= 0.31, p = 0.0001) and with results obtained by immunohistochemistry (n = 115, R = 0.53, p = 0.0001). By using the sensitivity and specificity curves, with amplification as reference, a cut-off value of 200 arbitrary units was chosen to appreciate overexpressed cases. With this cut-off, 34% (361/1065) of the cases were overexpressed. So, this kit appears as a good tool to quantitatively determine c.erbB.2 protein and our populations will be followed up to appreciate the prognostic value of this parameter.

PP-1-6 Expression of BCL-2 in Node-Negative Breast Cancer is Associated with Various Prognostic Factors, but does not Predict Response to Peri-Operative Chemotherapy

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Bcl-2 expression may be related to response to chemotherapy and a number of pathologic and biologic tumor parameters in premenopausal, lymph node-negative (N^-) breast cancer (BC) patients. Expression of Bcl-2 was determined using immunohistochemistry on paraffin-embedded sections in a series of 441 premenopausal, N^- BC's from patients randomized to receive peri-operative chemotherapy (PeCT) (5-fluorouracil, doxorubicin, cyclophosphamide) or no PeCT in EORTC Trial 10854. Strong positive correlations were found between high Bcl-2 expression and estrogen and progesterone receptor positivity and low tumor-grade, whereas high Bcl-2 expression was negatively correlated with p53 and c-erb*B*-2 positivity, high Ki-67 index, mitotic index and large tumor-size. Patients with tumors expressing high levels of Bcl-2 had a significantly better disease-free (p = 0.004) and overall (p = 0.009) survival. However, in a multivariate model this association no longer remained significant. There was a trend for an effect of PeCT on disease-free survival both for patients with Bcl-2 positive (HR = 0.61, 95% C.I. 0.35–1.06, p = 0.07) and negative (HR = 0.55, 95% C.I. 0.27–1.12, p = 0.09) BC's at a median follow-up of 49 months. *Conclusions:* The level of Bcl-2 expression does not seem to predict response to PeCT in premenopausal, N^- BC patients. High levels of Bcl-2 are preferentially expressed in well-differentiated tumors and associated with favorable prognosis.

PP-1-7 Accumulation of TP53 as Predictor of Response to Chemotherapy of Recurrent Breast Cancer

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We have shown that TP53 protein accumulation predicts a poor response to hormonal therapy in recurrent breast cancer. To evaluate whether TP53 protein accumulation can predict the response to chemotherapy in patients with recurrent breast cancer, TP53 protein levels were measured in routinely prepared cytosols from primary breast tumors, using a quantitative luminometric immunoassay (Sangtec Medical). Patients who developed recurrent disease received either first-line chemotherapy (n = 92; 48% premenopausal, 30% ER/PgR-positive, 60% with a disease-free interval [DFI] > 12 months), or first-line hormonal therapy followed by chemotherapy (n = 180; 27% premenopausal, 67% ER/PgR-positive, 67% with a DFI > 12 months). In univariate analysis, TP53 protein accumulation does not predict response to first-line chemotherapy. With respect to chemotherapy after tamoxifen therapy, TP53 protein accumulation only showed a relation with progression free-survival when analyzed as a dichotomized (cut-off value 1.6 ng/mg protein) variable (p = 0.02), but not as a continuous variable, with a relative hazard rate (95% confidence limits) of 1.5 (1.1–2.2). In conclusion: patients with high TP53 protein levels, as measured by LIA, respond poorly to chemotherapy only after failure to tamoxifen therapy.

PP-1-8 Cyclin D1 Expression and Response to Tamoxifen Treatment for Metastatic Breast Cancer

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Cyclin D1 is a cell cycle associated protein active in the G1 phase of the cell cycle. Amplification of the gene is found in approximately 20% of mammary carcinomas and immunohisto-chemistry has revealed that overexpression of cyclin D1 protein occurs more frequently. It is present in 40–50% of breast cancers suggesting oncogenic activity which could be associated with poor clinical outcome. Surprisingly, we found the reverse to be true; in primary breast cancer the highest levels of cyclin D1 expression occur in well differentiated ER positive tumours, usually associated with a good prognosis. We have also investigated the relationship between cyclin D1 protein expression and response to first line tamoxifen treatment for metastatic disease in 149 women. Response to treatment was assessed in a standard manner, according to UICC criteria and was available on all patients. Women whose response was unassessable were excluded from the study. 95 (64%) of cases overexpressed cyclin D1, 78 (82%) were ER positive. Response (complete/partial) was seen in 55 (71%) of these double positive tumours. Conversely tumours which were negative for both proteins had only an 8% chance of responding. Tumours which were positive for only one of the proteins had an intermediate response rate ($x^2 = 31.97$, $p < 0.0001$). Suggesting that immuno-histochemical staining for cyclin D1 could be a useful adjunct to the measurement in ER in identifying women who are likely to respond to endocrine treatment. Furthermore, these results pose interesting questions concerning the role of cyclin D1 in the biology of breast cancer.

POSTER PRESENTATIONS

PP-1-9 Tumor-Associated Lymphomonocytes from Neoplastic Effusions of Patients with Different Primary Tumors Including Breast Cancers are Able to Release Cytokines

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We studied several "in vitro" activities of tumor-associated lymphomonocytes (TALM) and the levels of IL-1α, IL-1β, IL-2, IL-6, TNFα and soluble IL-2 receptor (sIL-2R) in neoplastic effusions and in the serum of advanced stage cancer patients. Comparisons were made with the behavior of autologous peripheral blood mononuclear cells (PBMC) using PBMC from normal subjects as controls. TALM were collected from 12 peritoneal and 15 pleural neoplastic effusions. The peritoneal effusions were mainly secondary to primary ovarian cancers and included 1 breast cancer as primary. The pleural effusions were secondary to primary lung and breast cancers. The blastic response to PHA and anti-CD3 monoclonal antibody (mAb) of TALM was lower than that of autologous PBMC, whereas proliferative response to recombinant IL-2 of both TALM and autologous PBMC was in the same range. Blastic responses of patient PBMC were lower than those of control PBMC. No significant differences were found for the expression of IL-2R subunities after PHA or anti-CD3 mAb stimulation between TALM and autologous PBMC, which, in both cases, was lower than that of control PBMC. After PHA stimulation, the levels of IL-1α, IL-1β and TNFα in culture media of TALM were lower than those of autologous PBMC, whereas, IL-2 and IL-6 levels were significantly higher. The cytokine production from patient PBMC was always lower than that of control PBMC. The levels of IL-6, TNFα and sIL-2R in neoplastic effusions were significantly higher than those of autologous serum. The levels of all cytokines were higher in patient than in control sera. Our data seem to suggest that a general impairment of the immune function is present in cancer patients with advanced disease, such as those with neoplastic effusions, involving not only TALM but also autologous PBMC.

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PP-1-10 Cytogenetic Analysis in Short Term Culture of Breast Cancer in Korea Women

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This study was to analyze the cytogenetic characteristics of 20 primary human breast cancer cells. Different growth media and procedures for tissue aggregation and culturing were tested with regarding to cell attachment, the type of cells in outgrowth, and the emergence of cytogenetically abnormal clones. We found out that optimal tissue disaggregation was obtained by combined mechanical and enzymatic treatment of the tumor samples. Use of the plastic flask coated with Vitrogen 100 and the serum free growth