PP-8-34 Metastatic Breast Cancer Management in France

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There is few available data about current practices in France in MBC and more especially in second line chemotherapy. Using a stratified poll plan, we therefore conducted a survey, building first a representative sample of 111 hospitals and clinics out of the 2051 French public and private centers involved in the management of the disease. 247 patients were then selected while receiving second line chemotherapy for MBC and the overall history of their disease was collected. *First occurrence of MBC:* Median delay from first diagnosis: 4.5 years; median age (54 years); First line treatment: chemotherapy 66%, hormonotherapy 34%; 71% were treated with an anthracycline-or anthracenediones based combinations; 72% were treated on a day-time hospitalization basis, 18% had a full hospitalisation, combined: 8%.

Second occurrence of MBC: Mean delay after the beginning of first line treatment: one year; sites: bone 61%, liver 34%, lung 31%, pleura 25%, nodes 24%; concerning hospitalization, repartition between in and out-care patients remained unchanged. At this stage, 30% of patients were treated with an anthracycline or anthracenediones based combinations; other dominant type of treatment was vinorelbine based combinations. Overall, no less than 68 different chemotherapy regimens were prescribed.

Conclusion: This wide diversity of second line regimens in MBC is explained by the lack of efficacious combinations and shows the need for new validated protocols.

PP-9. Prognosis 2: Molecular Markers (September 13)

ORAL PRESENTATIONS

PP-9-1 Immunohistochemical Analyses of Proliferative Activity in Breast Carcinomas with Medullary Features

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Medullary carcinoma is usually considered to have a more favourable outcome when compared to the other types of infiltrating breast carcinomas. This is a biological paradox, since its clinical behavior is not in agreement with its morphology and rate of mitosis. Concerning proliferative activity, it should be remembered that neoplastic growth equals cell production minus cell loss, the latter being achieved by a specific type of cell death called apoptosis. At present, bcl-2 oncogene (apoptosis-inhibitor) and p53 gene, are assumed to be involved in the regulation of cell death and tumor proliferation. Sixty breast carcinomas previously indexed as medullary carcinomas were re-classified using Ridolfi's criteria. This review yielded 13 typical medullary carcinomas (TMC), 24 atypical (AMC), and 23 non-medullary carcinomas (NMC). Following antigen retrieval by microwave treatment the immunohistochemical anlyses were performed on serial sections from formalin-fixed, paraffin-embedded tissue using MIB-1, p53 and bcl-2 monoclonal antibodies. The mean MIB-1 index of TMC (61%) was significantly higher than those of AMC and NMC (40% respectively). Intensive nuclear p53 staining in almost all tumor cells was found in 69% of TMC, 39% of AMC and 13% of NMC. TMC were all bcl-2 negative. In contrast 25% of AMC and 36% of NMC showed moderate to strong cytoplasmatic bcl-2 staining in the majority of tumor cells. The mean MIB-1 index in p53 positive tumors was significantly different from the mean MIB-1 index in p53 negative tumors (54% vs 39%).

Surprisingly, TMC revealed the highest incidence of intense p53 positivity, and the highest mean MIB-1 index, and absence of the apoptosis-inhibitor protein bcl-2. The results indicate a higher overall cell turnover in TMC compared with AMC and NMC. Increased apoptosis balancing the increased cell proliferation might explain the more favourable prognosis in typically medullary carcinomas. Despite the relatively small number of patients in each group, preliminary results indicate a more fovourable prognosis for TMC.

PP-9-2 The Value of nm23 Protein Expression and Tumor Angiogenesis as a Prognostic Indicator in Breast Cancer of Korean Women

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This study was designed to evaluate the value of nm23 protein expression and tumor angiogenesis as a prognostic indicator in breast cancer of Korean women, and to compare with established clinicopathological prognostic factors. We obtained surgical specimens from 59 patients who had undergone surgery for breast cancer between July, 1988 and July, 1994. By using immunohistochemical staining with anti-nm23 nucleoside diphosphate kinase A and CD31, we studied 59 paraffin block for the expression of nm23 protein and microvessel count (MVC), respectively. All the patients were female and the 22 (31%) cases were negative for nm23 protein, 37 cases (63%) were positive. The positive staining of nm23 was not correlated with age, tumor size, and lymph node metastasis, but with tumor grade ($p = 0.023$) and hormone receptor ($p = 0.006$ for ER, $p = 0.0001$ for PR). Also the overall survival and disease free survival rate was superior in the group of positive staining for nm23 ($p = 0.0026$ and $p = 0.0048$). The mean value of MCV in 59 paraffin block was 42 ($\pm$ 20), and this value was negatively correlate with overall survival and disease free survival rate ($p = 0.0001$ and $p = 0.0001$). But there is no correlation with other established prognostic parameters. So we conclude that nm23 expression and MVC can be used independent prognostic indicator and they may play a role in the tumor metastasis and growth.

PP-9-3 Prognostic Value of P21/WAF1 and P53 Expression in Breast Carcinoma: An Immunohistochemical Study on 261 Cases with Long Term Follow Up

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Background: p21/WAF1 is a critical downstream effector in the p53-specific pathway of growth control, is related to terminal differentiation, and its expression may be prognostically relevant. *Material and methods:* We investigated p21 immunoreactivity in 261 breast carcinomas (141 node-negative and 120 node-positive) with long-term follow-up (median = 73 months, range 37–119). *Results:* Sixty eight (32%) cases showed p21. p21 overexpression was associated with large tumor size, positive nodal status, high histological grade and high mitotic count, and was related to short disease free survival in the whole series of patients ($p = 0.04$), in the node-negative subgroup ($p = 0.004$), and in the group of patients which did not undergo systemic adjuvant therapy ($p = 0.003$). Bivariate analysis of the combined p21 and p53 phenotype showed that, in patients treated with systemic adjuvant therapy, p21+/p53+ tumors were associated with long DFS and overall survival (OS), while p21−/p53+ tumors had the worst prognosis. Multivariate analysis showed that, in treated patients, the p21−/53+ phenotype was independently associated with short DFS and OS. *Conclusions:* The p21−/p53+ phenotype could correspond to a situation where p53 function is disrupted, G1 checkpoint is impaired and DNA-damaging drugs may be not able to induce apoptosis.

PP-9-4 The Predictive Value of the Urokinase System of Plasminogen Activation in Recurrent Breast Cancer

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In a pilot study involving 235 patients we previously showed that the urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 were associated with a poor response to tamoxifen therapy in recurrent breast cancer (*J Natl Cancer Inst* 87 [1995] 751–756). The present study involves 534 patients who were treated with tamoxifen as first-line therapy for metastatic disease. The overall response rate was 51% (objective response: 15%, no change > 6 months: 36%). In these patients we have evaluated the predictive value of uPA, its receptor uPAR, and its inhibitors PAI-1 and -2. The parameters were measured in cytosols with ELISA*. In univariate analysis for progression-free survival (PFS) using continuous variables, ER and PgR were related with a favorable outcome, while uPA, uPAR and PAI-1, were associated with a poor response to therapy and a poor PFS

(all: $P < 0.01$). PAI-2 was not significantly related with response or PFS in univariate analysis. In multivariate analysis for PFS, using dichotomized variables (in addition to a basic model including age and menopausal status, disease-free interval, site of metastasis, ER and PgR), each factor when added separately contributed to the model. The relative hazard rate (RHR) for PAI-2 was 0.8, while for uPA, uPAR and PAI-1, the RHR varied from 1.23 to 1.35 (all: $P < 0.05$).

*In collaboration with N Brünner & K Danø (Copenhagen), M Schmitt, F Jänicke & H Graeff (München), and MD Kramer (Heidelberg).

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PP-9-5 Expression of uPA, c-erbB2, EGF-R and p53 in 488 Primary Breast Cancer

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The prognostic impact of uPA, EGF-receptor, p53 and c-erbB2 proteins, was studied in 488 primary breast cancer patients treated at the Centre R Huguenin (median: 6.2 years). p53 and uPA were measured on classical hormone receptors cytosols using a novel luminometric immunoassay (Byk Sangtec, Sweden). EGF-R and c-erbB2 were detected on membrane extract using an ELISA method (Ciba Corning Diagnostics, USA). The candidate variables of the Cox model were: age, menopausal status, clinical and macroscopic tumor sizes, nodal status, SBR grade, modified SBR grade, ER, PR, uPA, p53, EGF-R and c-erb-B2. The cutoff points of the last 4 factors were determined using a minimum corrected p-value. In the overall population, for metastasis-free survival (MFS), the most significant factors were respectively uPA, nodal status, c-erbB2 and modified SBR grade; for overall survival (OS), nodal status, uPA, c-erbB2, modified SBR grade and clinical tumor size were selected as poor prognosis factors. In node negative patients, uPA was the only significant variable, thus confirming its prognostic impact.

PP-9-6 The Use of Molecular Markers to Predict Response to Preoperative Chemotherapy: Fact or Fiction?

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At present, no pathological or biological markers are available that can predict whether or not an individual breast cancer (BC) patient will benefit from adjuvant chemotherapy. Previously, we investigated the relationship between a number of prognostic factors and response to chemotherapy (CT) in patients randomised to receive one course of peri-operative chemotherapy (EORTC Trial 10854). P53 accumulation was found to be associated with a lack of response to CT, as determined by disease-free survival, whereas other factors, like Bcl-2, did not predict responsiveness. Material from patients treated with pre-operative CT offers the unique possibility to directly monitor the response of the primary tumor to CT. For this reason, a project was started to assess the relationship between a number of molecular markers and response to CT in patients randomised to receive 4 courses of pre-operative CT (5-Fluorouracil, Epi-adriamycin and Cyclophosphamide) in EORTC Trial 10902. Both biopsy and tumor material were collected, as well as mammographies taken pre- and post-CT. Tumor response (WHO-criteria) will be evaluated using the mammographies as well as the histological changes following CT. Data will be presented on the predictive value of 15 prognostic markers, including markers associated with proliferation, resistance to apoptosis and multi-drug resistance, currently under investigation.

POSTER PRESENTATIONS

PP-9-7 Immunohistochemical Expression of c-erbB-2, p53, and bcl-2 Oncoprotein and Response to Endocrine Therapy in Advanced Breast Cancer

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To determine whether oncoprotein expressions in advanced breast cancer may regulate the response to endocrine therapy, we investigated immunohistochemical expression of c-erbB-2, p53, and bcl-2 protein in 92 advanced breast cancer patients, treated with adreno-oophorectomy as the 1st line treatment. There were no relations between the expression of these oncoproteins and clinical background factors, except the inverse correlation between p53 and ER. Univariate and multivariate analyses showed that ER was the first, and p53 was the second determinant of the response to endocrine therapy, as well as the time to progression. These results suggested that the combination of these 2 covariates might be useful for the prediction of response to total estrogen blockade treatment.

PP-9-8 Fatty Acid Synthetase (FAS) and Survival in Breast Cancer

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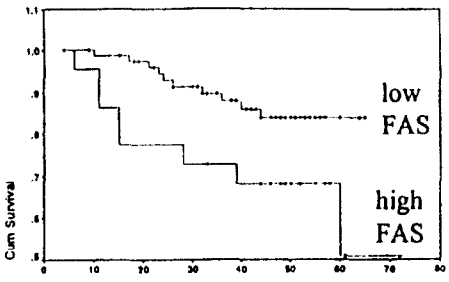
Ninety-five patients with primary breast carcinoma had FAS-mRNA determined in the tumour by Northern blots. Subsequently the patients were followed for an average of 47 months. Patients with carcinomas expressing high levels of FAS-mRNA had a significantly shorter survival than patients with low level FAS-mRNA (Kaplan-Meier test). Fewer patients with low FAS-mRNA had recurrent disease (21%) than those with high FAS-mRNA, but the difference was not significant.



PP-9-9 DNA Image Cytometry (ICM), Oncogenes and Prognostic Factors in Breast Cancer

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Objective: Aneuploidy, high S-phase fraction (SPF%) and alteration of oncogenes mean an unfavorable prognosis. Goal of the investigation was 1. to determine the frequency of diploid-neardiploid breast cancers. 2. to calculate SPF (%) 3. to demonstrate relations between DNA cytometric data (DNA Index, grade of malignancy), erb B oncogenes, c-myc and prognostic factors.

Methods: Imprint cytology of 125 primary breast cancers, Feulgen technique, interactive cell analysis, Hilgers, Koenigswinter, Germany. Detection of oncogenes by differential polymerase chain reaction.

Results: 1. Low frequency of diploid-neardiploid breast cancers (24%). 2. ICM allows only determination of SPF (%) of diploid-neardiploid breast tumors. 3. No correlations between cytometric data and oncogenes. 4. Correlations between DNA data and conventional prognostic factors.

Conclusion: Numbers of frequency of diploid-neardiploid breast cancers examined by flow cytometry seem to be too high (50–60%). Proliferation fraction (S + G2M) could be calculated in diploid and aneuploid tumors instead of SPF (%). Oncogenes seem to be independent prognostic factors.