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