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

F. Castiglione, I. Sarotto[1], M. Destefanis, M.T. Ricco, G. Porcile. [1]*Medical Oncology Service and Histopathology Service, Civic Hospital, Alba, Italy*

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

S. Braun, C.R.M. Kentenich, W. Janni, F. Hepp, J. de Waal, H.L. Sommer. *Ludwig-Maximilians-University, München, Germany*

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

J.M. Silva, R. Gonzalez, G. Dominguez, J.M. Garcia, G. Martinez, F. Navarro, M. Provencio, P. España, F. Bonilla. *Department of Medical Oncology, Clinica Puerta de Hierro, Madrid-28035, Spain*

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

F. Jänicke[1], C. Thomssen[1], N. Harbeck[2], A. Prechtl[2], U. Berger[3], K. Ulm[3], P. Dettmar[4], L. Pache[2], M. Schmitt[2], H. Graeff[2]. [1]*Frauenklinik und Poliklinik, Universitätskrankenhaus Eppendorf, Hamburg, Germany;* [2]*Frauenklinik und Poliklinik, Germany;* [3]*Institut für Medizinische Statistik und Epidemiologie, Germany;* [4]*Institut für Allgemeine Pathologie und Pathologische Anatomie der Technischen Universität, Klinikum rechts der Isar, Munich, Germany*

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

with high values of uPA and/or PAI-1 are randomized to 6 cycles CMF versus observation. Patients with low content of both uPA and PAI-1 are distributed to observation only. In this trial, 13 German clinical centers take part; to date 628 patients are recruited. It is now going to be extended to an European trial, supported by the BIOMED-2 program.

P46 15 year update of the naples GUN trial of adjuvant breast cancer therapy: Evidence of interaction between c-erb-B2 expression and Tamoxifen efficacy

A.R. Bianco, M. De Laurentiis, C. Carlomagno, R. Lauria G. Petrella, L. Panico, G. Pettinato, F. Perrone, C. Gallo[1], A. Marinelli, S. De Placido. *University Federico II, Italy; [1]II University, Naples, Italy*

From February 1978 to December 1983, 433 patients (pts) were enrolled in the GUN trial. Ten year results have already been reported (Bianco et al., Lancet, 1988). Postmenopausal pts (n = 308) with (N+) or without (N−) lymph node metastases were randomized to receive either Tamoxifen (TAM), 30 mg qd for 2 years or no therapy. Premenopausal N+ pts (n = 125) were randomized to receive either CMF × 9 cycles or CMF × 9 cycles plus Tamoxifen (TAM), 30 mg qd for 2 years. Overall 206 pts were randomized to receive TAM versus 227 pts not receiving the antioestrogen (no TAM). c-ErbB2 expression was evaluated on 245 paraffin-embedded specimen by immunohistochemistry. At 15 years, when the median follow-up was about 11 years, TAM was effective in improving both Disease Free Survival (DFS) (p = 0.0008) and Overall Survival (OS) (p = 0.05). When pts were stratified according to menopausal and lymph-node status DFS and OS benefits were observed in all subgroup of pts receiving TAM. More interestingly, when we evaluated the c-erbB2*TAM interaction the following results were found:

	Relapses Obs/Exp		Deaths Obs/Exp	
	c-erbB2− (n = 182)	c-erbB2+ (n = 63)	c-erbB2− (n = 182)	c-erbB2+ (n = 63)
TAM	0.83	1.17	0.85	1.56
no TAM	1.18	0.89	1.15	0.64

According to these data TAM seemed to improve DFS and OS only in c-erbB2− pts, while showing a paradoxical detrimental effect in c-erbB2+ pts. A multivariate test for interaction adjusting by lymph node status, menopausal status, nuclear grade, estrogen receptor (ER) status and ER*TAM interaction confirmed the predictive value of c-erbB2 expression (OS p = 0.007; DFS p = 0.04). In conclusion, a) at 15 years adjuvant TAM reduces relapse and death rates independently by nodal and menopausal status; b) in our randomized trial c-erbB2 expression is a strong predictor of adjuvant tamoxifen failure independently by ER, ER*TAM interaction and other major prognostic variables.

P47 Locally advanced breast cancer (LABC): Prognostic variables affecting results

E. Baldini[1], G. Gardin[2], R. Lionetto[2], E. Montanaro[2], B. Salvadori[1], R. Massa[1], R. Rosso[2], P.F. Conte[2]. *[1]Dipartimento di Oncologia U.C. di Oncologia Medica, Pisa, Italy; [2]Istituto Scientifico Tumori (IST), Genova, Italy*

Purpose: To evaluate the prognostic significance of clinical and hystopathological indicators on disease free survival (DSF), overall survival (OS) and objective response (OR) rates in Locally Advanced Breast Cancer (LABC) patients (pts) treated with a multimodality therapy.

Patients and Methods: Three-hundred sixty-seven assessable LABC pts entered onto two consecutive randomized trials performed in our Medical Oncology Dept. and North-West Oncology Group (GONO) cooperative centers. In the first study 117 pts were randomly allocated to receive either 3 courses of primary FAC (5-FU 600 mg/sqm, ADM 50 mg/sqm, 5-FU 600 mg/sqm day 1 every 21) followed by local-regional treatment (surgery and/or radiotherapy) and 6 courses of adjuvant chemotherapy consisting of 3 FAC alternated with 3 CMF (CTX 600 mg/sqm, MTX 40 mg/sqm, 5-FU 600 mg/sqm day 1 every 21) or the same program in which chemotherapy was preceded by oral Dethylstilbestrol (DES 1 mg/day for 3 consecutive days). In the second study 150 pts were randomized to receive either a standard primary FEC (5-FU 600 mg/sqm, EpiDX 60 mg/sqm, CTX 600 mg/sqm day 1 every 21 days) or an accelerated FEC every 2 weeks with GM-CSF (5 um/kg/day for 10 days s.c.).

Results: ORs to primary chemotherapy was 64% (95% C.I. 58–70%). On univariate analysis, performed on pts characteristics at diagnosis, pts with inflammatory breast cancer (IBC) had a significantly lower probability of response than pts who did not (p = 0.04); no other differences in response rates were observed between pre-menopausal and post menopausal pts, estrogen (ER) an/or progesterone (PgR) receptor positivity, stages of disease (IIIA vs IIIB). The median PFS and OS of the whole group were 3.5 and 5.1 years respectively. On univariate analysis, performed at surgery, no correlations were observed between age, menopausal status, stage of disease (IIIA vs IIIB), response to primary chemotherapy (pathological complete response, residual disease >/≤1 cm) and DFS or OS. Pts with. ER and/or PgR receptor positivity had a better OS rates compared to pts with ER and/or PgR negative tumors (p = 0.02 and p = 0.03 respectively): hormonal receptor positivity did not affect DFS. Pathological evidence of IBC significantly correlates with OS (p = 0.0005) but not with DFS (0.07). The number of positive nodes at surgery significantly predicts both DFS and OS (p = 0.0003 and p = 0.003 respectively).

Conclusion: in LABC pts treated with a multimodality therapy hormonal receptor positivity at surgery significantly correlates with a better OS;IBC significantly correlates with a poor outcome however is nodal status at surgery the strongest prognostic factor associated with PFS and OS. A multivariate analysis will be presented.

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P48 Prognostic value of estrogen receptor (ER) status in breast cancer patients with five or more axillary lymph nodes (LN) involved

L. Estévez, B. Marcos, V. Casado, L. Robles, J. Andrade, J.M. San Román, C. Rivas, A.G. Ageitos, M. Dómine, J. Vicente, F. Lobo. *Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Madrid, Spain*

Objective: To find parameters influencing the risk of relapse in breast cancer patients with five or more positive axillary LN treated with adjuvant intensive chemotherapy.

Methods: 31 patients up to 65 years of age received six cycles of an antracycline containing chemotherapy regimen followed by intensive chemotherapy with peripheral blood progenitor cell rescue and local irradiation. Patients with ER+ tumors continued on adjuvant therapy with tamoxifen later. Survival analysis identified ER status as prognostic factor for relapse in our series. ER+ and ER− patients were homogeneous for age, menopausal status, number of involved axillary LN, and expression of Ki-67, c-erb-b2, and p53.

Results: Median number of positive axillary LN was 10. Median relapse free survival was 23 months for ER− patients and no ER+ patient has relapsed with a median follow up of 23.5 months (p = 0.0008). ER+ patients tended to have lower stages of the disease but the adjusted analysis yielded ER status as an independent factor for risk of relapse.

Conclusion: Our preliminary data show that in our series ER status appears to be the main predictor of relapse. We can not rule out that adjuvant treatment with tamoxifen is responsible for the better outcome of ER+ patients.

P49 Predictive parameters of response in primary CMF chemotherapy of breast cancer

A. Escobedo, A. Moreno, B. Loveras, A. Figueras, F. Riu, C. Gimenez, L. Prieto. *Unitat Funcional Mama, Institut Català d'Oncologia, L'Hospitalet, Barcelona, Spain*

Introduction: In breast cancer, primary chemotherapy has proved to be useful in the evaluation of drugs effectiveness. It also permits a greater rate of conservative surgical procedures.

Objective: To assess factors that may predict the response to primary chemotherapy.

Design: Longitudinal study.

Patients and Methods: From January 1990, 150 patients with breast cancer were treated with primary chemotherapy. Conditions to join the study were tumor size greater than 3 cm. and age less than 66. Tumor diameter was measured by mammography. All patients had a positive cytology or a minute open biopsy that was also used for the evaluation of nuclear grade and the immunocytochemical determination of hormone receptors and c-erb-B2 expression. Patients were treated with CTX, 600 mgr/m2; MTX, 40 mgr/m2; and 5-FU, 600 mgr/m2; on days 1 and 8, for three cycles.

The tumor response was evaluated by mammography.

Results: A good response was assessed in 52% of cases (6% CR, 46% PR ≥50%). The proportion of responses was 30% in grade 1 tumors; 49% in grade 2 tumors and 73% in grade 3 tumors (p < 0.01). Negative/positive estrogen receptors were associated with responses of 66% vs. 41% (p < 0.002).

Tumor diameter, progesterone receptors and c-erb-B2 expression were not related to response.

Conclusion: High nuclear grade and lack of estrogen receptors expression are predictive parameters of good response to primary CMF chemotherapy in operable breast cancer.

P50 Effectiveness of postoperative radiotherapy in controling subclinical locoregional disease in breast cancer patients with positive axillary nodes

S. Korzeniowski. *Department of Radiotherapy, Centre of Oncology, Kraków, Poland*

Background: Results of randomized trials indicate that postmastectomy radio-