= 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

medium containing EGF resulted in being the best growth of epithelial cells. Chromosomal abnormalities were found in all 20 tumors. The changes were clonal in 10 tumors (50%). Karyotypic analysis of first or second passage cultures yeilded predominantly diploid cells. Among the clonal aberrations, clones with 15q deletion (q13–q15, q12–q15, q?) were observed in 4 cases. In addition, there were two clones involving the chromosome 1 (1p31, q21 deletion). In conclusion, the most common cytogenetic abnormalities in Korean women affect chromosome 15 compared to chromosome 1 in caucasian women.

PP-1-11 Transferrin Receptor (CD71) Expression by Human Breast Cancer Cells

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The expression of transferrin receptor by malignant cells was studied in cryostat tumor sections of 44 patients with breast cancer using MoAbs.. HLA-I and II, HMFG1, adhesion molecules (CD29) were also estimated as well as tumor infiltration with leukocytes (CD45+), T-lymphocytes ($CD7^+$) and macrophages ($D11^+$, $GHI/61^+$, $MAC387^+$).

CD71 was detected in 66% of cases, HLA-I in 43%, HLA-Dr in 22%, CD29 in 64%, HMFG1 – in 66% of cases. In CD71-positive groups compared to CD71-negative tumor cells revealed a statistically significantly higher proportion HLA class I (66% (19/29) vs 0% (0/15), $p < 0.01$) and of HLA-Dr (31% (9/29) vs 6% (1/15), $p = 0.05$).

No associations of CD71 with other markers were detected. $CD71^+$ and $CD71^-$ groups did not have statistically significant differences as regarts tumor infiltration levels with T-cells.

PP-1-12 Chemotherapy-Induced Diversification of DNA Profiles in Breast Cancer

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DNA flow cytometry was used to estimate spontaneous and therapy-induced changes in DNA profiles of breast carcinomas by comparing the findings from pre-treatment core needle biopsies and resection samples. Spontaneous diversification from primary diploid tumor to aneuploid node metastases was a rather rare event occuring in only 2% of 45 cases, 38% (17/45) of which expressed p53 protein in immunohistology. However, following chemotherapy (4–6 cycles, FAC regimen) 7 out of 20 originally diploid tumors (35%) were found to contain aneuploid population(s). No such diversification (0/27) was observed after radiotherapy and/or endocrine treatment. Since an aneuploid DNA profile is considered to be associated with more aggressive tumor behavior, chemotherapy may not be beneficial in individual cases which express newly-developed, chemotherapy-induced aneuploid populations. Whether p53 protein expression and/or other factors may predict increased tumor instability remains unclear.

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PP-1-13 Breast Cancer Cell Mediated Control of Human Stromelysin 3 (ST3) Promoter Activity

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ST3 is a matrix metalloproteinase overexpressed in stromal fibroblasts at the tumour-stromal interface of invasive breast cancers (Basset et al, Nature, 1990; 348: 699–704) and levels of expression of its mRNA have been correlated with both degree of invasion of primary breast cancers and clinical outcome (Engel et al, International Journal of Cancer, 1994, 58 (6): 830–835). We have shown, in transient transfection assays, that breast cancer cell factors modulate ST3 promoter activity. To characterize further the factors important in ST3 gene expression, clones of NIH3T3 fibroblasts stably expressing the firefly luciferase reporter gene under the control of 2 different lengths (0.46 kB and 3.4 kB) of the 5'flanking sequence of the human ST3 gene were created.

Co-culture of the human breast cancer cell lines MCF-7 and MDA-MB231, but not ZR75, BT20 or T47D cell lines with the stable fibroblast clones resulted in a consistent 2–3 fold upregulation of luciferase activity in the clones driven by 3.4 kB but not 0.46 kB of the ST3 promoter. These data suggest that certain breast cancer cell lines either by a cell-cell contact or diffusible factor mechanism can switch on human ST3 promoter activity, and furthermore that the putative response element in the promoter can be found between 0.46 and 3.4 kB upstream of the transcription start site.

PP-1-14 Integrins $\alpha v\beta 1$ and $\alpha v\beta 5$ Function as Breast Cancer Cell Vitronectin Receptors

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The classical vitronectin receptor ($\alpha v\beta 3$) is absent from most breast cancer cells suggesting that an alternative integrin may subserve a similar role to this receptor. In order to examine this possibility we have studied the expression and composition of αv-containing heterodimers on a range of human breast cancer cell lines (including ZR75, BT20, MB468, MCF7, MDA231, BT474). FACS analysis with heterodimer-specific monoclonal antibodies (MAbs) revealed cell surface $\alpha v\beta 5$ on all lines examined, no expression of $\alpha v\beta 8$, expression of $\alpha v\beta 6$ on 1 out of 6 and low levels of $\alpha v\beta 3$ on 1 out of 6 of the cell lines (BT20 and MDA231 respectively). These results were confirmed by immunoprecipitation analysis of cell surface ^{125}I-iodinated cells. Using anti-αv MAb (P2W7) as the immunoprecipitating antibody a protein of similar size to $\beta 1$ was brought down in conjunction with the αv subunit in ZR75, MCF7 and MDA231 cells. The $\beta 1$ identity of this band was confirmed by immunodepletion analyses. Adhesion assays to immobilised vitronectin with ZR75 cells were performed in the absence and presence of anti-αv (17E6), anti-$\alpha v\beta 5$ (P1F6) and anti-$\beta 1$ (P4C10) MAbs. Adherence to 5 μg/ml vitronectin (21%) was reduced by anti-αv to 6%. ($p \leq 0.01$) Anti-$\alpha v\beta 5$ and anti-$\beta 1$ alone did not reduce adherence (20–30%) but in combination reduced adhesion to 6%.

These results show that in breast cancer cells $\alpha v\beta 1$ and $\alpha v\beta 5$ serve as major functional vitronectin receptors in the absence of $\alpha v\beta 3$.

PP-1-15 Is Cowden Disease Gene a Tumor Suppressor Gene or Not?

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Cowden disease (CD) or multiple hamartoma syndrome is a cancer associated genodermatosis with a dominant autosomal pattern of inheritance. It was named for the first patient described in 1963 by Llyod and Dennis.

Its clinical features include many abnormalities but the main characteristics are hamartoma of the skin, breast, thyroid, oral mucosa and intestinal ephitelium. By performing linkage analysis in a total of 12 families, the gene for Cowden disease has been localized to 10q22–23 with a significant lod score of 8.92 at $\theta = 0.02$ with the marker D10S 573 [1].

It is observed that clinical features of CD are similar to certain manifestations of phakomatosis such as neurofibromatosis, Von Hippel-Lindau disease and tuberous sclerosis. Considering that the genes involved in these diseases behave as tumour suppressor genes, we can suppose that such a gene could be also involved in CD.

To begin to answer to this question, we performed loss of heterozygosity analysis on 10q, in 3 breast tumors [2 carcinomas, 1 adenofibroma], with markers located in Cowden disease gene region.

In the aim of searching an eventual involvement of the CD gene in sporadic breast cancer, 30 invasive breast carcinomas were also studied.

[1] Nelen et al. Localization of the gene for Cowden disease to 10q22–23. Nature Genet, in press. The data obtained will be presented and discussed.

PP-1-16 Apoptosis-Loss and Expression of Death-Related Genes in Breast Carcinomas

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We recently suggested that apoptosis-loss contributes to the metastatic progression of breast cancer by extending cell survival and thus, allowing the acquisition of gene mutations. To study whether the apoptotic death pathway is altered by the expression of proteins such as Bcl-2 or Bcl-x which have an antiapoptotic activity and the interactions with Bax and Bak proteins that can repress their apoptosis-blocking ability, we analyzed the expression of those genes in a serie of 124 T1 (< 2 cm) breast cancer tumors. Indeed, Bcl-2 overexpression was found to be correlated with apoptosis-loss ($p < 0.001$) in those T1 tumors with wild-type p53. In contrast, Bcl-x was not related to the apoptosis-loss in spite of its co-expression with Bcl-2 gene in 53% of tumors. The analysis of the antiapoptotic effect of Bcl-2 overexpression was greatly demonstrated in Bak positive tumors ($p = 0.019$), indicating its direct blocking effect on Bak death promoting activity.

Nonetheless, the overexpression of Bak in breast cancer tumors, was correlated with Bcl-x overexpression which suggests that it might be also an effective mechanism to the apoptotic death regulation.