Paik S, Bryant J, Tan-Chiu E et al (2000) HER2 and choice of adjuvant chemotherapy for invasive breast cancer: National Surgical Adjuvant Breast And Bowel Project Protocol B-15. J Natl Cancer Inst 92:1991–1998

Reed W, Hannisdal E, Boehler PJ et al (2000) The prognostic value of p53 and c-erbB-2 immunostaining is overrated for patients with lymph node negative breast carcinoma: a multivariate analysis of prognostic factors in 613 patients with a follow-up of 14–30 years. Cancer 88:804–813

Slamon DJ, Clark GM, Wong SG et al (1987) Human breast cancer. Correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. Science 235:177–182

Sorlie T, Perou CM, Tibshirani R et al (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 98:10869–10874

Van de Vijver MJ, Peterse JL, Mooi WJ et al (1988) *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 319:1239–1245

van't Veer LJ, Dai H, van de Vijver MJ et al (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature (Lond) 415:530–536

Wright C, Nicholson S, Angus B et al (1992) Relationship between c-erB-2 protein product expression and response to endocrine therapy in advanced breast cancer. Br J Cancer 65:118–121

Yang W, Klos Ks, Zhou X et al (2003) ErbB2 overexpression in human breast carcinoma is correlated with p21Cip1 up-regulation and tyrosine-15 hyperphosphorilation of p34Cdc2: poor responsiveness to chemotherapy with cyclophosphamide, methotrexate, and 5-fluorouracil is associated with ErbB2 overexpression and with p21Cip1 overexpression. Cancer 98:1123–1130