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Is ERBB-2 A predictive marker for response to primary chemotherapy for operable breast cancer: A prospective study in a phase II randomized trial of doxorubicin/cyclophosphamide (AC) and doxorubicin/paclitaxel (AT)

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The association between amplification of erbB-2 and poor outcome of patients with N+ primary breast cancer is well known. Our aim was to determine if erbB-2 activation may predict the response to primary chemotherapy.

T2, T3, N0, N1, M0 breast cancers were randomized in AC arm (Doxorubicin 60 mg/m^2, CPM 600 mg/m^2) versus AT (Dox 60 mg/m^2, Taxol 200 mg/m^2) every 3 weeks for 4 cycles, then surgery and radiotherapy. Primary objective was the pathological complete response (pCR); secondary objectives: objective response rate (ORR), disease-free survival, and overall survival, erbB-2 determination was performed at molecular and protein level on AFA-fixed, paraffin embedded biopsies, by competitive quantitative PCR and immunohistochemistery using CB11 monoclonal antibody.

To date, tumor samples of 89 patients were analyzed (64 in AT arm, 25 in AC). Patients characteristics were: T2 (59%) T3 (41%), SBR I 8%, SBR II 39% SBR III 52%, Hormone Receptor (HR) 64% (ER+ and PR+), 19% (ER+ or PR+) 18% (ER− and PR−), median S Phase 4% (range 0.2–23.4%). erbB-2 was overexpressed in 21% and erbB-2 amplified in 29% of informative samples. There was a highly significant concordance between erb-B2 gene amplification status and protein expression (93%, chi2 test $p < 0.001$). In 88 pts evaluable for response, we observed 75% ORR (6 Complete Responses and 60 Partial Responses) and 4 pCR. ORR was correlated with S Phase ($p < 0.001$) and erbB-2 overexpression ($p < 0.02$).

In conclusion, at the time of the analysis, we observed a correlation of erbB-2 expression with ORR.

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Her 2 positivity and ER negativity predicts the complete pathological response of breast cancers treated with primary docetaxel

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Background: Complete pathological resolution of tumour following primary chemotherapy is of considerable prognostic importance in patients with breast cancer. The addition of docetaxel to an anthracycline based primary chemotherapy regimen has been shown to result in significantly improved pathological breast cancer response. The identification of predictors of treatment response will permit cytotoxic regimens to be tailored to individual patient requirements and permit pathological response rates to be improved. Oestrogen receptor (ER) status and tumour grade are known prognostic factors for survival, although their ability to predict the response of breast cancers to chemotherapy is uncertain. Expression of the oncogene her-2 has been shown to predict susceptibility to doxorubicin and resistance to cyclophosphamide. However, the predictive value of her-2 expression in patients who receive docetaxel is unknown.

Aim: To identify any relationship between ER status, tumour grade, her-2 expression and complete pathological response (PathCR) in patients with breast cancer following treatment with docetaxel.

Method: Histopathological parameters were studied on core biopsies taken from 34 patients with breast cancer prior to receiving primary docetaxel. Tumours were typed and graded. Immunohistochemistry was carried out using a standard three-stage avidin biotin peroxidase complex technique. Antigen retrieval employed microwave technology. Specific monoclonal antibodies (with appropriate controls) were used to detect oestrogen receptors and Her-2 oncoprotein. Pathological response was determined from operative specimens.

Results: Univariate and multivariate analysis (UA and MVA) (logistic regression) were used to assess the predictive power of each variable. UA revealed that ER negativity ($p = 0.023$) and Her-2 positivity ($p = 0.023$) distinguished patients with complete pathological response. MVA suggested that her-2 positivity ($p = 0.045$) and ER negativity ($p = 0.010$) independently predict a PathCR. High tumour grade did not predict a PathCR on UA or MVA.

Conclusion: Breast cancers that express Her-2 oncoprotein and/or are oestrogen receptor negative are more likely to achieve a PathCR to primary docetaxel.

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Expression of endothelial and inducible nitric oxide synthase in benign and malignant lesions of the breast

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Background: Nitric oxide synthases (NOS) exists in different human solid tumours and tumour cell lines. Increased NO-concentration, catalysed by NO-synthases (NOS) might be cytotoxic and can promote apoptosis. Expression of endothelial (e-) and induced (i-) NOS was examined in various breast tissues.

Methods: An immunohistochemical staining with a monoclonal antibody (Ab) against e-NOS and a polyclonal Ab against i-NOS was performed on paraffin-embedded tissue of 41 benign, 7 in-situ and 54 invasive breast lesions. Functionality was confirmed indirectly by detection of dinitrosyl-iron complexes (DNC) using electron-spin-resonance (ESR)-spectroscopy.

Results: e-NOS expression was found in 5% of the benign and in 57% of in-situ and 61% of invasive lesions. One fibroadenoma and one proliferative mastopathia stained positive for e-NOS. No benign lesion was positive for i-NOS but 73% of in-situ and 61% of invasive cancers showed staining of endothelial and epithelial tumour cells. Some regions in the tumours showed no staining whereas especially capillaries embedded in lymphocytic stroma showed a positive reaction. ESR-spectrum of 5 invasive carcinomas was axial symmetric to standard DNC representing approximately 50 nM DNC. e-NOS positive tumours appeared more often in younger patients, were more frequently highly or moderately differentiated, more often invasive ductal subtypes, and showed a lower proliferation rate. e-NOS and i-NOS positive tumours were more likely to be node negative. Both, i- and e-NOS showed a strong co-expression.

Conclusions: NOS are predominantly detected in in-situ and invasive but rarely in benign breast lesions. NOS are more frequently found in low malignant invasive carcinomas.

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Immuncytochemical detection of cytokeratin (CK)-positive micro-metastases in lymph nodes (LN) and bone marrow (BM) from node-negativ breast cancer patients with stage I–II disease

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Purpose: Since the detection of BM micrometastases at the time of first diagnosis has been described as independent predictor of poor prognosis in node-negative breast cancer patients, we investigated in the present study whether hematogenous tumor cell dissemination is paralleled by lymphatic spread of single tumor cells.

Methods: At the time of this analysis, we have screened 599 axillary LNs of level I and 63 BM samples obtaind from 63 node-negative patients. Tumor cells in BM and LN were detected using the anti-CK antibodies A45-B/B3 and 5D3, respectively. To avoid interference with CK$^+$ mesenchymal cells present in LN, we applied double-labeling with anti-vimentin and anti-CD45 antibodies to LNs paraffin sections. Of each single LN, adjacent sections were analysed immuncytochemically and compared to independently screened hematoxylin-eosin (HE) stainings. Median follow-up was 30 months (range, 5–50).

Results: We found tumor cells in 27 (5%) of 599 axillary LNs, which resulted in 22 (35%) of 63 patients with CK$^+$ tumor cells in LNs, whereas all HE stainings revealed no tumor cells. Hematogenously disseminated tumor cells were found in 18 (29%) of 63 patients. Neither LN nor BM micrometastasis correlated with established risk factors for recurrence. In 22 patients with CK$^+$ LNs, 3 (14%) relapses had occurred, while 9 (22%) relapses were observed in 41 patients with CK$^-$ LNs ($P = 0.42$). In 18 patients with CK$^+$ BM, 3 (17%) relapses had occurred, while 9 (20%) relapses were observed in 45 patients with CK$^-$ LNs ($P = 0.76$). A single recurrent patient had CK$^+$ tumor cells in both BM and LNs. Distant

metastasis had occurred in one patient with CK+ micrometastases in BM, but not in LN.

Conclusion: With the short follow-up, our analysis remains descriptive at the present time. However, there appears to be no correlation between lymphatic and hematogenous tumor cell spread. This suggests the existence of biologic differences between these two tumor cell populations that might be elucidated by molecular characterization of these cells, which is part of further studies.

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A real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to detect breast carcinoma cells in peripheral blood

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Background: The detection of occult carcinoma cells in patients with breast cancer has been shown to predict disease recurrence and metastasis. To improve on existing methods of immunocytologic and molecular detection we have developed a sensitive and quantitative assay to detect breast carcinoma cells in blood, using real-time quantitative RT-PCR identifying transcripts of the cytokeratin-19 (CK19) gene.

Methods: The ABI Prism 7700 (Taqman®) technology is based on the cleavage of a probe labelled with both a fluorescent reporter and a quencher dye, by the 5, −3, exonuclease activity of Taq polymerase during strand elongation of PCR. This cleavage results in an increase in reporter emission intensity, which corresponds to the target sequence concentration. The fluorescent signal is measured during the exponential phase of product amplification, which ensures sensitive real-time quantification. The cycle at which the emission intensity rises above baseline is referred to as Ct (threshold cycle). The Ct values decrease linearly with increasing target quantity. To evaluate the sensitivity of the assay, we measured CK19 mRNA concentrations after RT-PCR in MDA-231 and EFM-19 breast cancer cells spiked in the CK19 negative AML14 cell line. Parallel amplification of the b-actin house keeping gene allowed normalisation of the target concentration.

Results: Primers and probe were developed specifically for the detection of CK19 transcripts with this technique using Primer Express software. Amplification was specific for CK19 mRNA, no amplification of pseudogenes was observed. CK19 transcripts were still detectable when 0.5 MDA-231 and 1 EFM-19 cell were diluted in 106 CK19 negative cells. The Ct for 100% positive cells was 19.25 for MDA-231 and 15.5 for EFM-19; for 0.5 MDA-231 cells/106 AML14 cells the Ct was 36.8 and for 1 EFM-19 cells/106 AML14 cells the Ct was 30. The correlation coefficient of the standard curve (target cell quantity versus Ct value) was at least 0.98.

Conclusion: We have developed a sensitive, accurate real-time quantitative RT-PCR with high reproducibility within a wide dynamic range, which permits simultaneous analysis of samples with varying input concentrations. The procedure offers several technical advantages over classic quantitative PCR methods (competitive RT-PCR, Northern blotting) such as decreased likelihood of contamination due to absence of post-PCR manipulations, high sample throughput because of absence of post-PCR processing time (no agarose gel electrophoresis). Analyses using this real time quantitative RT-PCR for CK19 mRNA may prove to have clinical implications in the assessment of circulating tumour cells in peripheral blood, micrometastases in bone marrow or lymph nodes in breast cancer patients. Validation of application of this technique in a clinical population may improve diagnosis and monitoring of metastatic breast cancer.

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HER2 interaction with intermediate filament proteins and the influence on prognosis in breast cancer

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Purpose: The oncoprotein HER2 seems to affect cell adhesion and metastasis via structural proteins. In this context we investigated the influence of intermediate filament proteins (IF) on prognosis in relation to HER2 in breast cancer.

Methods: Paraffin embedded specimens of 80 primary invasive ductal breast carcinomas were analyzed immunhistochemically (APAAP) for the expression of *keratin* (K) *8*, characterizing normal breast epithelia, *K19*, inconstantly expressed in normal breast epithelia, *vimentin*, characterizing mesenchymal epithelia and the oncoprotein *HER2*. The results were compared with disease-free (DFS) and overall survival (OS) over a ten-years-follow-up.

Result: Strong expression of K8 was seen in 27.5% of the tumors and was correlated with excellent prognosis (DFS/OS: $p < 0.004$). K19 showed a stronger expression than K8 (35%), but did not correlate with prognosis. Aberrant expression of vimentin was seen in 21.3% of the tumors and was associated with poor prognosis (DFS: $p < 0.003$/OS: $p < 0.006$). HER2-overexpression was noticed in 35% and was associated with short survival rates (DFS: $p < 0.01$/OS: $p < 0.02$). HER2 overexpression was significantly correlated with expression of K19 ($p < 0.0004$) and vimentin ($p < 0.0005$).

Conclusions: HER2-overexpression is associated with fundamental changes of the IF-pattern in breast carcinoma cells. Beside the loss of the K8 and K18, expression of K19 and aberrant expression of vimentin mark structural alterations especially in HER2 overexpressing cells, which obviously leads to early metastasis and poor prognosis in breast cancer. IF-changes therefore may play an important role in the malignant functioning of HER2.

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C-ERB-B2 expression as a predictor of outcome in a randomized trial comparing adjuvant CMF vs single-agent epirubicin in stage I–II breast cancer (BC) patients

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Purpose: c-erbB-2 abnormalities either gene amplification or protein overexpression are associated with worse prognosis of pts with N+ BC but the relationship for pts N− is weak at best. Some studies, mostly retrospective, have reported that pts whose tumors have amplified erbB-2 genes or overexpress c-erbB-2 protein benefit from anthracyclines-containing regimen.

Methods: We completed a randomized study comparing CMF versus weekly epirubicin (E) in the adjuvant treatment of 348 stage I and II BC pts (Proc ASCO 19976:142a). We evaluate retrospectively the expression of c-erbB-2 and its interaction with the treatment.

Results: At a median follow-up of 5.6 yrs c-erbB-2 expression was measured by IHC in 266 pts (76%) using the monoclonal antibodies CB11. 133 pts were in CMF arm and 133 in E arm. A baseline significant excess of erbB-2 positive tumors was observed in E arm (41% vs 28%; HR = 2.78; 95% CI 1.13–6.88; $p = 0.03$). DFS and OS were calculated by the Kaplan Meier method. Long rank and Cox models were used to compare DFS and OS for c-erbB-2 status regarded both as a continuous or binary variable. A significantly worse OS was observed for c-erbB-2 positive pts ($p = 0.02$). A Cox regression model including menopausal status, T and n° of positive nodes, treatment and interaction term between arm and c-erbB-2 showed that c-erbB-2 overexpression is a predictor of a poorer OS (HR = 2.78; 95% CI 1.13–6.88; $p = 0.03$). No statistically significant difference was observed between the two arms.

Conclusions: The E treatment had no significant impact on the outcome of pts with erbB-2 tumor positive ($p = 0.12$), but in our study E (30 mg/m^2) was administered weekly for 16 wks. The small number of events (39 deaths) prevents from definitive conclusions.

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Predictors of axillary lymph node involvement in breast cancer <20 mm

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Purpose: Because the frequency of metastatic involvement of axillary lymph node (ALN) in breast cancer (BC) <20 mm is low, the necessity of ALN dissection in these patients (pts.) is under discussion today. If it would be possible to predict the ALN status by parameters of the tumor, a lot of pts. could potentially be spared ALN dissection.

Method: The data of 386 pts. with BC and a tumor size (TS) of 3–20 mm and non palpable and non suspicious ALN by ultrasound were studied retrospectively. Potential predictive histopathological parameters, hormone receptor status and newer factors (HER/2-neu, EGF, Cathepsin D, pS2, DNA-index, S-phase, p53, uPA, PAI-1) were examined with regard to their correlation with the ALN status.