(p = 0.037). The classification was in good agreement with IHC data for ER and CK5/6. PCA revealed that only 35% of the differences between IBC and nIBC can be explained by the presence of the cell-of-origin subtypes. 12/16 IBC specimens correlated with the quiescent fibroblast signature centroid compared with 5/18 nIBC specimens (p = 0.006). When only significant correlations were taken into account, 6/6 IBC and 3/8 nIBC specimens correlated with the quiescent fibroblast signature centroid (p = 0.03). These data are currently confirmed using real-time qRT-PCR.

Discussion: These data sustain our previous findings that IBC and nIBC are two distinct biological entitities. Different cell-of-origin subtypes in IBC were identified, but cannot fully explain the specific phenotype. Other processes must determine the biology of IBC, as shown by the differential expression of the wound healing response signature.

270 Poster

Effect of tibolone on breast cancer cell proliferation in postmenopausal ER+ patients: results from a double-blind, placebo-controlled, randomized clinical trial (STEM)

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Experimental design: Postmenopausal women with stage I or II, ER+ primary breast cancer, were randomly assigned to 14 days of placebo or 2.5 mg/day tibolone. Core biopsies of the primary tumor were obtained before therapy, and a representative sample of the excised tumor was obtained from the operative specimen after treatment. For each patient, Ki67 and, apoptosis index were analyzed in both baseline and the corresponding post-treatment specimen.

Results: Of 102 enrolled patients, 95 had evaluable data. Baseline characteristics were comparable between both treatment groups. Most breast cancer cases were invasive (99%), stage I or II (42% and 50% respectively) and ER+ (99%). Median intratumoral Ki67 expression at baseline was 13.0% in the tibolone group and 17.8% in the placebo group, and decreased to 12.0% after 14 days of tibolone while increasing non-significantly to 19.0% in the placebo group. Similarly, no significant differences were observed between the treatment groups when the median baseline apoptosis index (1.4% in both groups) was compared to the corresponding post-therapeutic indices of 1.6% (tibolone) and 1.7% (placebo). No differences between tibolone and placebo were observed with respect to the incidence of adverse effects.

Conclusion: 2.5 mg/day tibolone given for 14 days has no significant effect on tumor cell proliferation and apoptosis in ER+ tumors.

271 Poster

Expression of FOXP3 and vascular endothelial growth factor in human breast cancer: its correlation with angiogenesis and disease progression

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Background and Objective: The Forkhead/winged helix transcription factor FOXP3 is positively associated with the induction of $CD4^+CD25^+CD45RA^-$ T regulatory cells, which have a suppressive effect on the effector T-cells. To determine whether FOXP3 might be involved in the progression of breast carcinoma, we measured the expression of FOXP3 in infiltrating breast carcinoma along with their corresponding normal breast tissues and a smaller number of ductal carcinoma *in situ* (DCIS) specimens and correlated it with the expression of Vascular Endothelial Growth Factor (VEGF, an invasogenic and angiogenic growth factor) and intratumoral microvessel density (IMD, a prognostic marker for angiogenesis).

Methods: FOXP3 and VEGF mRNA expression was semiquantitated by RT-PCR assay in 20 biopsies of infiltrating breast carcinoma, 5 biopsies of DCIS along with 20 biopsies of normal breast tissues and analyzed their correlation with each other and with the IMD, determined by immunohistochemical staining using anti-CD34 antibody. We also analyzed whether FOXP3 mRNA expression correlated with other pathological variables like tumor size, histological grade and lymph node status, the prognostic indicators of breast carcinoma.

Results: Invasive cancers had nearly three times greater FOXP3 mRNA expression than did ductal carcinoma *in situ* and nearly eight times greater than normal tissue and the difference was statistically significant ($P < 0.05$; $P < 0.02$, two tailed t-test respectively). There appeared to be a trend towards increasing FOXP3 mRNA expression with increase in tumor size, with the larger tumors (≥ 2.0 cm) having approximately two-fold higher FOXP3 expression than the smaller tumors (< 2.0 cm), although the difference was statistically insignificant. FOXP3 expression was also found to be increased in higher grade tumors ($0.05 < P < 1.0$). There was a clear trend towards increasing VEGF mRNA expression with increase in FOXP3 expression, however, the statistical comparison revealed no significance. There was no significant correlation between FOXP3 mRNA expression with IMD and lymph node status.

Conclusion: These findings suggest that the expression of FOXP3 transcription factor has a direct correlation with other clinicopathological indicators of aggressive tumor behavior, consistent with the hypothesis that FOXP3 is a biological factor that may play a role in breast cancer progression.

272 Poster

Real-time RT-PCR of CD146 and VE-cadherin mRNA to detect circulating endothelial cells in peripheral blood of patients with breast cancer

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Angiogenesis is a fundamental process in tumour growth and metastatic dissemination. The number of circulating endothelial cells (CECs) in peripheral blood (PB) of patients with cancer reflects the amount of proceeding angiogenesis and can therefore be used as a surrogate marker to monitor antiangiogenic therapy. The standard quantification method of CECs is currently based on a complex four-color flow cytometry. Real-time RT-PCR technology to quantify EC-specific mRNA in PB samples has been shown to be a promising alternative approach. This study aimed to compare mRNA expression levels of EC-specific markers (CD146 and VE-cadherin) in PB of healthy volunteers and patients with breast cancer using real-time RT-PCR.

PB samples have been collected from 18 healthy volunteers and 18 metastatic breast cancer patients. RNA was subsequently isolated with the PAXgene Blood RNA isolation kit. Real-time PCR analysis was performed with primers and TaqMan probes for both CD146 and VE-cadherin mRNA. Ct values were normalised for beta-actin mRNA expression and gene expression levels were calculated relative to a reference sample (RGE).

VE-cadherin mRNA was increased in patients with breast cancer in comparison to healthy volunteers: the median VE-cadherin mRNA expression level in PB of healthy volunteers was 1.20 (range 0.50–4.18); this was 2.45 (range 0.69–25.80) for patients with breast cancer (p = 0.040). However, the difference in CD146 mRNA expression levels between healthy volunteers and patients with breast cancer did not reach statistical significance: the median CD146 mRNA expression level in PB of healthy volunteers was 0.037 (range 0.020–0.058); this was 0.058 (range 0.013–0.488) for patients with breast cancer (p = 0.077). CD146 and VE-cadherin mRNA expressions were significantly correlated (r = 0.401, p = 0.017). A cut-off value was determined as the 95^{th} percentile of the RGE values of the healthy volunteers: this value was 0.058 for CD146 and 4.184 for VE-cadherin mRNA. 9 out of 17 patients with breast cancer had a RGE of CD146 above the cut-off value;for VE-cadherin 7 out of 18 patients with breast cancer had increased RGEs.

Our preliminary results suggest that the quantitative evaluation of EC-specific mRNA by real-time RT-PCR could indeed be a promising tool to monitor the efficiency of antiangiogenic therapy in patients with breast cancer but a larger study population and a comparison with flow cytometry is necessary to confirm this. These studies are ongoing.

273 Poster

Local aromatase and sulfotransferase protein expression in malignant breast tumors vs adjacent and distant breast tissue

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The suppression of local estrogens levels is of key importance in the treatment of ER positive breast cancer. Most endocrine strategies now

act by either suppressing the local estrogen formation or by competitively inhibiting receptor binding. Nevertheless, little is known about the local expression of aromatase and sulfotransferase, which are key modulators of intratumoral estrogen levels.

We have performed immunohistochemostry to investigate the expression of aromatase and sulfotransferase in 42 samples obtained directly from malignant breast tumors, and compared it to biopsies obtained from uninvolved tissue in the vicinity of the front and to distant breast tissue. We found that aromatase was equally detectable in both tumor epithelial and stroma, but was mostly confined to the epithelium in non-malignant tissues ($p = 0.00008$, Fisher's Exact Test). Also, aromatase protein expressin was significantly more common in tumoral stroma when compared to peritumoral and distant breast stroma ($p = 0.00005$, and $p < 0.00001$, respectively). By contrast, sulfotransferase protein was only detectable in epithelial tissues, regardless of the location within the diseased breast. Epithelial sulfotransferase was, however, correlated with epithelial aromatase ($r = 0.35461$, $p = 0.0009$, Spearman's Rho test) and with the epithelial ER status ($r = 0.29313$, $p = 0.005$).

Taken together, we have demonstrated a differential aromatase and sulfotransferase protein expression pattern that is dependent of the spacial relation to a malignant breast tumor. Our results indicate a net increase in intratumoral active estrogen levels through increased stromal aromatization, while physiological local inactivation by sulfotransferase activity remains essentially unchanged.

Thursday, 23 March 2006 16:00–16:45

POSTER SESSION

Predictive and prognostic factors

274 Poster

Prognostic factors and impact of contralateral cancer on survival of hereditary breast cancer

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Introduction: A high incidence of contralateral breast cancer (CBC) has been reported in BRCA1 mutation carriers and high risk familial patients. Data are scarce on the influence of hereditary CBC on survival, while 10- 25% of new familial patients opt for a risk-reducing contralateral mastectomy. Here we assess differences in CBC incidence, ipsilateral recurrence (ILR) and breast cancer specific survival (BCSS) in 3 risk groups and which factors influence prognosis.

Methods: We assessed tumour characteristics, CBC incidence, ILR and BCSS in 223 BRCA1 mutation carriers with invasive BC and 311 BC patients with $\geqslant 3$ breast and/or ovarian cancers in the family but no BRCA1/2 gene mutation (non-BRCA1/2). They were matched to 759 sporadic controls for year and age at detection.

Results: Median follow-up was 5.4 yrs. Tumours were $\leqslant$ T1 in 50% of the BRCA1, 60% of non-BRCA1/2 and 45% of sporadic patients ($p = 0.02$), node-negative in 66%, 53% and 48% respectively ($p < 0.001$), grade 1 or 2 in 8%, 30% and 26% respectively ($p < 0.001$). Risk-reducing contralateral mastectomy was performed in 23%, 11% and 1% respectively ($p < 0.001$.

After correction for the selection bias of offering DNA-testing with preference to patients with CBC and longer living patients, by exclusion of the patients with a DNA test performed 2 years or more after their diagnosis, 10 years metachronous CBC incidence was 25% in 170 unselected-BRCA1 patients, 6% in 238 unselected-non-BRCA1/2 patients and 5% in the sporadic patients ($p < 0.001$).

After correction for age, stage, grade, estrogen receptor and adjuvant therapy there was no significant difference in BCS survival between the 3 groups (unselected-BRCA1 vs. sporadic HR 1.1; p0.6) (unselected-non-BRCA1/2 vs. sporadic HR 0.9; p0.7), nor did ILR differ (multivariate HR 0.81 for BRCA1 vs. sporadic $p = 0.6$; HR 1.5 for non-BRCA1/2 vs. sporadic $p = 0.2$).

Independent prognostic factors for BCS survival in the total BRCA1, non-BRCA1/2 and sporadic group were tumour size (HR T2 vs. T1: 2.3; $p < 0.001$) nodal status (HR+ vs. HR−: 3.2; $p < 0.001$), age (HR 0.98 per year increase; $p = 0.009$), adjuvant therapy (HR 0.5; $p < 0.001$), and positive estrogen receptor (HR 0.6; $p < 0.001$) Metachronous CBC was associated with favourable BCSS,using follow-up from first diagnosis HR 0.6;p0.01, reflecting longevity before CBC. After CBC, BCSS was comparable (H 1.1; $p = 0.7$)

Conclusion: After correction for selection bias, stage and treatment factors we found no significant difference in BCS survival between both hereditary groups and sporadic breast cancer patients. Stage at detection of the first BC and adjuvant therapy are also in hereditary patients key determinants of prognosis, whereas the occurrence of metachronous contralateral breast cancer is not. We will discuss the impact of CBC and risk-reducing contralateral mastectomy. Decisions on breast-conserving treatment can be made on the same grounds in hereditary and sporadic patients.

275 Poster

Expression of the HOXB13-to-IL17BR-gene ratio in oestrogen receptor positive primary breast carcinomas: Relation with tumour aggressiveness and response to tamoxifen

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Using a genome-wide screening, Ma et al. [(1)] identified the HOXB13-to-IL17BR expression ratio to predict clinical outcome of breast cancer patients treated with adjuvant tamoxifen. However, in the adjuvant setting this ratio may predict both a tumour's response to tamoxifen and its intrinsic aggressiveness. Therefore, we evaluated the two-gene expression ratio in retrospectively collected frozen specimens from 917 oestrogen receptor (ER) positive primary breast tumours. Using a quantitative RT-PCR assay we have assessed: 1) the relation with tumour aggressiveness and 2) the association with response to first-line tamoxifen monotherapy. Patients who received adjuvant systemic therapy were excluded in this study.

To investigate the relation with tumour aggressiveness, 619 tumours were analysed from 468 lymph node-negative and 151 node-positive patients of whom 332 patients showed a recurrence. The association with therapy response was determined in 193 tumours from patients treated with first-line tamoxifen for advanced disease. Expression levels were compared to housekeeper genes and correlated with clinical outcome. The hazard ratio (HR) and 95% confidence interval (95% CI) were calculated and all statistical tests were two-sided.

As continuous variable, the two-gene ratio had a statistically significant correlation in univariate analysis with disease-free survival (DFS) and progression-free survival (PFS), irrespective of lymph-node status. When dichotomised, high expression levels of HOXB13-to-IL17BR ratio showed a strong association with a shorter DFS for both node-negative (HR = 1.52 [95% CI: 1.16–1.99]; P = 0.002) as well as node-positive patients (HR = 1.66 [95% CI: 1.14–2.44]; P = 0.009). In addition, a shorter PFS for patients treated with first-line tamoxifen (HR = 3.43 [95% CI: 2.18–5.40]; P < 0.0001) was observed.

In conclusion, these results indicate that the HOXB13-to-IL17BR ratio is able to identify 1) patients at risk for earlier recurrence as well as 2) patients who fail to respond to first-line tamoxifen monotherapy for advanced disease. As a consequence, these patients may benefit more from other treatment modalities.

References

[1] Ma XJ, Wang Z, Ryan PD, Isakoff SJ, Barmettler A, Fuller A, et al. A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. Cancer Cell 2004; 5: 607–616.

276 Poster

Risk of second non breast cancer in relation to BRCA1 and BRCA2 mutation status following breast-conserving treatment

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Purpose: *BRCA1* and *BRCA2* germline mutations are associated with a strong risk of breast and ovarian cancer. The increased risk of other cancers is not clearly established. We investigated whether mutation status influenced the rate of second non breast malignancies (SNBM).

Patients and Methods: *BRCA1* and *BRCA2* genes were screened for germline mutations in 131 patients with a family history of breast and/or ovarian cancer, treated with breast conserving surgery and radiotherapy. The 131 patients with familial history were matched to 261 patients without, according to age at diagnosis and year of treatment. The follow-up of controls was at least equal to the time-interval between diagnosis and genetic testing in familial cases. SNBM free interval was calculated from the