(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.

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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.

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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.

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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.

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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