metastasis had occurred in one patient with CK+ micrometastases in BM, but not in LN.

Conclusion: With the short follow-up, our analysis remains descriptive at the present time. However, there appears to be no correlation between lymphatic and hematogenous tumor cell spread. This suggests the existence of biologic differences between these two tumor cell populations that might be elucidated by molecular characterization of these cells, which is part of further studies.

283 POSTER DISCUSSION

A real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to detect breast carcinoma cells in peripheral blood

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Background: The detection of occult carcinoma cells in patients with breast cancer has been shown to predict disease recurrence and metastasis. To improve on existing methods of immunocytologic and molecular detection we have developed a sensitive and quantitative assay to detect breast carcinoma cells in blood, using real-time quantitative RT-PCR identifying transcripts of the cytokeratin-19 (CK19) gene.

Methods: The ABI Prism 7700 (Taqman®) technology is based on the cleavage of a probe labelled with both a fluorescent reporter and a quencher dye, by the 5, −3, exonuclease activity of Taq polymerase during strand elongation of PCR. This cleavage results in an increase in reporter emission intensity, which corresponds to the target sequence concentration. The fluorescent signal is measured during the exponential phase of product amplification, which ensures sensitive real-time quantification. The cycle at which the emission intensity rises above baseline is referred to as Ct (threshold cycle). The Ct values decrease linearly with increasing target quantity. To evaluate the sensitivity of the assay, we measured CK19 mRNA concentrations after RT-PCR in MDA-231 and EFM-19 breast cancer cells spiked in the CK19 negative AML14 cell line. Parallel amplification of the b-actin house keeping gene allowed normalisation of the target concentration.

Results: Primers and probe were developed specifically for the detection of CK19 transcripts with this technique using Primer Express software. Amplification was specific for CK19 mRNA, no amplification of pseudogenes was observed. CK19 transcripts were still detectable when 0.5 MDA-231 and 1 EFM-19 cell were diluted in 106 CK19 negative cells. The Ct for 100% positive cells was 19.25 for MDA-231 and 15.5 for EFM-19; for 0.5 MDA-231 cells/106 AML14 cells the Ct was 36.8 and for 1 EFM-19 cells/106 AML14 cells the Ct was 30. The correlation coefficient of the standard curve (target cell quantity versus Ct value) was at least 0.98.

Conclusion: We have developed a sensitive, accurate real-time quantitative RT-PCR with high reproducibility within a wide dynamic range, which permits simultaneous analysis of samples with varying input concentrations. The procedure offers several technical advantages over classic quantitative PCR methods (competitive RT-PCR, Northern blotting) such as decreased likelihood of contamination due to absence of post-PCR manipulations, high sample throughput because of absence of post-PCR processing time (no agarose gel electrophoresis). Analyses using this real time quantitative RT-PCR for CK19 mRNA may prove to have clinical implications in the assessment of circulating tumour cells in peripheral blood, micrometastases in bone marrow or lymph nodes in breast cancer patients. Validation of application of this technique in a clinical population may improve diagnosis and monitoring of metastatic breast cancer.

284 POSTER

HER2 interaction with intermediate filament proteins and the influence on prognosis in breast cancer

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Purpose: The oncoprotein HER2 seems to affect cell adhesion and metastasis via structural proteins. In this context we investigated the influence of intermediate filament proteins (IF) on prognosis in relation to HER2 in breast cancer.

Methods: Paraffin embedded specimens of 80 primary invasive ductal breast carcinomas were analyzed immunhistochemically (APAAP) for the expression of *keratin* (K) *8*, characterizing normal breast epithelia, *K19*, inconstantly expressed in normal breast epithelia, *vimentin*, characterizing mesenchymal epithelia and the oncoprotein *HER2*. The results were compared with disease-free (DFS) and overall survival (OS) over a ten-years-follow-up.

Result: Strong expression of K8 was seen in 27.5% of the tumors and was correlated with excellent prognosis (DFS/OS: $p < 0.004$). K19 showed a stronger expression than K8 (35%), but did not correlate with prognosis. Aberrant expression of vimentin was seen in 21.3% of the tumors and was associated with poor prognosis (DFS: $p < 0.003$/OS: $p < 0.006$). HER2-overexpression was noticed in 35% and was associated with short survival rates (DFS: $p < 0.01$/OS: $p < 0.02$). HER2 overexpression was significantly correlated with expression of K19 ($p < 0.0004$) and vimentin ($p < 0.0005$).

Conclusions: HER2-overexpression is associated with fundamental changes of the IF-pattern in breast carcinoma cells. Beside the loss of the K8 and K18, expression of K19 and aberrant expression of vimentin mark structural alterations especially in HER2 overexpressing cells, which obviously leads to early metastasis and poor prognosis in breast cancer. IF-changes therefore may play an important role in the malignant functioning of HER2.

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C-ERB-B2 expression as a predictor of outcome in a randomized trial comparing adjuvant CMF vs single-agent epirubicin in stage I–II breast cancer (BC) patients

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Purpose: c-erbB-2 abnormalities either gene amplification or protein overexpression are associated with worse prognosis of pts with N+ BC but the relationship for pts N− is weak at best. Some studies, mostly retrospective, have reported that pts whose tumors have amplified erbB-2 genes or overexpress c-erbB-2 protein benefit from anthracyclines-containing regimen.

Methods: We completed a randomized study comparing CMF versus weekly epirubicin (E) in the adjuvant treatment of 348 stage I and II BC pts (Proc ASCO 19976:142a). We evaluate retrospectively the expression of c-erbB-2 and its interaction with the treatment.

Results: At a median follow-up of 5.6 yrs c-erbB-2 expression was measured by IHC in 266 pts (76%) using the monoclonal antibodies CB11. 133 pts were in CMF arm and 133 in E arm. A baseline significant excess of erbB-2 positive tumors was observed in E arm (41% vs 28%; HR = 2.78; 95% CI 1.13–6.88; $p = 0.03$). DFS and OS were calculated by the Kaplan Meier method. Long rank and Cox models were used to compare DFS and OS for c-erbB-2 status regarded both as a continuous or binary variable. A significantly worse OS was observed for c-erbB-2 positive pts ($p = 0.02$). A Cox regression model including menopausal status, T and n° of positive nodes, treatment and interaction term between arm and c-erbB-2 showed that c-erbB-2 overexpression is a predictor of a poorer OS (HR = 2.78; 95% CI 1.13–6.88; $p = 0.03$). No statistically significant difference was observed between the two arms.

Conclusions: The E treatment had no significant impact on the outcome of pts with erbB-2 tumor positive ($p = 0.12$), but in our study E (30 mg/m^2) was administered weekly for 16 wks. The small number of events (39 deaths) prevents from definitive conclusions.

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Predictors of axillary lymph node involvement in breast cancer <20 mm

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Purpose: Because the frequency of metastatic involvement of axillary lymph node (ALN) in breast cancer (BC) <20 mm is low, the necessity of ALN dissection in these patients (pts.) is under discussion today. If it would be possible to predict the ALN status by parameters of the tumor, a lot of pts. could potentially be spared ALN dissection.

Method: The data of 386 pts. with BC and a tumor size (TS) of 3–20 mm and non palpable and non suspicious ALN by ultrasound were studied retrospectively. Potential predictive histopathological parameters, hormone receptor status and newer factors (HER/2-neu, EGF, Cathepsin D, pS2, DNA-index, S-phase, p53, uPA, PAI-1) were examined with regard to their correlation with the ALN status.