seen in patients positive for both 2E11 and one of the two additional markers (UPA or cathepsin D).

The detection of tumor cells in bone marrow is an excellent prognostic marker for the development of metastatic disease. Patients with positive UPA/cathepsin D detection in micrometastatic cells have a worse prognosis and should be considered for a more aggressive adjuvant systemic therapy.

P42 Prognostic significance of mutant p53 in breast cancer patients

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The p53 oncosuppressor gene has a negative influence on cell proliferation. Expression of mutant p53 is frequently seen in breast tumors and has been significantly related with clinical outcome.

At the moment its prognostic role is discussed and this biological parameter is not currently included in decision making about adjuvant therapy in breast cancer.

In order to verify its prognostic role we assessed p53 status in 90 operable breast cancer patients (pts), with a median age of 64 years and a mean follow up period of 31 months. Metastases in axillary nodes were present in 45% of cases; positive estrogen receptor status in 64% and median Ki-67 index was 20.2%. Cut-off level for p53 status was fixed at 5%.

Mutant p53 immunohistochemistry was carried out on formalin-fixed, paraffin embebbed tumor specimens. Surgical and medical adjuvant therapy followed the guidelines of the most important international consensus conferences.

Our data are summarized in the following table and show a significant relationship between p53 status and relapse of disease ($p = 0.01$, Fisher's exact test).

Mutant p53	Relapsed PTS	Disease-free PTS
positive	12	30
negative	4	44

The small number of deaths in our series is not sufficient for statistical analysis but an interesting trend is confirming a negative impact of mutant p53 status on survival.

In our experience mutant p53 status influenced the risk of relapse and, probably, the overall survival. The increasing value of prognostic factors needs a more relevant role in the choice of medical adjuvant therapy of breast cancer.

P43 Evaluation of bone marrow micrometastases as potential surrogate marker for efficacy of adjuvant treatment

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Early occult dissemination of tumor cells to bone marrow (BM) which is detectable in approximately one third of breast cancer patients at the time of diagnosis is assumed a main reason for metastatic relapse. This may provide a rationale for adjuvant treatment. However, currently no concise data are available on the treatment-susceptibility of BM micrometastases. In an ongoing prospective randomized adjuvant trial, node-positive (>4 nodes involved) breast cancer patients were treated with 6 courses of DE (q21d), docetaxel (75 mgm^{-2})-epirubicin (60 mgm^{-2}), *vs* 4 courses of EC (q21d), epirubicin (90 mgm^{-2})-cyclophosphamide (600 mgm^{-2}) followed by 3 courses of CMF (q21d), cyclophosphamide (600 mgm^{-2})-methotrexate (40 mgm^{-2})-fluorouracil (600 mgm^{-2}). Our hypothesis was that clearance of BM from micrometastatic tumor cells is a surrogate marker for treatment efficacy translating into improved disease-free survival. In follow-up BM asapirations, disseminated tumor cells were detected using anti-cytokeratin (CK) monoclonal antibody A45-B/B3. To date, 17 patients have been randomized. Prior to treatment CK^+ cells were detected in 6 of 17 patients (35%). After treatment all previously CK^+ patients treated with DE experienced elimination of CK^+ cells; two of three patients treated with EC/CMF remained CK^+. One, respectively two negative-to-positive transitions were notified in patients treated with DE and EC/CMF. In a total of 53 DE courses (10 patients) and 41 EC/CMF courses (7 patients), side-effects of NCI grades 3 or 4 included hematoxicity (83% *vs* 39%; $P < 0.0001$), alopecia (100% *vs* 63%; $P < 0.0001$), allergic reactions (2% *vs* 0%; P = N.S.). No early cardiotoxicity, fluid retention, febrile neutropenia from chemotherapy as well as no osteomyelitis or hemorraghia from BM aspirations have been observed so far. G-CSF was administered for neutropenia (<500/μl) in 44 DE courses (83%) and 16 EC courses (39%; $P < 0.0001$). In conclusion, our preliminary data suggest that DE might be more effective to eliminate CK^+ cells from BM than EC/CMF. Of course, the low number of patients analysed so far, revealed no significant difference, but an early trend in favor of our hypothesis. Overall, DE was well tolerated in the adjuvant setting, although hematoxicity appeared to be a limitating factor of this combination. Follow-up BM aspirations did not add any notable risk for patients' health. Clinical follow-up will have to demonstrate whether persistence and disappearance of micrometastases are related to treatment-resistance and -susceptibility, respectively.

P44 Age is not a prognostic factor in breast cancer patients with combined losses of heterozygosity (LOH) in BRCA1 and BRCA2 regions

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Aim: Old patients with breast cancer can exhibit a more indolent behavior than young patients. However, this assertion can be changed by alterations in specific genes or chromosomic regions. The cloning of susceptibility genes for breast cancer, BRCA1 and BRCA2 and the study of their chromosomic regions, offer the opportunity of to know their implications like prognostic factors.

Methods: We investigated the presence of LOH in 17q21 and 13q12–13 regions. The molecular findings were correlated with the following pathological parameters: histologic type, tumor size, lymph node metastases, oestrogen receptors, progesterone receptors, histologic grade and peritumoral vessel involvement, in 98 patients with sporadic breast cancer. For the allelic deletion, four microsatellite markers were studied in BRCA1 region (D17S855, D17S856, D17S1323, D17S1327) and two in BRCA2 region (D13S310, D13S260), they were amplified by PCR method and the products were run in 12% nondenaturing polyacrilamide gels and stained with a commercially available silver method.

Results: After analyze the normal tissue of all patients, 49 cases resulted informative for both regions. Fifteen women did not show LOH in any microsatellite marker of both regions; 9 patients displayed LOH at least in one marker of the BRCA1 region; 10 cases in the BRCA2 region and 15 patients exhibited LOH concomitantly in some marker of the two regions. When we compare the pathological parameters between patients with LOH at both regions and those without LOH we observed statistically significant differences in lymph node metastases ($p = 0.0002$), oestrogen receptors ($p = 0.02$), progesterone receptors ($p = 0.003$), histologic grade ($p = 0.02$) and peritumoral vessel involvement ($p = 0.0009$). For to know if old age in the patients offer a protective effect, respect to these poor pathologic characteristics observed in patients with combined losses at two regions, we divided these positive patients in two subgroups, <50 years old and >50 years old, and analyzed the distribution of these parameters in both subgroups. No differences in the distribution of the parameters studied were found.

Conclusions: The data presented in this study suggest that combined losses of the 17q21 and 13q12–13 regions is associated with a poor tumor pathophenotype in patients with breast cancer, and probably with a poor prognosis. Also, these alterations can influence in the similar pathological behavior between patients older and younger than 50 years.

P45 Tumorbiological factors (uPA, PAI-1) as selection criteria for adjuvant chemotherapy in axillary node-negative breast cancer patients

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Morbidity and mortality in breast cancer are caused by the capability of the tumor cells for invasion and metastasis. Tumor-derived proteases are a prerequisite for the dissolution of the tumor surrounding structures enabling the tumor cell to invade and metastasize. Evidence has accumulated that the urokinase-type plasminogen activator (uPA) and its specific inhibitor PAI-1 play a central role in tumor-related proteolysis, invasion and metastasis.

uPA and PAI-1 were quantified (ELISA) in tissue extracts of 316 breast cancer patients. The median follow-up was 77 months (41–108). Optimized cutoff-levels were used for uPA (3 ng/mg protein), PAI-1 (14 ng/mg protein), cathepsin D (45 pmol/mg protein), and S-phase-fraction (7%).

In the multivariate analysis only nodal status (RR = 3.1; $p < 0.0001$), and PAI-1 (RR = 2.5; $p < 0.0001$) were of independent prognostic significance. In *147 node-negative patients* PAI-1 (RR: 3.6; $p < 0.0001$) and uPA (RR: 2.1; $p < 0.049$) were found to be the only independent prognostic factors for disease-free survival (DFS). S-phase, hormone receptors, cathepsin D and tumor size did not add prognostic information in the Cox model. Since uPA and PAI-1 are independent factors, node-negative patients can be grouped further by a combination of these two variables. Node-negative patients with tumors of low content of both uPA *and* PAI-1 have an especially good outlook (93.1% 5-year DFS) in contrast to patients with high content of uPA and/or PAI-1 (67.6% 5-year DFS).

Based on these results a prospective randomized study supported by the "Deutsche Forschungsgemeinschaft" (DFG) was initiated, in which patients