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Prognostic impact of HER-2/neu gene copy number determination by fluorescence in situ hybridisation (FISH) versus immunohistochemically detected overexpression in node positive primary breast cancer

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Purpose: The prognostic impact of HER-2/neu membrane staining in CMF-treated node-positive breast cancer patients is discussed in the literature. FISH represents the latest methodological approach to HER-2/neu copy number quantification in interphase nuclei. We compare the prognostic impact of both techniques on disease-free (DFS) and overall survival (OS).

Methods: A total of 205 node-positive breast cancer patients were drawn from the GBSG trials 2 and 3. After mastectomy all patients received 3 or 6 cycles of CMF (subgroups additionally Tamoxifen or radiotherapy). The median follow-up was more than 8 years. A unique sequence probe (Oncor) was used for amplification analysis. Overexpression was determined immunohistochemically.

Results: The impact of HER-2/neu copy number seemed to exhibit characteristics of a discontinuous function leading to a dichotomization based on a threshold of > = 8 copies for amplification. An amplification was found in 29% of cases, an overexpression in 23%. amplification and overexpression were strongly correlated (r = 0.72). However, a discrepancy was observed in 11% for amplification without overexpression and 12% vice versa. In univariate analysis both membrane staining and amplification showed a highly significant effect for DFS and OS. In an unadjusted bivariate analysis, membrane staining was the dominating factor for both criteria (RR ~ 2.0). Amplification showed a non-significant tendency (RR ~ 1.5). Adjustment for standard prognostic factors (tumor size, grade, age, number of positive nodes, estrogen receptor, progesterone receptor) resulted in a slight reduction of the estimated effects. Interestingly, analysis of amplification in the absence of overexpression might provide additional information regarding both DFS and OS.

Conclusion: In CMF-treated node-positive patients HER-2/neu amplification as well as membrane staining exhibited prognostic impact on DFS and OS. The additional value of amplification after inclusion of membrane staining in the model remains to be further clarified in a larger study.

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Lack of correlation between *c-erbB*-2 overexpression and the effect of dose-intensification of adjuvant chemotherapy in high-risk breast cancer patients

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Purpose: Overexpression of *c-erbB-2* is discussed as a new and important prognostic and predictive factor in breast cancer. Within a prospective, randomised trial on dose-intense adjuvant chemotherapy, we have studied the significance of *c-erbB2* expression for the effect of dose-intensification in high-risk breast cancer patients.

Methods: Patients (n = 182) with (10 tumor-infiltrated axillary lymph nodes or extranodal infiltration were randomly treated or by 4 dose-intense courses of EC 120/600 [mg/m2] q2wks plus G-CSF (HDI-EC), or four courses of EC 90/600 [mg/m2] q3wks, followed by 3 courses of CMF (EC/CMF). Overexpression of *c-erbB2* in the primary tumors was assessed by immunohistochemistry using the monoclonal antibody 3B5 (Oncogene Science).

Results: In 118 evaluable patients, 28% showed an overexpression of *c-erbB2*. During a median follow-up period of 27 months (mo.), 33 recurrences occurred. Treated by EC/CMF, the median disease-free interval (DFS) was significantly worse in patients with overexpression of *c-erbB2* as compared to patients with no overexpression (31 vs 42 mo.; p = 0.04). A significant improvement of the DFS by dose-intensification (applying HDI-EC) could be achieved only in *c-erbB-2* negative tumors (46 vs 37 mo., p = 0.04), while in patients with *c-erbB-2* overexpression only a slight improvement was shown (33 vs 28 mo., p > 0.25).

Conclusion: In *c-erbB-2* negative tumors dose-intensification may result in a better effectiveness of adjuvant chemotherapy. Overcoming chemoresistance by dose-intensification (applying HDI-EC) in patients with *c-erbB-2* overexpression may not be possible.

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A study of molecular markers with potential prognostic and predictive value (c-erb b-2, p53, cyclin D1, MIB1, ER and PgR) in locally advanced breast cancer treated with neo-adjuvant dose intensive chemotherapy in an EORTC-NCIC-SAKK randomized phase III study

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Patients and Methods: From 05/93 to 04/96, 448 patients with locally advanced breast cancer were randomized into a study comparing 2 anthracycline based neoadjuvant regimens with dose-intensity (DI) in arm B (Epirubicin 120 mg/m^2 iv d1, Cyclophosphamide 830 mg/m^2 iv d1 q 2w × 6 and G-CSF: Filgrastim®) being twice DI in arm A (Canadian FEC d1, d8 × 6 q 4w, without G-CSF). Locoregional therapy was flexible and tamoxifen was administered to all patients until progression. Paraffin-embedded tumour-biopsies taken prior to neo-adjuvant chemotherapy from 155 patients included in this study were collected and paraffin sections were analyzed for c-erbB-2, p53, cyclinD1, MIB1, ER and PgR expression by immunohistochemistry. In the statistical modelling these markers were analyzed both as dichotomous and continuous variables using the Cox proportional-hazard model in the multivariate analysis.

Results: Of the 155 patients 70 relapsed and 40 died.

	Univariate analysis		Multivariate analysis					
	PFS	OS	PFS			OS		
	p-value	p-value	p-value	HR	95%CI	p-value	HR	95%CI
C-erbB-2	NS	NS						
p53	0.0001	0.0003	0.008	2.2	(1.3–3.6)	0.04	2	(1.02–3.8)
Cyclin D1	0.0018	0.01	NS			NS		
MIB1	NS	0.03	NS			NS		
ER	0.0001	0.0001	0.001	0.4	(0.23–0.7)	0.001	0.2	(0.1–0.6)
PgR	0.008	0.005	NS			NS		

Discussion: In our study p53 accumulation and ER negativity are associated with a shorter PFS and OS. We have not been able to demonstrate any prognostic value for the other markers. We will present data regarding the predictive value of p53 which has been proposed as a marker of chemoresistance in the literature.

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Heregulin and doxorubicin activate a p53 dependent pathway in cancer cells whereas taxol depends on activation of the MAP kinase pathway

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Purpose: We investigated the activity of the differentiation factor Heregulin/NDF which elevates tyrosine phosphorylation of its receptors erbB-3, erbB-4, and erbB-2 (through heterodimer formation) on breast cancer cells when treated with NDF alone or with combination of doxorubicin and taxol.

Methods: We used immunohistochemistry staining combined with image analysis, western blot analysis and northern blot analysis to investigate p53 and the MAP kinase pathways after treating breast cancer cells with NDF.

Results: We show that NDF/HRG up-regulates expression of p53 and p21. The induction of p21 is further enhanced when cells are treated with both NDF/HRG chemotherapeutic agents (i.e. doxorubicin and taxol). The erbB mediated induction of p21 by NDF/HRG and doxorubicin is dependent on wild type p53. Cellular killing and upregulation of p21 by taxol was independent of p53, but dependent of activation of the MAP kinase pathway.

Conclusion: These observations suggest that the mechanism of cell killing by Taxol is associated with activation of the MAP kinase pathway whereas doxorubicin cell killing depends on the p53 pathway.