

Impact of Tumor Biological Factors on Response to Pre-operative Epirubicin and Paclitaxel Chemotherapy in Primary Breast Cancer

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Abstract. *Background:* The objective of this study was to determine the predictive impact of several established tumor biological factors (progesterone receptor, estrogen receptor, HER2/neu, and Ki-67) on response to pre-operative combination chemotherapy with epirubicin and paclitaxel in a representative group of primary breast cancer patients. *Patients and Methods:* Thirty-eight primary breast cancer patients (metastasis-negative) received pre-operative chemotherapy with epirubicin and paclitaxel. The response characteristics analyzed included pathological complete response and partial remission determined by 3D ultrasound, as well as down-staging and breast conserving surgery. *Results:* Pathologically complete response occurred in six patients. Overexpression of HER2/neu, low (negative) hormone receptors and high Ki-67 were all significant positive predictive factors for response to pre-operative chemotherapy. *Conclusion:* HER2/neu overexpression is predictive for response not only to trastuzumab, but also to epirubicin and paclitaxel.

In randomized trials, relapse-free and overall survival after pre-operative vs. postoperative therapy are comparable for a given chemotherapy regimen (1-4). Moreover, by down-staging the primary tumor, pre-operative chemotherapy generally increases the proportion of breast cancer patients eligible for breast conserving therapy. Factors that could help to predict response (long-term survival) to adjuvant chemotherapy are

also likely to have an impact on response to chemotherapy in the pre-operative setting. Hence, in view of the rapidly developing tumor biological approaches to breast cancer treatment, identification of tumor biological processes determining pre-operative therapy response could also accelerate progress towards adjuvant therapy individualization and thus contribute to a long-term patient survival benefit. To this end, the impact of several established tumor biological factors (estrogen and progesterone hormone receptors, HER2/neu, and Ki-67) on the response to pre-operative combination chemotherapy with epirubicin and paclitaxel in a representative group of primary breast cancer patients was the focus in this study.

The anthracycline-based agent epirubicin is less toxic than doxorubicin at the same dosage and is widely used in Germany for the treatment of both primary and metastasized breast cancer, as well as for ovarian cancer and soft-tissue sarcoma (5). Though less intensive than doxorubicin (6), the side-effects of epirubicin include hematological and cardiac toxicity, as well as mucositis, nausea and vomiting, reversible hair loss and local skin reactions. The combination of epirubicin with paclitaxel here is based on evidence of its efficacy in adjuvant as well as pre-operative trials and on a basic understanding of its chemotherapeutic action on the tumor (7-9). Adjuvant taxanes have improved overall survival (OS) in several studies (10, 11), in particular the Geicam study (12, 13). The side-effects include allergic reactions, bone marrow and cardiac toxicity, as well as possible cumulative liver complications such as neutropenia, leukocytopenia, thrombocytopenia, anemia and neurological toxicity. Combination chemotherapy is generally considered to be more effective than monotherapy (14-16) and is recommended by the NIH in most cases of primary breast tumors exceeding 1 cm in size (17). The Early Breast Cancer

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Key Words: her2/neu, pre-operative therapy, prediction of therapy response, epirubicin / paclitaxel.

Trialists' Collaborative Group (14, 15) found an advantage of polychemotherapy, including anthracyclines, compared to CMF with respect to disease-free survival (DFS) and OS. However, although the overall efficacy of epirubicin and paclitaxel is supported by evidence, there is much to be learned about the impact of tumor biological factors on the efficacy of these treatments alone and especially in combination (15). As in any treatment regimen with side-effects, it would be of enormous benefit to classify groups of patients most likely to benefit from these therapies. In order to gain as much insight as possible into the processes involved, the pathological and sonographic measures of response were considered primary indicators and down staging and breast conserving operations were considered secondary indicators.

Patients and Methods

This prospective uncontrolled study was conducted in the department of OB/GYN at Cologne University initially on 41 consecutive primary breast cancer patients (metastasis-negative) between 2000 and 2003, who were selected for treatment with combination pre-operative chemotherapy with epirubicin and paclitaxel (Taxol®, Bristol Meyers Squibb, Munich, Germany). The study power was 80% for distinguishing pathologically complete remission (pCR) of 13% from a null hypothesis of 3%. The diagnosis of primary breast cancer included mammography, breast ultrasound and magnetic resonance imaging (MRI). Prior to chemotherapy, high-speed core biopsies were evaluated (blinded assessment) to determine her2/neu (DAKO-Test), Ki-67 and hormone receptor percentages (immunohistochemistry), as well as histology and differentiation. Three of the patients were lost to follow-up for reasons unrelated to their disease stage or treatment, leaving a total of 38 for the end-point analysis. The patient characteristics, including TNM classification, are summarized in Table I. The median age was 49.7 years (range 30-69). Of the 33 patients reporting menopausal status, 17 were premenopausal and seven had undergone hysterectomies.

The study protocol specified that patients should receive up to six cycles of chemotherapy with epirubicin (intravenous one-hour infusion, dose 90 mg/m²) and paclitaxel (dose 175 mg/m²). The dose modification criterion was evidence of progression after 2 or 4 cycles. In this case, a new core biopsy was taken, chemosensitivity was evaluated and a new chemotherapy regimen was determined. Deviations from the chemotherapy protocol were implemented for two out of the 38 patients. Eleven patients received supporting erythropoietin (Erypo®, Ortho Biotech, Bridgewater, NJ, USA) therapy administered according to hemoglobin levels. After completion of the pre-operative therapy, patients who were considered appropriate candidates for breast conserving surgery were offered segmental mastectomy (lumpectomy). Patients who were considered inappropriate for breast conserving surgery or who did not desire it underwent total mastectomy.

Initially, the primary tumor and lymph nodes were classified (18) according to palpitation, ultrasound, mammography and MRI. Lymph node classification prior to surgery was possible in 35/38 cases. Grade (differentiation) and lymph node class were determined initially and after surgery by breast pathologists at the Institute of Pathology, Cologne University. Pre-operative grade refers to core biopsies, post-operative to resected specimens.

End-points and Statistics

Primary end-points. Pathologically clinical complete remission (pCR) was defined as a binary variable (disappearance of all clinical evidence of active tumor as assessed by a pathologist following chemotherapy). Two additional continuous primary variables were defined using sonographic and pathological measurements, which are analogs of the conventional WHO criteria: relative tumor size reduction according to sonography Δ_{sono} was defined as:

$$\Delta_{\text{sono}} = [(V_{\text{sono}}(\text{after}) - V_{\text{sono}}(\text{before}) / V_{\text{sono}}(\text{before}))]^{1/3}$$

relative tumor size reduction according to pathology Δ_{patho} was defined as:

$$\Delta_{\text{patho}} = [(L_{\text{patho}}(\text{after}) - L_{\text{patho, recon}}(\text{before})) / L_{\text{patho, recon}}(\text{before})]$$

The quantities $V_{\text{sono}}(\text{before})$, $V_{\text{sono}}(\text{after})$ refer to the volume of the tumor computed from ultrasound examinations prior to and following chemotherapy, respectively. $L_{\text{patho}}(\text{after})$ refers to the 1-dimensional tumor size reported by the pathologist after chemotherapy. Finally, the quantity $L_{\text{patho, recon}}(\text{before})$ is a surrogate for the 1-dimensional tumor size that a pathologist would have reported if the tumor had been removed prior to chemotherapy. Of course, this quantity is impossible to measure directly, but in our laboratory it was possible to reconstruct this quantity approximately by calibrating the tight empirical relationship between pathological and sonographic size determinations (19). (Incidentally, since the distribution-free statistical tests performed here involve ranks, taking the cube root in the definition of Δ_{sono} actually makes no difference in the analysis performed below.) If tumor size increased according to one of these measures, the corresponding variable was correspondingly negative. The value $\Delta_{\text{patho}} = 1$ corresponds to pCR = 1.

Secondary end-points. The characteristics of down-staging were derived from a comparison of lymph node class and tumor stage before (pN, pT) and after (ypN, ypT) chemotherapy, respectively. To this end, we defined:

$$\Delta pT = ypT - pT \text{ (tumor } in \text{ situ counted as 0.5)}$$

$$\Delta pN = ypN - pN.$$

Defining tumor stage and lymph nodes as metric variables is useful in a small group due to the increased sensitivity of the statistical tests used.

Breast conserving surgery was defined as a binary variable (yes/no).

Statistical methods. Since none of the tumor biological measurements were normally distributed, distribution-free tests or rank statistics were used whenever these variables

Table I. Patient characteristics.

	Ductal	Lobular	Ductolobular	Medullar	Inflammatory	Missing
Histology						
Number	29	5	1	1	1	1
%	76.3	13.2	2.6	2.6	2.6	2.6
pT	T1	T2	T3	T4		
Number	3	30	1	4		
%	7.9	78.9	2.6	10.5		
pN	N0	N1	N2			
Number	18	19	1			
%	47.4	50.0	2.6			
Grade	G2	G2-3	G3	GX		
Number	24	3	10	1		
%	63.2	7.9	26.3	2.6		
Chemotherapy	3 cycles	4 cycles	5 cycles	6 cycles		
Number	1	9	5	23		
%	2.6	23.7	13.2	60.5		
Estrogen receptor status	positive (>10 %)	negative				
Number	27	11				
%	76.3	23.7				
Progesterone receptor status	positive (>10 %)	negative				
Number	20	18				
%	52.6	47.4				
Her2/neu status	positive (3+ by IHC)	negative (all others)				
Number	9	29				
%	23.7	76.3				

were studied as continuous variables. The Wilcoxon test was used for overall improvement in nodal and tumor status. The association of tumor biological factors with the binary variables pCR and breast conservation was analyzed by the Mann-Whitney *U*-test (2-sided). Spearman’s correlations were computed among the continuous variables. *P*-values as reported here do not include corrections for possible multiple testing. The influence of tumor biological factors on the continuous primary measures Δ_{sono} and Δ_{patho} was also evaluated by the Mann-Whitney *U*-test, but here binary variables were defined in the usual way for estrogen and progesterone receptor status, as well as HER2/neu status (see Table I). Ki-67 was treated as a continuous variable in all analyses. Associations between Ki-67 and Δ_{patho} or Δ_{sono} were characterized by Spearman’s correlations.

Results

Correlations and associations. The significant correlations among the tumor biological factors were as follows: Ki-67 was negatively correlated with both estrogen (ER) and progesterone receptor (PgR) percentages, in both cases with Spearman’s correlation $R=-0.42$ ($p=0.01$); ER and PgR were positively correlated at $R=0.505$ ($p=0.001$).

Overall response characteristics. In six of 38 patients, pCR was achieved by pre-operative epirubicin and paclitaxel. The

median sonographically determined tumor reduction Δ_{sono} was about 50% (mean 52%). The median relative tumor size reduction according to pathology Δ_{patho} was also about 50% (mean 47%). These two measurements correspond to approximately a factor of eight reduction in tumor volume. The median improvement in tumor stage was one unit. The improvements reflected in Δ_{sono} , Δ_{patho} and ΔpT were significantly different from zero, $p<0.001$. The improvement in ΔpN was borderline ($p=0.07$) significant, with zero median.

Tumor biological impact on primary measures of response to pre-operative therapy. The impact of the tumor biological factors on pCR is summarized in Table II, which provides the first example of two persistent trends: the patients benefiting from pre-operative chemotherapy tended to be those with lower progesterone receptor measurements but higher levels of Ki-67.

The impact of tumor biological factors on response was computed by studying relative tumor size reduction Δ_{sono} and Δ_{patho} defined above, which in contrast to pCR are continuous variables. The results for HER2/neu and the receptors are summarized in Table III. Both negative ER status and negative PgR status were associated with better response, *i.e.*, significantly larger tumor reductions according to Δ_{sono} and Δ_{patho} . This response advantage for ER-negative and PgR-negative patients is quantified in Table III in terms of the fractional rank within the population, amounting to 7

Table II. Impact of tumor biological factors on pathologically complete remission (pCR). Average fractional rank of each factor in group with pCR is compared to corresponding average in group without pCR.

Factor	N	pCR=1	Ave. fractional rank difference between groups	p	Association
HER2/neu overexpression	36	6		n.s.	
Estrogen receptor % (ER)	36	6		n.s.	
Progesterone receptor % (PgR)	36	6	-0.25	0.045	lower PgR → better pCR
KI67	33	6	0.30	0.033	higher KI67 → better pCR

pCR: pathological complete remission; n.s.: not significant.

Table III. Impact of HER2/neu and receptors on relative tumor size reduction Δ_{sono} and Δ_{patho} . For each factor, average fractional rank of Δ_{sono} and Δ_{patho} in the group with positive status is compared to corresponding average in the group with negative status.

Factor	Measure of response							
	Δ_{sono}				Δ_{patho}			
	N	Ave. fractional rank difference	p	Association	N	Ave. fractional rank difference	p	Association
HER2/neu status	37	0.29	0.008	HER2+ → tumor reduction	36	--	n.s.	
ER status	37	--	n.s.	--	36	-0.25	0.017	ER- → tumor reduction
PgR status	37	-0.20	0.036	PgR- → tumor reduction	36	-0.20	0.045	PgR- → tumor reduction

Sono: sonography; patho: pathology; ER: estrogen receptor; PgR: progesterone receptor; n.s.: not significant.

or 8 ranks out of 36 or 37, respectively. Table III also shows that positive HER2/neu status was significantly associated with better response (as characterized by Δ_{sono}), with a difference corresponding to about 11 ranks out of 37. Finally, there was a significant Spearman's correlation of $R=0.345$ ($p=0.049$) between Ki-67 and Δ_{patho} . This correlation is consistent with Table II in the sense that higher levels of Ki-67 are associated with better partial response. Ki-67 was significantly and positively correlated with grade (Spearman's correlation $R=0.430$, $p=0.011$), but the tumor grade itself was not significantly correlated with response by any of the measures available here.

From a knowledge of the continuous indicators, Δ_{sono} and Δ_{patho} , response was also classified according to WHO response evaluation criteria (19, 20). The above statistical tests were repeated using these classifications and led to the same qualitative results as those in Table III.

Down-staging. The two down-staging indicators, ΔpT and ΔpN , were significantly correlated (Spearman's $R=0.42$, $p<0.001$). The data for ΔpT were available in all 38 patients (Table II). Considering ΔpT as a down-staging indicator, the impact of the tumor biological factors on down-staging was quantified in terms of Spearman's correlations (Tables

IV, V). Again, the directions of all effects were consistent with those of the primary response indicators. The relationships were also significant according to the Mann-Whitney test for PgR status ($p=0.001$) and HER2/neu status ($p=0.049$). The improvement of lymph node status ΔpN was computed in the 38 cases available (see Table VI) as a second down-staging indicator. None of the tumor biological factors led to significant effects according to Spearman's correlations, but negative ER status was significantly associated with down-staging according to the Mann-Whitney test ($p=0.047$). The remaining tumor biological trends observed in relation to lymph node status, though not statistically significant, were consistent with the trends for the primary response indicators. Tumor grade was not a significant factor for down-staging.

Breast conserving surgery. Breast conserving therapy was carried out in 24/38 patients (63.2%). In the case of the PgR, lower receptor levels were significantly associated with favorable results ($p=0.022$). The trends of all four tumor biological factors (ER, PgR, HER2, Ki-67) on the secondary end-point, breast conserving surgery, indicated a similar effect as their respective influence on the primary variables. Tumor grade had no significant influence.

Table IV. Relationship between tumor stage before (pT) and after (ypT) pre-operative chemotherapy.

	pT=1	pT=2	pT=3	pT=4
ypT=0	1	4	0	0
ypT=Tis	0	1	0	0
ypT=1	1	11	0	2
ypT=2	1	13	0	1
ypT=3	0	0	1	1
ypT=4	0	1	0	0

Tis: Tumor *in situ*.

Safety data, side-effects. There were no severe adverse events, such as cardio-toxicity or neutropenia; the frequencies of less severe side-effects were not unusual.

Discussion

Regarding the group as a whole, our study confirms the efficacy of pre-operative chemotherapy with the combination epirubicin and paclitaxel at the dosages given here. The overall characteristics of response, down-staging and breast conserving surgery were consistent with what was expected from many previous studies (16, 18-32). Of key interest is the relative efficacy of this therapy regimen in the pre-operative setting. Our pre-operative study supports such a classification and could shed light on the long-term survival issues in the adjuvant setting as well. With other chemotherapy regimens, previous studies have mostly shown either no difference or an advantage for pre-operative treatment (1-2, 28, 33-37). For example, the NSABP B-18 study showed comparable DFS, metastasis-free survival and OS when the same chemotherapy regimen was compared in the pre-operative and adjuvant setting (2). To the extent that they tip the balance between the survival advantage of pre-operative and adjuvant chemotherapy, the evaluation of tumor biological factors such as those studied here could be a key determinant of optimal individualized primary treatment in the future. Specifically, our results have provided evidence that a stronger response to pre-operative epirubicin and paclitaxel chemotherapy occurred in patients with Her2/neu overexpression (3+), high Ki-67 levels, low levels of progesterone receptor (PgR-negative) and (to a lesser extent) low levels of estrogen receptor (ER-negative). This is in agreement with findings (3, 38) that negative hormone receptor status is a predictive factor for pre-operative chemotherapy. Our results also are consistent with the findings of the GEPARTRIO study (38) that negative hormone receptor status and high levels of the proliferation factor Ki-67 are associated with higher complete remission rates for pre-operative chemotherapy

Table V. Impact of the tumor biological factors on down-staging (ΔpT) in terms of Spearman's correlations.

Factor	N	R	p	Association
HER2/neu expression	38	-0.40	0.013	overexpression → down-staging
Estrogen receptor % (ER)	38	0.36	0.026	lower ER → down-staging
Progesterone receptor % (PgR)	38	0.51	0.001	lower PgR → down-staging
KI67	35	-	n.s.	

n.s.: not significant.

Table VI. Improvement of lymph node status.

	pN=0	pN=1	pN=2
ypN=0	15	8	0
ypN=1	3	11	1
ypN=2	0	0	0

pN: lymph node class before chemotherapy; ypN: lymph node class after chemotherapy.

in breast cancer (41). To our knowledge, this study provides the first evidence for the predictive role of these factors for the pre-operative chemotherapy combination of epirubicin and paclitaxel.

Our study contributes to the existing evidence (41) that HER2/neu overexpression is a (positive) predictive factor not only for response to trastuzumab, but also for pre-operative chemotherapy, here using epirubicin and paclitaxel. A pre-operative study (41) demonstrated an increased benefit with respect to response for the combination of chemotherapy with trastuzumab compared to chemotherapy alone in patients overexpressing HER2/neu. This increased benefit is all the more remarkable in light of our finding of HER2/neu overexpression as a predictive factor for response to chemotherapy even without trastuzumab. Our findings of a stronger response to pre-operative epirubicin and paclitaxel chemotherapy in patients with low levels of hormone receptor are consistent with the results of the NASBP, GBG and ECTO studies (21, 31, 56). Higher hormone receptor levels (or positive status) have a favorable predictive impact on long-term survival with respect to adjuvant endocrine therapy. This predictive impact manifests itself statistically as a (possibly time-varying) factor interaction between endocrine therapy and hormone receptors in (mostly older) patient groups in

which some patients receiving endocrine therapy were receptor-negative and some receptor-positive patients received no endocrine therapy (42, 43). However, there is little evidence for statistical factor interactions between hormone receptors and chemotherapy in the adjuvant setting, *i.e.*, a predictive role of hormone receptors for response to chemotherapy. Hence, our study would provide support for pre-operative chemotherapy at least in patients with both hormone receptors low/negative. For patients with either ER- or PgR-positive status, other studies considered the potential advantages of pre-operative endocrine therapy (44, 45). If PgR status is negative and ER status positive, the evidence points to a favorable response to either pre-operative endocrine therapy or pre-operative chemotherapy. There is as yet no evidence available regarding the benefits of a combination of both therapy regimens in the pre-operative setting for this patient group. A significant survival benefit (DFS and OS) was demonstrated, regardless of hormone receptor status, for those patients who do achieve pCR (46). Nonetheless, if the probability of achieving pCR is higher for receptor-negative patients, then inclusion of receptor status in the decision between pre-operative chemotherapy *vs.* adjuvant therapy is still important.

Highly proliferating tumors (*e.g.*, those with high Ki-67, S-phase or MIB-1) are generally associated with decreased DFS and OS (47-50). In particular, although the proliferation marker Ki-67 is an established prognostic factor in breast cancer with unfavorable *prognostic* impact, it is quite intriguing that the *predictive* impact in the pre-operative setting for our study was favorable, *i.e.*, higher values were associated with a better response. Incidentally, although Ki-67 was significantly and positively correlated with tumor grade ($R=0.43$, $p=0.011$), this association of proliferation with therapy response does not appear to be an artifact of a mutual association with grading, since tumor grade itself was not significantly associated with response. The clinical response to pre-operative therapy would be expected to reflect substantial reductions in the proliferation of breast cancer cells. Our results for pre-operative epirubicin and paclitaxel chemotherapy confirm the generally observed positive association between high cell proliferation and "chemosensitivity", particularly the response to pre-operative chemotherapy (51, 53). Hence, our results directly support the use of pre-operative chemotherapy for patients with high levels of Ki-67 and suggest the possible utility of testing other proliferation markers such as S-phase fraction and MIB-1 for their predictive impact in the pre-operative regime, particularly for epirubicin and paclitaxel chemotherapy.

Different tumor biological processes important for breast cancer metastasis are known to act on different time scales, as reflected in time-varying effects of factors such as uPA/PAI-1 (54). Hence, although the evidence at present does not clearly

distinguish between pre-operative and adjuvant benefit from a given chemotherapy regimen, a closer look at the time-scales of different processes might indeed aid in choosing subgroups of patients for whom pre-operative therapy is preferred or not. For instance, it has been found (55) that the latency time to breast cancer after known exposure to ionizing radiation is inversely related to proliferation. Since some tumors proliferate quickly but are less dangerous in terms of invasion, angiogenesis and other metastatic processes, one can imagine that such tumors might be excellent candidates for pre-operative therapy, which would slow down the dominant metastatic process in these tumors (proliferation) and help confine disease at an early stage.

The evidence from this and other studies thus points toward a predictive effect of proliferation markers for favorable response to pre-operative chemotherapy. This response might be strong enough to provide a long-term survival advantage to this group of patients compared to those receiving the same chemotherapy regimen in the adjuvant setting. A trial of breast cancer patients with positive proliferation markers (S-phase fraction or Ki-67), randomized between pre-operative and adjuvant chemotherapy treatment arms would be helpful. Trials to test the predictive impact of negative receptors, especially of PgR, on response to pre-operative chemotherapy as well as on long-term survival compared to adjuvant chemotherapy would also be useful. Larger pre-operative chemotherapy trials could allow more precise multivariate analysis and possibly improved identification of patient subgroups benefiting from pre-operative chemotherapy, based on combinations of tumor biological factors rather than on individual factors.

References

- 1 Fisher B, Brown A, Mamounas E, Wieand S, Fisher E, Robidoux A, Margolese R, Cruz A, Wickerham DL and Wolmark N: Effect of preoperative therapy for primary breast cancer (BC) on local-regional disease, disease-free survival (DFS) and survival (S): results from NSABP B-18. *Proc Am Soc Clin Oncol 16*: abstr 449, 1997.
- 2 Fisher B, Brown A, Mamounas E, Wieand S, Robidoux A, Margolese RG, Cruz AB, Fisher ER, Wickerham DL, Wolmark N, DeCillis A, Hoehn JL, Lees AW and Dimitrov NV: Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: Findings from National Surgical Adjuvant Breast and Bowel Project B-18. *J Clin Oncol 15*(7): 2483-2493, 1997.
- 3 Untch M, Ditsch N, Bauerfeind I, Georges B, Kahlert S and Konecny G: Primäre Chemotherapie beim Mammakarzinom. *In: Diagnostik und Therapie des Mammakarzinoms - State of the Art - (eds.)*. W. Zuckerschwerdt Verlag München, 2002.
- 4 Veronesi U, Bonadonna G, Zurrida Stefano, Galimberti V, Greco M, Brambilla C, Luini A, Rilke F, Raselli R, Merson M, Sacchini V and Agresti R: Conservation surgery after primary chemotherapy in large carcinomas of the breast. *Ann Surg 222*(5): 612-618, 1995.

- 5 Glueck S: The expanding role of epirubicin in the treatment of breast cancer. *Cancer Control* 9(2): 16-27, 2002.
- 6 Fumoleau P, Bonnetterre J and Luporsi E: Adjuvant chemotherapy for node-positive breast cancer patients: which is the reference today? *J Clin Oncol* 21(6):1190-1191, 2003.
- 7 Schiff PB, Fant J and Horwitz SB: Promotion of microtubule assembly *in vitro* by taxol. *Nature* 227: 665-667, 1979.
- 8 Dye RB, Fink SP and Williams Jr RC: Taxol-induced flexibility of microtubules and its reversal by MAP-2 and Tau. *J Biol Chem* 268: 6847-6850, 1993.
- 9 Bartsch V, Höffken K, Kreienberg R and Bamberg M: *Das Taxol® - Buch*, Thieme Verlag, Stuttgart - New York, 2000.
- 10 Ring AE and Ellis PA: Taxanes in the treatment of early breast cancer. *Cancer Treat Rev* 31: 618-627, 2005.
- 11 Nabholz JM and Gligorov J: The role of taxanes in the treatment of breast cancer. *Expert Opin Pharmacother* 6: 1073-1094, 2005.
- 12 Martin M, Rodriguez-Lescure A, Ruiz A *et al*: Multicenter, randomized phase III study of adjuvant chemotherapy for node-positive breast cancer comparing 6 cycles of FEC *versus* 4 cycles of FEC followed by 8 weekly paclitaxel administrations: interim analysis of GEICAM 9906 trial. *Breast Cancer Res Treat* 94(suppl 1): abstr 39, 2005.
- 13 Martin M, Pienkowski T, Mackey J, Pawlicki M, Guastalla JP, Weaver C, Tomiak E, Al-Tweigeri T, Chap L, Juhos E, Guevin R, Howell A, Fornander T, Hainsworth J, Coleman R, Vinholes J, Modiano M, Pinter T, Tang SC, Colwell B, Prady C, Provencher L, Walde D, Rodriguez-Lescure A, Hugh J, Loret C, Rupin M, Blitz S, Jacobs P, Murawsky M, Riva A and Vogel C; Breast Cancer International Research Group 001 Investigators: Adjuvant docetaxel for node-positive breast cancer. *N Engl J Med* 352(22): 2302-2313, 2005.
- 14 Early Breast Cancer Trialists' Collaborative Group: Multi-agent chemotherapy for early breast cancer. *Cochrane Database Syst Rev* 1: CD000487 (review), 2002.
- 15 Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 365: 1687-1717, 2005.
- 16 Thomas A, Ohlinger R, Hauschild M, Mustea A, Blohmer JU and Kümmel S: Option and limit of surgery after preoperative chemotherapy in breast cancer. *Anticancer Res* 26(2C): 1677-1682, 2006.
- 17 Eifel P, Axelson JA and Costa J: National Institutes of Health Consensus Development Conference statement: adjuvant therapy for breast cancer Nov 1-3, 2000. *J Natl Cancer Inst* 93: 979-989, 2001.
- 18 Schmidt-Matthiesen H, Bastert G and Wallwiener D: *In: Gynäkologische Onkologie* (eds.). Schattauer Verlag, S. pp: 128-131, 2002.
- 19 Sinn HP, Schmid H, Junkermann H *et al*: Histologische Regression des Mammakarzinoms nach primärer (neoadjuvanter) Chemotherapie. *Geburtsh u Frauenh* 54: 552-558, 1994.
- 20 Kimura M and Tominaga T: Outstanding problems with response evaluation criteria in solid tumors (RECIST) in breast cancer. *Breast Cancer* 9(2): 153-159, 2002.
- 21 Bonadonna G, Veronesi U and Brambilla C: Primary chemotherapy to avoid mastectomy in tumors with diameters of three centimeters or more. *J Natl Cancer Inst* 82: 1539-1545, 1990.
- 22 Anderson ED, Forrest AP and Hawkins RA: Primary systemic therapy for operable breast cancer. *Br J Cancer* 63: 561-566, 1991.
- 23 Jacquillat C, Weil M and Baillet F: Results of neoadjuvant chemotherapy and radiation therapy in the breast-conserving treatment of 250 patients with all stages of infiltrative breast cancer. *Cancer* 66: 119-129, 1990.
- 24 Smith IE, Jones AL and O'Brien ME: Primary medical (neoadjuvant) chemotherapy for operable breast cancer. *Eur J Cancer* 29A: 1796-1799, 1993.
- 25 Smith IE, Walsh G, Jones A and Prendiville J: High complete remission rates with primary neoadjuvant infusional chemotherapy for large early breast cancer. *J Clin Oncol* 13: 424-429, 1995.
- 26 Chollet P, Charrier S and Brain E: Clinical and pathological response to primary chemotherapy in operable breast cancer. *Eur J Cancer* 33: 862-866, 1997.
- 27 Bilchert-Toft M, Smola MG, Cataliotti L and O'Higgins N: Principles and guidelines for surgeons - management of symptomatic breast cancer. *European Society of Surgical Oncology. Eur J Surg Oncol* 23: 101-109, 1997.
- 28 Fisher B, Bryant J, Wolmark N, Marmounas E, Brown A, Fisher ER, Wickerham DL, Begovic M, DeCillis A, Robidoux A, Margolese RG, Cruz AB, Hoehn JL, Lees AW, Dimitrov NV and Bear HD: Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol* 16: 2672-2685, 1998.
- 29 Bonadonna G, Valgussa P, Brambilla C, Ferrari L, Moliterni A, Terenziani M and Zambetti M: Primary chemotherapy in operable breast cancer: eight-year experience at the Milan Cancer Institute. *J Clin Oncol* 16: 93-100, 1998.
- 30 Hortobagyi G and Budzar AU: Locally advanced breast cancer: a review including the M.D. Anderson experience. *In: High Risk Breast Cancer*. Ragaz J, Ariel I (eds.). Springer Verlag, Berlin, pp: 382-415, 1991.
- 31 von Minckwitz G: Taxane in der primären systemischen Therapie des Mammakarzinoms. *Onkologie* 26(7): 21-25, 2003.
- 32 Kaufmann M, von Minckwitz G, Smith R, Valero V, Gianni L, Eiermann W, Howell A, Costa SD, Beuzebec P, Untch M, Blohmer JU, Sinn HP, Sittke R, Souchon R, Tulusan AH, Volm T and Senn HJ: International Consensus Panel on the primary systemic treatment of breast cancer-meeting highlights and recommendations. *J Clin Oncol* 21: 2600-2608, 2003.
- 33 Fisher B, Anderson S, Redmond CK, Wolmark N, Wickerham DL and Cronin WM: Reanalysis and results after 12 years of follow-up in a randomised clinical trial comparing total mastectomy with lumpectomy with or without irradiation in the treatment of breast cancer. *N Engl J Med* 345: 1378-1387, 2001.
- 34 Untch M, Konecny G, Ditsch N, Sorokina Y, Moebus V, Muck B, Kuhn W, Bastert G, Werner C, Thomssen C, Wallwiener D, Albert U, Bothmann G, Kreienberg R and Lück HJ: Dose-dense sequential epirubicin-paclitaxel as preoperative treatment of breast cancer: Results of a randomised AGO study. *Proc Soc Clin Oncol* 16: abstr 133, 2002.
- 35 Mauriac L, Durand M, Avril A and Dilhuydy JM: Effects of primary chemotherapy in conservative treatment of breast cancer patients with operable tumors larger than 3 cm. Results of a randomized trial in a single centre. *Ann Oncol* 2(5): 347-354, 1991.

- 36 Scholl SM, Fourquet A, Asselain B, Pierga JY, Vilcoq JR, Durand JC, Dorval T, Palangié T, Jouve M, Beuzeboc P, Garcio-Giralt E, Salmon RJ, de la Rochefordière A, Campana F and Pouillart P: Neoadjuvant versus adjuvant chemotherapy in premenopausal patients with tumours considered too large for breast conserving surgery: preliminary results of a randomised trial: S6. *Eur J Cancer* 30A: 645-652, 1994.
- 37 Jakesz R for the ABCSG: Comparison of pre- vs. postoperative chemotherapy in breast cancer patients: four-year results of Austrian Breast & Colorectal Cancer Study Group (ABCSG) Trial 7. *Proc Am Soc Clin Oncol* 20: abstr 125, 2001.
- 38 von Minckwitz G, Blohmer JU, Raab G, Lohr A, Gerber B, Heinrich G, Eidtmann H, Kaufmann M, Hilfrich J, Jackisch C, Zuna I and Costa SD; German Breast Group: *In vivo* chemosensitivity-adapted preoperative chemotherapy in patients with early-stage breast cancer: the GEPARTRIO pilot study. *Ann Oncol* 16(1): 56-63, 2005.
- 39 Euler U, Buehner M, Tio J, Dresel V, Rinas N, Schulze W and Tulusan A: Primary systemic chemotherapy (PST) with dose and time intensified epirubicin (E) and cyclophosphamid (C) in locally advanced breast cancer (BC) - an attempt to identify predictive markers. *Proc Am Soc Clin Oncol* 21: abstr 126, 2002.
- 40 Ross JS, Fletcher JA, Bloom KJ, Linette GP, Stec J, Symmans WF, Puzstai L and Hortobagyi GN: Targeted therapy in breast cancer: the HER-2/neu gene and protein. *Mol Cell Proteomics* 3: 379-398, 2004.
- 41 Buzdar AU, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, Puzstai L, Green MC, Arun BK, Giordano SH, Cristofanilli M, Frye DK, Smith TL, Hunt KK, Singletary SE, Sahin AA, Ewer MS, Buchholz TA, Berry D and Hortobagyi GN: Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. *J Clin Oncol* 23: 3676-3685, 2005.
- 42 Schmitt M, Thomssen C, Ulm K, Seiderer A, Harbeck N, Höfler H, Jänicke F and Graeff H: Time-varying prognostic impact of tumor biological factors urokinase (uPA), PAI-1, and steroid hormone receptor status in primary breast cancer. *Br J Cancer* 76(3): 306-311, 1997.
- 43 Kates R, Schmitt M and Harbeck N: Advanced statistical methods for the definition of new staging models. *Recent Results in Cancer Research* 162: 101-113, 2003.
- 44 Cheung KL, Howell A and Robertson JF: Preoperative endocrine therapy for breast cancer. *Endocr Relat Cancer* 7: 131-141, 2000.
- 45 Paepke S, Jacobs VR, Paepke D, Euler U, Blohmer JU, Warm M, Ohlinger R, Fischer T, Kiechle M and Harbeck N: Critical appraisal of primary systemic endocrine therapy in receptor-positive postmenopausal breast cancer: an update. *Onkologie* 29(5): 210-217, 2006.
- 46 Guarneri V, Broglio K, Kau SW, Cristofanilli M, Buzdar AU, Valero V, Buchholz T, Meric F, Middleton L, Hortobagyi GN and Gonzalez-Angulo AM: Prognostic value of pathologic complete response after primary chemotherapy in relation to hormone receptor status and other factors. *J Clin Oncol* 24(7): 1037-1044, 2006.
- 47 Wintzer HO, Zipfel I, Schulte-Monting J, Hellerich U and von Kleist S: Ki-67 immunostaining in human breast tumors and its relationship to prognosis. *Cancer* 67: 421-428, 1991.
- 48 Pinder SE, Wencyk P, Sibbering DM, Bell JA, Elston CW, Nicholson R, Robertson JF, Blamey RW and Ellis IO: Assessment of the new proliferation marker MIB1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. *Br J Cancer* 71: 146-149, 1995.
- 49 Brown RW, Allred CD, Clark GM, Osborne CK and Hilsenbeck SG: Prognostic value of Ki-67 compared to S-phase fraction in axillary node-negative breast cancer. *Clin Cancer Res* 2: 585-592, 1996.
- 50 Railo M, Lundin J, Haglund C, von Smitten K, von Boguslawsky K and Nordling S: Ki-67, p53, Er-receptors, ploidy and S-phase as prognostic factors in T1 node negative breast cancer. *Acta Oncol* 36: 369-374, 1997.
- 51 Bonetti A, Zaninelli M, Rodella S, Molino A, Sperotto L, Piubello Q, Bonetti F, Nortilli R, Turazza M and Cetto GL: Tumor proliferative activity and response to first-line chemotherapy in advanced breast carcinoma. *Breast Cancer Res Treat* 38: 289-297, 1996.
- 52 Chevillard S, Pouillart P, Beldjord C, Asselain B, Beuzeboc P, Magdelenat H and Vielh P: Sequential assessment of multidrug resistance phenotype and measurement of S-phase fraction as predictive markers of breast cancer response to neoadjuvant chemotherapy. *Cancer* 77: 292-300, 1996.
- 53 MacGrogan G, Mauriac L, Durand M, Bonichon F, Trojani M, de Mascarel I and Coindre JM: Primary chemotherapy in breast invasive carcinoma: predictive value of the immunohistochemical detection of hormonal receptors, p53, c-erbB-2, MiB1, pS2 and GST pi. *Br J Cancer* 74: 1458-1465, 1996.
- 54 Harbeck N, Kates R and Schmitt M: Clinical relevance of invasion factors uPA and PAI 1 for individualized therapy decisions in primary breast cancer is greatest when used in combination. *J Clin Oncology* 20(4): 1000-1009, 2002.
- 55 Olsson H, Baldetorp B, Ferno M and Perfekt R: Relation between the rate of tumour cell proliferation and latency time in radiation associated breast cancer. *BMC Cancer* 3: 11, 2003.
- 56 Gianni L, Baselga J, Eiermann W, Porta VG, Semiglazov V, Garcia-Conde J, Zambetti M, Valagussa P and Bonadonna G: First report of the European Cooperative Trial in operable breast cancer (ECTO): Effects of primary systemic therapy (PST) on local-regional disease. *Proc Am Soc Clin Oncol* 21: abstr 132, 2002.

Received September 29, 2006

Revised February 6, 2007

Accepted February 7, 2007