observed despite 65% of C-alone patients crossing over to receive H at disease progression. A previous analysis demonstrated that 89% of 3+ tumors and 24% of 2+ tumors show HER2 gene amplification. We sought to determine how the clinical benefit of H relates to HER2 amplification in this trial.

Methods: Histologic material from 458 of 469 enrolled subjects was available for FISH testing. This consisted of archived, unstained tissue sections (44%) or previously immunostained tissue sections (56%). The PathVysion dual probe FISH assay system was used to determine the HER2:CEP17 signal ratio in these samples. Amplification was prospectively defined as a ratio of >2.

Results: FISH results were obtained in 451/469 enrolled patients (96.2%). Amplification was detected in 76% of the study population (89% of 3+ and 31% of 2+ cases). The addition of H to C improved the RR in the FISH-positive subgroup from 30.8% to 54.0% (p<0.0001). There was no improvement in the FISH-negative subgroup (37.5% vs 38.0%, p=NS). Furthermore, the addition of H to C in the FISH-positive group resulted in a survival benefit (odds ratio 0.71, 95% CI: 0.54, 0.92, p=0.009) that was not detected in the FISH-negative subgroup (odds ratio 1.11, 95% CI: 0.70, 1.80, p=NS).

Conclusions: The survival benefit in FISH-positive patients is significant. Patient selection based on HER2 amplification as determined using FISH may accurately identify patients who obtain clinical benefit from H. These data support the use of FISH testing to select patients for H therapy.

971 POSTER DISCUSSION

Over-representation of a polymorphism/missense mutation in the ataxia telangiectasia, mutated (ATM) gene in breast cancer patients versus controls

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Purpose: Mothers of children diagnosed with ataxia telangiectasia have been reported to be at increased risk for breast cancer development. To test whether germline mutations in the ATM gene are associated with breast cancer, we compared the frequency of ATM cDNA sequence changes in breast cancer patients and controls.

Methods: We sequenced ATM cDNA in 91 breast cancer patients and compared sequence changes in these patients to the frequency of these alterations in a control set of 996 individuals with no cancer history. An allele specific oligonucleotide assay was used to study the specific polymorphisms of interest in the ATM cDNA for the control set. The frequency of identified base changes was also tested across ethnic groups and gender.

Results: No mutations that would lead to protein truncation were identified, but several polymorphisms were found in the cDNA of the breast cancer patients. The three polymorphisms that were found in two or more patients cause amino acid substitutions in the ATM protein of the following type: Ser49Cys, Pro1054Arg, and Asp1853Asn. The Ser49Cys polymorphism was found in 6.7% (5/75) of the breast cancer patients compared to 1.6% (12/946) of the control group (P=0.006, Fisher's 2-sided exact). The subgroup of patients with bilateral breast cancer had a frequency rate of 11.8% (2/17) which again was significantly different from the control group (P=0.025, Fisher's 2-sided exact). None of the 9 breast cancer patients that had a normal tissue complication following radiation treatment had the Ser49Cys change. The allelic frequencies of the other two polymorphisms were not different between cases and controls.

Conclusion: Breast cancer patients, particularly those with bilateral disease, are more likely to have a polymorphism in the ATM gene that results in a Ser49Cys change in the protein compared to controls. These data suggest Ser49Cys may be a functional polymorphism that contributes to breast cancer development or a polymorphism that is linked to another causative genetic factor.

972 POSTER DISCUSSION

Comparison of the prognostic significance of occult metastatic cells in the bone marrow (OMC-BM) and HER2-status in patients with stage I-III breast cancer (BC)

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Purpose: Both HER2neu gene amplification and protein over-expression as well as the presence of OMC-BM are significant biological factors for the survival of patients with BC. Recent studies have supported their potential clinical role to stratify patients for adjuvant therapy. In this ongoing study, the prognostic influence of OMC-BM in 378 BC patients (stage I-III) was compared to the prognostic impact of the HER2 status of the primary tumor tissue.

Methods: HER2/neu-status in the paraffin-embedded primary tumor tissue was determined by either immunostaining with antibodies CB11 and A0485, and automated cellular imaging (ACIS>), or FISH and confocal laser scanning microscopy. Occult tumor cells in the bone marrow aspirates were detected with immunocytochemistry, using the anti-cytokeratin antibody A45 B/B3, and screening of 2 x 106 cells per aspirate in bright field microscopy.

Results: OMC-BM were found in 112/378 (30%) patients, HER2 overexpression (2/3) in 62/299 (21%) and amplification (HER2/chromosome-17 ratio greater than 2) in 54/235 (23%) patients. HER2 status was associated with lymph node metastasis (p=0.04 for immunostaining and p=0.033 for FISH), while the presence of OMC-BM was related to an increasing tumor size (p=0.006), but not to HER2 overexpression and amplification. After 40 (12-72) months of median follow-up, OS was significantly reduced in patients with OMC-BM (p<0.0001), while the HER2 status only reported a statistical trend towards poor OS (p=0.052 and p=0.11). The presence of OMC-BM was found to be an independent prognostic factor with a 2.9-fold increased relative risk of cancer-related death (p=0.028) in the multivariate analysis.

Conclusion: The direct identification of metastatic precursor cells in the bone marrow could help to improve current stratification of stage I-III breast cancer patients at high risk of relapse. In contrast to HER2 status of the primary tumor, OMC-BM can also be used to monitor patients during specific adjuvant therapy (e.g. antibody therapy), as previously shown by our group.

Cell biology/Genetics II

973 POSTER DISCUSSION

Pamidronate (P) induces modifications of circulating angiogenetic factors in cancer patients

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Purpose: This study was designed primarily to investigate the potential antiangiogenic role of P in patients with malignancies.

Methods: 16 consecutive patients (11 males, 5 females), aged 49-77 years (median 65), with bone metastases from solid cancer, were included in the study. Exclusion criteria were: a) the presence of acute or chronic inflammatory diseases or infections; b) previous radiotherapy, chemotherapy, immunotherapy or haemopoietic growth factors administration during the 4 weeks before accrual in the study; c) recent or simultaneous administration of steroids. Patients received 90 mg i.v. of P over a 2 hours infusion. Blood samples for cytokines assessment (VEGF, gamma-IFN, IL-6 and IL-8) were collected as follows: before and after 24, 48 hours and 1 week from P administration.

Results: The mean value of basal VEGF was 762,46 pg/ml (Standard Deviation (SD): 291,04). 24 hours after single P infusion the mean value of VEGF decreased to 515,93 pg/ml (SD: 186,91) (p=0.006), and after 48 hours persisted lower with a mean value of 485,57 pg/ml (SD: 237,13) (p=0.001). The effect of P on VEGF persisted after 1 week with a mean value of 596,47 pg/ml (SD: 385,71) (p=0.028). The mean value of basal gamma-IFN was 11,12 pg/ml (SD: 4,34). After 24 hours the P infusion the mean value significantly increased to 21,93 pg/ml (SD: 14,26) (p=0.019). Otherwise, after 48 hours gamma-IFN (12,66 pg/ml; SD: 6,11l) did not significantly differ from the basal value (p= 0.701) and persisted stationary also after 1 week (12,25 pg/ml; SD: 8,34) (p=0.929). The mean basal value of IL-6 was 9,88 pg/ml (SD: 12,1). 24 hours after the P administration the

mean value increased to 14,10 pg/ml (SD: 14,10) (p=0.007). Moreover, IL-6 decreased to 10,78 pg/ml (SD: 12,38) after 48 hours (p=0.263) and persisted stable (14,97 pg/ml; SD: 14,86) after 1 week (p=0.362). IL-8 levels did not show any statistically significant modification from the basal values after P administration. Statistical analysis showed a significant negative correlation between VEGF values and gamma-IFN values (p= 0.016) and a significant positive correlation between VEGF and IL-8 (p=0.040).

Conclusions: Our data show that P has a powerful antiangiogenic effect mediated by a statistically significant increase in VEGF levels persisting also after one week from the administration of the drug.

974 POSTER DISCUSSION

The expression of vascular endothelial growth factor(VEGF) is a highly significant prognostic factor in stage IB carcinoma of the cervix

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Purpose: The aim of this study was to clarify the role of VEGF expression as an independent prognostic factor and to identify the patients at high risk for poor prognosis in stage IB cervical cancer.

Methods: A total of 118 patients with stage IB cervical cancer who had radical hysterectomy and pelvic lymph node dissection were included in the study. All known high risk factors of the patients were pathologically confirmed from the surgical specimen. Of the 118 patients, 88 patients were treated with postoperative radiotherapy and/or chemotherapy. VEGF expression was examined using immunohistochemistry in formalin-fixed, paraffin-embedded specimens of post-hysterectomy surgical materials. A semiquantitative analysis was made using a scoring system of 0, 1, 2, and 3 for increasing intensity of stain. We classified the patients with scores from 0 to 2 as low staining intensity and the patients with a score of 3 as high staining intensity.

Results: Of the 118 patients, 35 patients (30%) showed high staining intensity (3) of VEGF. Strong correlations were found between the high staining intensity of VEGF and both deep stromal invasion (p=0.009) and the positive pelvic node (p=0.024). The 5-year overall and disease-free survival rates for all 118 patients were 94.6% and 92%. The 5-year overall (p=0.01) and disease-free survival (p=0.0014) rates were 97.1% and 98.6% for low intensity (0, 1, and 2) of VEGF and 90.7% and 81.7% for high intensity of VEGF, respectively. Pelvic and distant failures for low versus high intensity of VEGF were 1.2% versus 17.1%, (p=0.003) and 0% versus 14.3% (p=0.002), respectively. In a Cox multivariate analysis of survival, the high staining intensity of VEGF (p=0.008) and the positive pelvic node (p=0.013) were significant prognostic factors for overall survival. The high staining intensity of VEGF (p=0.013), vascular invasion (p=0.02), and bulky mass (p=0.034) demonstrated as significant prognostic indicators for disease free survival.

Conclusion: These results showed that the intensity of VEGF expression was a highly significant predictor for pelvic and distant failure and the most significant prognostic factor of overall and disease free survival for the patients with stage IB cervix cancer treated with radical surgery. We strongly suggest that the immunohistochemistry for VEGF expression be performed in a routine clinical setting in order to identify patients at high risk for poor prognosis in cervical cancer.

975 POSTER DISCUSSION

Inhibition of neuroblastoma-induced angiogenesis by fenretinide

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Purpose: Retinoids partecipate in the control of cell proliferation, differentiation and foetal development. The synthetic retinoid fenretinide (HPR) inhibits carcinogenesis in various animal models and it has been suggested that retinoids are effective inhibitors of angiogenesis.

Methods: The effects of HPR on certain endothelial cell (EC) functions were investigated in vitro, and in vivo, by using the chorioallantoic membrane (CAM) assay.

Results: HPR inhibited VEGF- and FGF-2-induced EC proliferation without affecting endothelial motility; moreover, it inhibited growth factors-induced angiogenesis in the CAM assay. A significant anti-angiogenic potential of HPR has been observed also in neuroblastoma (NB) biopsies induced angiogenesis in vivo. We previously demonstrated that supernatants derived from NB cell lines stimulated EC proliferation. Here, we show that this effect is abolished when NB cells were incubated in the presence of HPR. VEGF- and FGF-2 specific ELISA assays, performed on both NB-cells derived conditioned medium and cellular extracts, indicated no effect of HPR on the level of these angiogenic cytokines. Moreover, RT-PCR analysis of VEGF and FGF-2 gene expression confirmed the above lack of effect. HPR was also able to significantly repress the spontaneous growth of EC, requiring at least 48-72 h of treatment with HPR, following by a progressive accumulation of cells in G1 at subsequent time points. Finally, immunohistochemistry experiments performed in the CAM assay demonstrated that endothelial staining of both VEGF receptor-2 and FGF-2 receptor-2 was reduced after implantation of HPR-loaded sponges, as compared to control CAM's.

Conclusion: These data suggest that HPR exerts its antiangiogenic activity through both a direct effect on EC proliferative activity and an inhibitory effect on the responsivity of the EC to the proliferative stimuli mediated by angiogenic growth factors.

976 POSTER DISCUSSION

Pancreatic tumor growth is regulated by the balance of positive and negative modulators of angiogenesis

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There is increasing evidence for the implication of tumor-derived angiogenic and anti-angiogenic factors in tumor growth in vivo. In this study we examined how changes in the balance between these factors regulate the growth of a tumor in a pancreatic cancer model in vivo. The pancreatic cancer cell line Hs-776T (HS-W) displays slow growing tumors and we have isolated a natural occurring variant (HS-R), which grew tumors more rapidly. In vitro, HS-W and HS-R produce low amounts of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) but only HS-W cells produce endostatin. In order to examine the effect of over-expression of angiogenic factors on pancreatic tumor growth in vivo, we have transfected HS-W cells with human VEGF165 cDNA. Upon injection of VEGF overexpressing cells into immune deficient mice, rapidly growing tumors were observed. In contrast, mock transfected cells, HS-PCP, formed small tumors similar to parental cells. Tumors of VEGF-transfected clones were highly vascularized with many dilated blood vessels, lined by a single layer of CD31-positive endothelial cells. PCNA staining showed high proliferation index in VEGF producing tumors and conversely, apoptosis, as determined by TUNEL staining, was higher in HS-W, moderate in HS-R and low in VEGF producing tumors. Collectively, our study confirms that tumor growth is dependent on its ability to increase the angiogenic stimulus or to reduce the amounts of endogenous antiangiogenic factors.

977 POSTER DISCUSSION

Increased angiogenesis in bone metastases of patients with metastatic breast cancer

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Introduction: In a great proportion of patients with metastatic breast cancer, the disease metastasizes to the bone causing pain, hemopoietic insufficiency and a reduction in the quality of life. However, the mechanisms that contribute to bone metastasis are poorly understood. Therefore, the aim of this study is to clarify the mechanisms of tumor-endothelial-cell-interaction and to evaluate the role of angiogenesis for the development of bone metastases in patients with breast cancer.

Methods: Specimens from patients with metastatic breast cancer and non-malignant bone tissue were studied. Paraffin embedded sections were stained with hematoxylin and eosin and examined for the presence or absence of malignant tissue. Furthermore, an indirect immunoperoxidase staining with an monoclonal antibody against the CD31 epitope was used to identify vascular endothelium. Microvessel density (MVD) was counted in hot spots as well as in representative areas of the adherent section.

Results: To date 27 bone metastases and 26 non-malignant bone tissue specimens have been examined. The number of microvessels in bone