sis-related genes *c-myc*, *p53* and *bcl2* in breast cancers (BC) in association with the main prognotic markers after a long-term follow-up.

Methods: Total RNAs and paraffin-embedded tissue sections were obtained from 175 primary tumors and analysed for *c-myc* transcripts by Northern blot and *p53* and *bcl-2* proteins by immunohistochemistry. All patients were treated by surgery and by adjuvant therapy according to their clinicobiological characteristics. The risk of death associated with the expression of these genes was evaluated by multivariate analysis taking into account the main prognostic factors, namely, tumor size, histoprognostic grade (HG), hormonal receptors (HR) and nodes, after a mean follow-up of 9.5 ± 2 yrs.

Results: *c-myc* overexpression was observed in 35% of BC and *p53* and *bcl2* overexpression was found in 23 and 63% of BC, respectively. A strong association between *c-myc* overexpression and positive nodes ($p = 0.0005$), *p53* expression and both high HG ($p = 0.0001$) and HR-negative tumors ($p = 0.0003$) was shown. In contrast, *bcl-2* expression was found to be associated with favorable prognostic factors including HR-positive tumors ($p = 0.0001$). Multivariate analysis showed that only positive nodes ($p = 0.0001$) and *bcl-2* expression ($p = 0.008$) were independent factors correlated to a higher or lower risk of death, respectively.

Conclusion: Both *c-myc* and *p53* genes favor the development of more aggressive BC. In contrast, the anti-apoptotic function of *bcl2* seems to be most hypothetical in BC. Expression of *bcl2* identifies a particular phenotype of BC with a favorable long-term prognosis and thus may be a useful marker.

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Prognostic significance of p53 mutations in the zinc-binding domains (L2/L3) in lymph node-positive breast cancer patients

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Purpose: Mutations of the p53 tumor suppressor gene have been shown to be of prognostic significance in the node-negative breast cancer population. We investigated the prognostic value of p53 mutations in the functionally important L2/L3 zinc-binding domains in lmph node-positive breast cancer patients.

Methods: For detection of p53 mutations we used in vitro amplification by polymerase chain reaction and consecutively performed temperature gradient gel electrophoresis (PCR-TGGE). We evaluated if mutations in the L2/L3 domains provide prognostic information for node-positive breast cancer patients.

Results: Out of 213 tumor samples we found p53 mutations in 48 cases (23%). About a third of the p53 mutations ($n = 17$) were located in the L2/L3 domains. Univariate analysis revealed that patients with p53 mutated tumors had a statistically significant shorter relapse free survival (RFS) ($p = 0.034$). However, in multivariate analysis no significant correlation between p53 mutated tumors and reduced RFS could be detected. We could not detect any significant difference regarding RFS and overall survival (OS) for patients with p53 mutations in the L2/L3 domains.

Conclusion: p53 mutations in the L2/L3 domains in node-positive breast cancer patients do not provide information regarding the clinical outcome of these patients.

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mRNA determination of estrigen receptor, progestin receptor, pS2. PAI-1 by competitive reverse transcription-PCR in human breast cancer

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Purpose: Estrogen receptor (ER) and progestin receptor (PR) are important regulators of growth and differentiation of the mammary gland as well as in the development of malignant tumors. pS2 is an estrogen inducible protein, which is supposed to be involved in tissue differentiation. Urokinase inhibitor-1 (PAI-1) inhibits the activity of urokinase-type plasminogen activator (uPA) which contributes to the degradation of the extracellular matrix in tumor invasion and metastasis. ER, PR, pS2 and PAI-1 have been reported to be prognostoic parameters in primary breast cancer. We developed a competitive RT-PCR system to allow simultaneous quantitative determinations of ER, PR, pS2 and PAI-1 mRNA in tissue samples. We evaluated 100 breast cancer specimen for mRNA expression. Results were compared with protein status and with clinical data.

Results: We found a statistically significant correlation between mRNA and protein levels of ER ($p < 0.00001$), PR ($p < 0.00001$), pS2 ($p < 0.00001$) and PAI-1 ($p < 0.0147$), respectively. ER, PR, pS2, and PAI-1 showed a statistically significant correlation to each other except to the mRNA expression of PAI-1. Furthermore the mRNA data of ER and PR showed an inverse correlation to tumor size and histological grade.

Conclusion: Our data are in agreement with reports about ER, PR concerning tumor size and grade. We did not find any correlation to lymph node involvement. We did not find any association of pS2 mRNA to the clinical data. PAI-1 was found to be independent of tumor size, grade or lymph node involvement. The present study shows, cRT-PCR is an appropriate method for the simultaneous determination of prognostic factors in breast cancer specimen, requiring small amounts of total RNA.

453 POSTER

Medullary breast carcinoma. Ten-year results in 108 patients treated in a single center

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Purpose: To analyse clinical presentation and outcome of patients with medullary breast carcinoma treated at the Institut Curie between 1981 and 1996.

Methods: 108 cases of medullary carcinoma (0.7%) were retrospectively identified in the breast cancer database. All charts were reviewed. All 108 patients were female. Median age was 50 years (24–82 yrs.). 64 pts. (59%) were premenopausal. Median clinical tumor size was 3 cm (0–15). There were 35 T1 (32%), 51 T2 (47%), and 22 T3T4 (20%); 57 were NO (53%), 47 N1 (43%) and 4 N2 (4%). Treatments varied according to tumor sizes. 62 pts. (58%) had a wide tumor excision and radiotherapy (mean tumor size: 2.4 cm), 24 pts. (22%) received irradiation alone (mean size: 6.4 cm) and 22 pts. (20%) had a mastectomy (mean size: 3.8 cm). Overall, 86/108 pts. (80%) had a breast-conserving treatment. 32 pts. (30%) received chemotherapy, either before (13 pts.) or after local treatment (19 pts. Treatment outcome was determined using Kaplan-Meier estimates.

Results: Median followup was 116 months (8–205). Ten-year survival and metastasis-free interval rates were 81% and 79%, respectively. 13 breast recurrences occurred and 8 contralateral breast cancer. 10-year breast recurrence rate was 17%. The 10-year overall breast conservation rate was 66%. It was 83% in patients who had an initial breast-conserving procedure. Survival and metastasis-free interval were similar in patients treated with mastectomy, or breast conservation.

Conclusion: This retrospective study of 108 patients with 10 year follow-up confirms that medullary breast cancer seems to carry a better prognosis than invasive ductal carcinoma. Medullary breast cancer seems very sensitive to chemotherapy and radiotherapy, which allow breast preservation in patients with large tumors. Biological characterization of this particular form of breast cancer is ongoing.

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Anti-apoptotic phenotype is associated with decreased loco-regional recurrence rate in breast cancer

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Purpose: Tumour and nodal status are the most important factors predicting loco-regional recurrence in breast cancer. We wanted to investigate the predictive value of some new molecular genetic markers, for the occurrence of a loco-regional recurrence in order to improve the selection of patients for loco-regional adjuvant therapy.

Methods: Bcl-2, p53, MIB-1, pS2, PCNA and CD44v6 were determined immunohistochemically on formalin-fixed and paraffin embedded tumour tissues of 163 patients treated by modified radical mastectomy between 1982 and 1987. Postoperative irradiation was given to only few patients. Node-positive patients had been given CAF adjuvant chemotherapy. A multivariate analysis was performed on a number of potential prognostic factors. The risk for loco-regional recurrence was estimated using the competing risk approach.

Results: After a median period of 7.5 years 28 patients developed a loco-regional recurrence. The cumulative incidence of loco-regional recurrence at 10 years was 17%. Bcl-2 and p53 were found to be independent