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)

in comparison to serum HER2/neu positive patients (mean: 31.3 weeks; p=0.018).

Conclusions: Our results indicate that an elevated serum HER2/neu level is a negative predictive factor for bad treatment outcome in terms of progression-free survival. This result, together with the putatively increased anthracycline sensitivity of HER2/neu positive patients, may help for patient selection to a more individualized mode of chemotherapy.

719 POSTER

Biological study of anastrozole in post-menopausal advanced breast cancer (ABC) patients: Effects on bone metabolism and oestrogen suppression

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Purpose: To study the short term biological effect of anastrozole on serum oestrogens, androgens, 17OH-progesterone (17OH-PGR), gonadotrophins, sex hormone binding globulin (SHBG) and bone metabolism markers.

Methods: 34 consecutive pts with ABC received anastrozole 1 mg/day treatment Blood samples were taken before and at 2, 4, 8 and 12 weeks during treatment to measure serum levels of: oestrogens (E1, E2 and E1-S), androgens (Δ4, DHT, TST, Free TST, DHEA and DHEA-S), 17OH-PGR, SHBG and gonadotrophins. As indicator of bone resorption we measured serum carbossi-terminal telopeptide of type I collagen (ICTP) and the cross-linked N-telopeptide of type I collagen (NTx), and for the osteoblastic activity intact osteocalcin (BGP) and bone alkalyne phosphatase (BAP).

Results: After 2 weeks E_1 and E_1-S levels decreased of an average of 56% (range 23.1–88.8) and 75.8% (range 52.4–87.2) respectively; E_2 decreased of an average of 62% (range 31.4–89.6). No significant changes were detected in androgens and 17OH-PGR. There was a significant increase of gonadotrophins over time ($p = 0.0001$ and $p = 0.0001$ for LH and FSH, respectively), and a significant decrease in SHBG $p = 0.0001$. A progressive significant increase in bone metabolism serum markers was detected in all pts: $p = 0.0394$ for BAP, $p = 0.0156$ for BGP, $p = 0.0021$ for ICTP and $p = 0.0013$ for NTx. In particular, pts with bone metastases had an increase statistically significant of bone resorption markers ($p = 0.0019$ for ICTP and $p = 0.0251$ for NTx) and borderline for bone formation markers. In pts without bone disease BAP, BGP and ICTP remained unchanged, whereas serum NTx significantly increased $p = 0.0186$.

Conclusion: Anastrozole is a selective aromatase inhibitor as it does not modify serum levels of androgens and 17OH-PGR. In our experience no relation was found in the short term period between serum oestrogen suppression and bone metabolism. The evaluation of bone metabolism markers seems to be helpful for the monitoring of bone disease during hormonal treatment.

720 POSTER

Safety and activity of Capecitabine in elderly patients with advanced breast cancer

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Purpose: Capecitabine is a selectively tumor activated fluoropyrimidine which is effective in a wide range of solid tumors. This study tested the safety and the activity of Capecitabine in elderly patients (pts) with advanced breast cancer (ABC).

Methods: From May 1999 to March 2001 forty consecutive pts were treated. The first thirty pts were treated using a dosage of 2500 mg/sqm/day b.i.d. for 2 weeks with a week of rest; than to improve the safety profile we are continuing the trial by reducing the dosage (2000 mg/sqm/day). The pts median age was 74 years (range 65–89). Pts could receive one prior chemotherapy and/or 2 hormonal regimens for metastatic disease. A previous therapy containing 5-fluorouracil was allowed but a 12 months withdrawal period was required, starting from the last dosage of the previous treatment. The metastatic sites were liver (19), lung (14), soft tissue (12), bone (9), other (9).

Results: Toxicity according to NCI-CTC Bethesda was: *grade 3–4* diarrhea (10%), *grade 3* vomiting (7%), *grade 2* (10%) and *grade 1* (26%) hand-foot syndrome, *grade 2–3* asthenia (13%), *grade 2* stomatitis (7%). One patient died for gastrointestinal toxicity and one patient developed deep venous thrombosis. The objective responses were 11/31 (35%), 3% complete remission, stabilizations of disease were 9/31 (29%), and progressions 11/31 (35%). The median time to progression was 6 months.

Conclusions: These results suggest that Capecitabine is safe and active in elderly pts with advanced breast cancer.

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721 POSTER

Paclitaxel-ifosfamide for anthracycline-resistant advanced breast cancer

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The combination of paclitaxel 135mg/m2 as a 3 hour infusion on day 1 and ifosfamide 1.7 g/m2 as a 4 hour infusion on days 2 to 4 every 22 days was given to patients (pts) with advanced breast cancer resistant to anthracyline containing regimen or patients relapsed after antracycline containing adjuvant chemotherapy. Pts had to have measurable or evaluable progressive metastases or local disease, and only one regimen for metastatic disease. Thirty one pts with a median age of 49 years (range, 30-69) entered the study. Nine (29%) had lung and seventeen (55%) liver metastases (mts), nineteen (61%) bone mts. Only seven (23%) had lymph node mts and four (13%) skin mts. Median of 7 cycles (range 1-18) were delivered. Responses were evaluated according to WHO guidelines and side effect according to NCI criteria. A panel of oncologist and one radiologist reviewed all responses. At baseline only three patients (10%) were free of the adverse consequenses of the prior therapy. During the treatment severe toxicities (grade >3) included nausea 3%, vomiting 3%, pulmonary 3%, neuromotor 3%, asthenia/fatigue 7%, pain 7%, neutropenia 90%, thrombocytopenia 10%, anaemia 10%, infection 7%, while alopecia was universal. Three complete responses (10%), 10 partial responses (32%), 8 (32%) stable disease and 8 progressive disease (26%) were documented. Median survival and progression free survivals after beginning of treatment were 19.3 months and 6.1 months, respectively.

Conclusion: Combination of paclitaxel and ifosfamide seems to be a promising regimen (objective response rate of 42% and a median survival time of 19 months) with acceptable side effects in advanced breast cancer patients relapsed after antracycline based adjuvant treatment or resistant to antracycline treatment.

722 POSTER

Salvage treatment with irinotecan and gemcitabine in breast cancer patients pretreated with taxanes and anthracyclines: a multicenter phase II study

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Purpose: A multicenter phase II study was conducted to evaluate the efficacy and toxicity of the irinotecan (I) - gemcitabine (G) combination in women with disease progression after initial chemotherapy for metastatic breast cancer.

Patients and Methods: Thirty-six breast cancer patients pretreated with regimens including taxanes and anthracyclines received G 1200 mg/m2 on day 1 and day 8 and I 300 mg/m2 on day 8, every 3 weeks. The median age was 58 years and the performance status (WHO) was 0-1 in 26 (72%) patients and 2 in 10 (28%). Nineteen patients had received one, and 17 two or more prior chemotherapy regimens.

Results: All patients were evaluable for toxicity and 28 for response. One-hundred forty treatment cycles were administered with a median of 3.5 cycles/patient. Complete remission was recorded in one (4%) patient and partial response in 5 (18%) for an overall response rate of 22% (95% CI: 6.23% - 36.63%). Nine (32%) patients had stable disease and 13 (46%) progressed. Responses were observed at all metastatic sites with a median duration of response of 5.5 months (range, 2.5 to 6.5), and a median time to progression of 7.5 months (range, 4.5 to 15.5). The median survival was 9 months (range, 1 to 13) and the one-year survival rate 37%. Grade 3 neutropenia occurred in 7 (19%) and grade 4 in 6 (17%) patients. Neutropenia was associated with fever in 3 (9%) patients without toxic deaths. Grade 3 thrombocytopenia developed in 4 (11%) patients and grade 4 in 1 (3%). Non-hematologic toxicity was mild with grade 2-3 diarrhea reported in 6 (17%) patients and grade 2-3 asthenia in 13 (35%).