the 10-day schedule we evaluated appears useful in reducing the incidence of neutropenic episodes and treatment delays, allowing an adequate dose-intensity of the drug with moderate toxicity.

715 POSTER

The upregulation of cellular adhesion proteins following transfection of the keratin 18 gene into human breast cancer cells is accompanied by a dramatic decrease of invasion and metastasis in vitro and in vivo

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In vitro experiments as well as clinical studies revealed that the expression of keratin 18 (K18) in breast cancer tumors is associated with a favorable prognosis and a less aggressive phenotype of the carcinoma. To prove the principle we transfected the human K18 gene into the aggressive MDA-231 cell line and isolated a permanently overexpressing clone. These cells grow in dense monolayers with epithelial morphology whereas wild type and mock transfected control are of the dedifferentiated, malignant type with cells being spindle shaped, motile, and only loosely attached. The K18-transfected clone is characterized by a high expression of the adhesion proteins plakoglobin, desmoglein and E-cadherin in contrast to wild type and control which are virtually devoid. In addition, keratin 8 the indispensable dimerisation partner of K18 in keratin filament formation is upregulated too. Conversely the mesenchymal filament protein vimentin, forming the intermediate filaments of the cytoskeleton in MDA-231 wild type and control, is completely downregulated in the K18 clone. The high invasiveness of the wild type in the Boyden chamber is dramatically reduced for the K18-clone. In the nude mouse no metastasis could be observed for the K18-cells whereas wt and control metastasized into lung, liver, and bone marrow. In epithelial cells the intermediate filaments of the cytoskeleton are formed by keratins and K18 is a marker of well differentiated mammary lumenal cells. The loss of K18 and its replacement by vimentin is part of a general loss of differentiation along with the malignant transformation. An additional aspect of this process is the loss of adhesion proteins. This dedifferentiation seems to be reversible, at least in part, by the re-expression of K18. To reconfirm this hypothesis by approaching from "the opposite" we established an epithelial subclone of the MDA-231 cells without gene transfer by selecting adhesive cells in weekly trypsinations over a period of 9 month. This clone is not only characterized by the expression of plakoglobin, desmoglein, and E-cadherin but also by the expression of keratins 8 and 18! In addition the same impressive drop in invasiveness and metastasis as for the K18-transfected cells could be observed.

716 POSTER

Five-day Infusion Fluorouracil and Vinorelbine as chemotherapy for advanced breast cancer patients previously treated with anthracyclines

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Vinorelbine (VNR) has proven to be effective drug in metastatic pretreated breast cancer patients. Particularly, no cross resistance with anthracyclines has been demonstrated. The long term continuous 5-Fluorouracil (5-Fu) infusion presents better pharmacological profile than its bolus administration.

Purpose: We investigated the combination of this two antitumor drugs in patients (pts) with MBC who were previously treated with anthracycline-containing regimens.

Patients and Methods: From February 1998 to January 2000, sixty five pts were enrolled into the study. The pts mean age was 48 years (range 31-70). The most important inclusion criteria was as follows: Karnofsky 70-100, measurable or evaluable disease, normal renal, hepatic, bone marrow and cardiac function.

Fourteen of the sixty five women have already received more than one chemotherapy line. Twenty three pts were previously treated with taxanes.

Sites of metastatic lesions were as follows (% of pts): lungs 50%; liver 37%; soft tissue 72%; bone 58%; other sites 32%.

Treatment consisted of VNR 25 mg/m2 administered on day 1 and 6 every 21 days and 5-Fu 700 mg/m2/day for five consecutive days (1-5) every 21 days. The total number of cycles was 340, (mean: 5 cycles).

Results: The scheme was well tolerated. Febrile neutropenia was observed in 4,6% of pts. 14% of pts experienced grade 3 or 4 neutropenia, and 3% grade 3 thrombocytopenia. Grade 3 stomatitis was observed in 9,2% of pts, grade 3 neurologic toxicity was observed in 1,5% of pts, and grade 3 cardiotoxicity in 4,6% of pts. Grade 3 local reaction occurred in 3% of pts.

Sixty pts were evaluated for response. One (1,7%) patient attained a complete clinical response and twenty eighth (46,7%) achieved a partial response. In twenty two (36,6%) cases stable disease was documented, and nine (15%) pts progressed while on treatment. Median time to progression was 24 weeks, median duration of response: 35 weeks, and median overall survival was 41 weeks.

Conclusion: VNR with five-days infusion of 5-Fu is an active and manageable scheme in MBC patients previously treated with anthracyclines. Overall response was 48,4% and median overall survival was 41 weeks.

717 POSTER

Serum IL-6 (sIL-6) predicts overall survival in patients with metastatic breast cancer (MBC)

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Purpose: IL-6 is a pleiotropic cytokine that is implicated in a variety of cellular functions in immune, hematopoietic and hepatic systems. IL-6-type cytokines have diverse actions on breast cancer cell lines. The prognostic value of sIL-6 in patients with MBC remains unclear.

Patients and Methods: IL-6 was measured by an ELISA in serum of 96 consecutive patients with progressive MBC. SIL-6 levels were correlated with clinicopathological variables and survival. Survival was calculated in days from the sampling date until time of dead or until the end of the study. The survival fractions are calculated using the Kaplan-Meier method and compared with the Mantel-Haenszel test.

Results: The median value sIL-6 value was 6.6 pg/ml (95% c.i.: 8.6-17 pg/ml; range: < 0.7-162.3 pg/ml). Median sIL-6 differed significantly between pts with two or more metastatic sites (8.15 pg/ml) and only one metastatic site (3.06 pg/ml,) ($p < 0.001$), between pts with or without liver metastasis, 8.3 pg/ml and 4.5 pg/ml, ($p = 0.001$), between patients with and without pleural effusion, 10.65 pg/ml and 5.45 pg/ml, ($p= 0.007$), between patients with dominant visceral disease (8.15 pg/ml) and dominant bone disease (4.5 pg/ml) ($p=0.0077$). No correlation between sIL-6 and age, menopauzal status, tumour grade, histiotype, receptor status, initial tumour staging, prior adjuvant therapy and number of prior therapies for metastatic disease. Patients with a sIL-6 above the median had a significant shorter survival ($p<0.001$) of 277 days, whereas the median survival for the low sIL-6 group has not yet been reached.

Conclusion: In patients with MBC sIL-6 levels are positively correlated with survival. Higher sIl-6 levels are observed in pts with more metastatic sites, with liver metastasis, pleural effusion or lymfangitis carcinomatosis and with dominant visceral disease. This suggests that sIL-6 adequately characterizes poor prognosis in patients with progressive MBC.

718 POSTER

Elevation of the serum HER2/neu level is associated with shorter progression-free survival after chemotherapy in metastatic breast cancer

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Purpose: Circulating levels of the shed, extracellular domain of HER2/neu have been shown to be a predictive parameter for response to (high-dose) cytotoxic and anti-hormonal therapy in HER2/neu positive patients [Harris et al., JCO 2001; Lipton et al., Breast Cancer Res Treat 2000].

Methods: We measured the serum HER2/neu baseline (and longitudinal) levels in 95 patients with metastatic breast cancer enrolled to different chemotherapy trials (mostly anthracycline- and/or taxane-based), and correlated the results to treatment outcome.

Results: Using a cut-off of serum HER2/neu positivity of 15 ng/ml for the Oncogene Science® (Cambridge, MA, USA) kit, we found that 63% of patients had elevated levels of the extrecellular domaine of HER2/neu which is in line with serum HER2/neu results for stage IV disease found by other investigators [Andersen et al., Acta Oncol 1995]. The overall response rate to chemotherapy was 31%. There was no statistically significant difference of the response rate to chemotherapy between serum HER2/neu positive patients (29%) and serum HER2/neu negative patients (33%). However, the progression-free interval after initiation of chemotherapy was significantly longer for serum HER2/neu negative patients (mean: 48.2 weeks)