and the disease-free patient group showed the same trend of differential expression as they did in the xenograft model. These trends could be confirmed for all of the 5 down-regulated as well as for 3 of the 6 up-regulated genes. Comparing the expression data for each gene in individual patients, distinct expression differences were observed for NNAT, IGFBP5 and HOXB13.

Conclusion: TaqMan® Low Density Arrays provide an efficient and reliable RealTime PCR method in a 2 µl reaction volume for the simultaneous analysis of multiple genes. Expression data for several genes in clinical samples reveal differences with respect to recurrence under tamoxifen therapy. We were able to define candidate genes possibly related to tamoxifen responsiveness that will be subjected to further functional evaluation.

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Gene profiling analysis of tissue-specific metastases from human breast cancer

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Breast cancers are prone to metastasise, particularly to the lymphatics, bone, liver, lungs and central nervous system. Once solid secondary tumours are established, the chances of long-term survival fall from over 90% to around 5%. The European project "MetaBre" investigates the molecular mechanisms of breast cancer organ-specific metastasis. The first step of this project has studied the gene expression profiles of tissue-specific metastases by use of microarray analysis (Affymetrix HG U133 plus 2.0, over 47,000 transcripts). To this aim, we hybridised 21 human breast cancer metastases resected from 5 different organs: lung (5), liver (6), bone (4), brain (4) and skin (2). We also analysed normal tissues from each corresponding target organ.

Statistical analyses were performed using BRB ArrayTools 3.2.2. "One class versus all" class prediction analysis was used to identify genes involved in organ-specific metastasis. 4 signatures of approximately 20 genes were established for lung, liver, bone and brain metastases. The validation of the signatures was performed by quantitative RT-PCR. These 4 signatures were able specifically to distinguish metastases from different sites from each other. Furthermore, our lung metastasis signature was able to discriminate breast cancer primary tumours relapsing to lungs from a series of tumours metastasizing to different organs. Thus, our expression profiling study allowed us to identify genes potentially involved in organ-specific metastasis.

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Gene copy numbers and expression of ErbB-1 and ErbB-2 in breast cancer

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Background: The family of ErbB oncogenes and their receptors play important role in breast cancer development, however prognostic relevance of ErbB-1 and ErbB-2 abnormalities is debatable. The aim of this study was to determine clinical impact of ErbB-1 and ErbB-2 gene copy numbers and expression in a large series of breast cancer patients.

Material and Methods: Study group included 225 consecutive stage I-III breast cancer patients treated between 1998 and 2002 in three Polish institutions. Average gene copy numbers (AGCN) of ErbB-1 and ErbB-2 were determined by double differential polymerase chain reaction (ddPCR). Expression of ErbB1 (63 patients) and ErbB-2 (171 patients) was assessed by tissue microarray immunohistochemistry (TMA-IHC) and by IHC-based HercepTest, respectively. Disease free survival (DFS) and overall survival (OS) were computed by the Kaplan-Meier method. Univariate and multivariate survival analysis was performed with log rank test and Cox proportional hazard model.

Results: ErbB-1 amplifications and deletions were found in 15% and 31% of cases, respectively, and ErbB-2 amplifications and deletions – in 26% and 3% of cases, respectively. Deletions of ErbB-1 occurred more frequently in node negative ($p = 0.03$) and in PgR negative cases ($p = 0.06$), whereas ErbB-2 AGCN was not related to major clinicopathological characteristics. Overexpression of ErbB-1 and ErbB-2 occurred in 17% and 18% of patients, respectively and both abnormalities were correlated with negative estrogen receptor status ($p = 0.007$ and $p = 0.02$, respectively). ErbB-1 was correlated with lymph node metastases ($p = 0.06$) and larger tumor size ($p = 0.027$). The correlation between expression and AGCN was strong for ErbB-2 ($p = 0.0003$) and insignificant for ErbB-1. ErbB-1 amplification was associated with shorter DFS and OS ($p = 0.03$ and 0.02, respectively) and overexpression – with shorter DFS ($p = 0.04$). ErbB-2 overexpression was associated with shorter OS ($p = 0.02$), whereas prognostic impact of ErbB-2 AGCN did not reach statistical significance. There was a strong correlation between AGCN of ErbB-1 and ErbB-2 ($p = 0.000036$). Patients with co-amplification of both genes tended to be node-positive, but small number of this subset did not allow for statistical analysis.

Conclusions: Overexpression of both ErbB-1 and ErbB-2, and amplification of ErbB-1 carry adverse prognosis in breast cancer patients. Strong correlation between ErbB-1 and ErbB-2 AGCN may indicate an important role of ErbB heterodimers in tumor progression. Clinical relevance of these findings warrant further studies.

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VEGF-D in assocation with VEGFR-3 promotes nodal metastasis in human invasive lobular breast cancer

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The aim of this study was to investigate the role of lymphangiogenesis in lymphatic dissemination in invasive lobular breast cancer by examining peri- and intratumoral lymph vessel density as well as the expression of VEGF-C, VEGF-D and VEGFR-3 in these tumors.

By performing immunohistochemistry stainings on human invasive lobular breast cancer tissue samples we assessed the expression of vascular endothelial growth factor C (VEGF-C) and vascular endothelial growth factor D (VEGF-D) in breast cancer cells and the density of lymph vessels and vascular endothelial growth factor receptor 3 (VEGFR-3) vessels in and around the tumor.

We found a significant correlation between peritumoral lymph vessel density and the presence of lymph node metastases ($P = 0.001$). Lymph vessel density also correlated with the number of metastatic lymph nodes ($P < 0.001$). Furthermore a significant correlation was detected between tumor cell VEGF-D expression and lymph node status ($P = 0.001$).VEGF-D expression also correlated with the density of LYVE-1 positive vessels ($P = 0.035$). Tumors positive for both VEGFR-3 and VEGF-D or both VEGFR-3 and VEGF-C had a significantly higher number of metatstatic lymph nodes than tumors with other staining patterns ($P < 0.001$). Finally, tumors that were neither VEGF-D nor VEGFR-3 positive had a lower density of LYVE-1 positive vessels compared with the tumors with other staining patterns ($P = 0.033$).

Our study represents the first simultaneous analysis of VEGF-C/-D expression with LYVE-1, CD34 and VEGFR-3 vessel densities in breast cancer. The results indicate that peritumoral lymph vessel density is associated with lymph node metastases in invasive lobular breast cancer. Moreover, these findings show, that invasive lobular cancer producing VEGF-D, surrounded by VEGFR-3 positive vessels, have a significantly higher peritumoral lymph vessel density as well as a higher number of metastatic lymph nodes.

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Real time RT-PCR detection of disseminated tumour cells in bone marrow has superior prognostic significance in comparison with circulating tumour cells in patients with breast cancer

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Purpose: This study assessed the feasibility of using real time RT-PCR analysis to detect disseminated epithelial cells (DEC) in peripheral blood (PB) and bone marrow (BM) of patients with breast cancer (BC). Detection of DEC in BM is an obvious choice in BC, but blood sampling is more

convenient. The aim of this study was to evaluate whether the detection of DEC in either PB or BM predicts overall survival.

Methods: PB and BM samples were collected from 148 patients with BC with stage I to IV disease prior to the initiation of any local or systemic treatment. PB of healthy volunteers and BM of patients without any malignancy served as the control group. DEC was detected by measuring relative gene expression (RGE) for CK-19 and MAM using a quantitative RT-PCR detection method. The mean follow-up time was 786 days (±487). Kaplan Meier analysis was used for predicting overall survival (OS).

Results: Taking the 95 percentile of the RGE of CK-19 (BM: 26.3 and PB: 58.7) of the control group as cut-off, elevated CK-19 expression was detected in 42 (28%) BM samples and in 22 (15%) PB samples. MAM expression was elevated in 20% (both PB and BM) of the patients witch BC. There was a 68% (CK-19) and 75% (MAM) concordance between PB and BM samples when classifying the results as either positive or negative. Patients with an elevated CK-19 or MAM expression in the BM had a worse prognosis than patients without elevated expression levels (OS: log-rank test, $p = 0.0045$ (CK-19) and $p = 0.025$ (MAM)). For PB survival analysis no statistical significant difference was observed between patients with or without elevated CK-19 or MAM expression (OS: log-rank test, $p = 0.551$ (CK-19) and $p = 0.329$ (MAM)).

Discussion and Conclusion: DEC, measured as elevated CK-19 or MAM mRNA expression, could be detected in both PB and BM of patients with breast cancer. Only the presence of DEC in BM was highly predictive for OS. The occurrence of DEC in the BM is probably less time-dependent and may act as a filter for circulating breast cancer cells. The use of either larger volumes of PB or performing an enrichment step for circulating tumour in blood cells, might improve these results.

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Potentiation of estrogen receptor-mediated transcription by steroid and xenobiotic receptor (SXR) in breast cancer cells

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Estrogen receptor (ER) is a key regulator of proliferation and differentiation in normal mammary gland and breast cancer cells. ER activity can be modulated by other nuclear receptors. On the other hand, steroid and xenobiotic receptor (SXR), an adapted orphan nuclear receptor, has been shown to mediate the genomic effects of steroid hormones, including estrogen and xenobiotics. This receptor regulates the expression of the cytochrome P-450 3A (CYP3A) gene family, which plays important roles in the metabolism of endogenous steroids and xenobiotics. It has been reported that SXR is expressed mainly in liver and small intestine, however, recent study showed that SXR is also expressed in both normal and neoplastic breast tissue. To study whether ER activity is altered by SXR, we investigated the effect of SXR on Estrogen(E_2)-induced transcription through ER using transient transfection-based reporter assays. SXR potentiated ER-mediated transcriptional activity of the estrogen responsive element (ERE)-containing promoter in the presence of E_2 in MCF-7 breast cancer cells. On the other hand, SXR alone did not affect ERE-containing promoter activity in ER negative CV-1 cells. In semi-quantitative RT-PCR studies, SXR up-regulates a classic E_2-dependent gene such as pS2. To study further the mechanism of SXR potentiation of ER-mediated transcription, we performed a series of experiments. Using GST pull down, mammalian two hybrid, and electropholetic mobility shift assays, we showed that (i) SXR did not interact with ER, (ii) SXR did not bind to ERE, and (iii) SXR did not alter the binding between ER and steroid receptor coactivator (SRC)-1. Thus we focus on the effect of SXR on the binding between ER and corepressors. It has been reported that corepressors nuclear receptor corepressor (N-CoR) and silencing mediator for retinoid and thyroid receptors (SMRT) are expressed in breast cancer, and may be recruited by ER in the presence of E_2 and tamoxifen. In reporter assays, increasing amounts of SMRT reversed the potentiation of ER activity by SXR. The binding of ER with SMRT was decreased by SXR in GST-pull down assay and mammalian two-hybrid assay. These results suggest that SXR induced ER-mediated transcriptional activity by squelching limiting amounts of SMRT corepressor. In conclusion, we demonstrate that SXR induces ER signaling, which may play crucial role for cell growth, cell differentiation, and xenobiotic metabolism in breast cancer cells.

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Insulin-like Growth Factor Binding Protein 3 (IGFBP-3) modifies Epidermal Growth Factor (EGF)-related breast cancer growth depending upon the extracellular-matrix (ECM)

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Introduction: Insulin-like growth factor binding protein-3 (IGFBP-3) is the most abundant IGFBP in serum and is able to modulate cell proliferation independently of its ability to bind IGF. Tumour-associated increases in IGFBP-3 levels relate to up-regulation of EGFR and HER-2 with increasing oestrogen-independence. Remodelling of the extracellular matrix with increased fibronectin expression in poor prognostic tumours further enhances EGFR levels and signaling. We have explored the potential interaction of these pathways using the EGFR/HER-2 tyrosine kinase inhibitor, Iressa.

Aims: We have examined the effects of IGFBP-3 on EGF-mediated growth in both normal breast and breast cancer cells in the presence and absence of fibronectin.

Material and Methods: Normal breast epithelial cells (MCF-10A) and breast cancer cells (T47D) were dosed with EGF (5 ng/ml & 10 ng/ml) or IGFBP-3 (100 ng/ml) or SPD (an IGFBP-3 peptide that mimics IGF-independent actions of IGFBP-3) or Iressa (0.25 μm) either alone or combinations of each, on plastic and on fibronectin (0.25 μg/ml). Cellular proliferation was evaluated by cell counting and thymidine incorporation (TLI).

Results: In MCF10A cells, EGF and IGFBP-3 each increased cell proliferation on their own (by 55.2% and 31.7%, respectively) and together synergistically enhanced cell growth relative to EGF alone (by 123%). We found that the proliferative effect of IGFBP-3 alone, like that of EGF, was completely abrogated in the presence of an effective dose of Iressa.

In T47D cells, EGF increased cell proliferation (by 204%), IGFBP-3 alone had no effect, but in combination, in contrast to the normal cells, IGFBP-3 markedly inhibited EGF-mediated cell proliferation (by 85% relative to EGF alone). The IGF-independent effects of IGFBP-3 were corroborated by SPD with the same results for both cell lines. MCF10A cells on fibronectin responded significantly to EGF (increased TLI by 285%), with IGFBP-3 suppressing EGF in contrast to plastic. On fibronectin, EGF increased cell growth (by 86%) of T47D, with IGFBP-3 enhancing EGF-induced growth (additional 27%) compared to its inhibitory effect on plastic.

Summary and Conclusion: IGFBP-3 has differential effects on EGF-mediated proliferation in normal and breast cancer epithelial cells that are switched when the cells are plated onto fibronectin, which is indicative of a more invasive phenotype. Future characterisation of breast tumours, in addition to EGFR/HER-2, may also include fibronectin and IGFBP-3 production to predict clinical responses to agents targeting the EGFR pathway.

'Iressa' kindly donated by Astra Zeneca.

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The age of women at which bilateral breast cancer was diagnosed; reference to the presence of germline mutations in BRCA1, BRCA2 and CHEK2 genes and their family history of neoplasm

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Background: 163 women with bilateral breast cancer were examined. 30.1% of women had synchronous and 69.9% metachronous cancer.

Material and Methods: The DNA of peripheral blood lymphocytes of patients was examined for the presence of selected germline mutations in BRCA1, BRCA2 and CHEK2 genes using molecular biology techniques. Patients' family history of neoplasm was also analysed.

Results: The following mutations in BRCA1 gene were identified: 185delAG in 1 patient, T300G in 2 patients, 5382insC in 17 patients and 3875del11ins7, C5370T, IVS20+60ins12 and IVS2−16G>A in 1 patient each. In BRCA2 gene, 9631delC mutation was found in 1 patient and IVS16−116ins3 mutation in another one. In CHEK2 gene, 430T>C mutation was identified in 10 patients and 1100delC in 2. BRCA1/2 mutations were identified in 16% of patients (26/163) and CHEK2 mutations in 7.4% (12/163). It was carried out that the presence of the mutations in BRCA1/2 genes among patients with bilateral breast cancer is associated with an earlier occurrence of the first and the second breast cancer than in patients without germline mutations in these genes (a difference of