

## 1303

## POSTER

**Effect of neoadjuvant chemotherapy on oestrogen receptor, progesterone receptor and HER 2 receptor expression in breast cancer**

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**Background:** Neoadjuvant chemotherapy (NC) is used in treating locally advanced operable breast cancer. After surgery, further adjuvant treatment is offered based on the estrogen receptor (ER), progesterone receptor (PR) and HER2 status. Treatment post operatively can be based on the ER/PR/HER2 status of the core biopsy taken preoperatively. It is not a usual practice in the United Kingdom to repeat these markers on the surgical specimen. However a change in ER/PR or HER2 status following NC could have a profound effect on adjuvant treatment with the real possibility of appropriate therapy being unknowingly withheld. The aim of our study was to determine the percentage of patients whose ER/PR, HER2 receptor expression change with NC and if these changes lead to change in their adjuvant treatment.

**Materials and Methods:** This is a retrospective study of 32 patients with locally advanced breast cancer who had NC followed by breast conservation surgery or mastectomy. Quick score (Q score) for ER/PR and the HER2 expression was measured both from the preoperative core biopsy and from the excision specimen following NC.

**Results:** After NC, 5 patients had complete pathological response and 2 patients had residual ductal carcinoma in situ. 25 (78%) patients had residual invasive malignancy. Quantitative change in Q scores for ER and PR was seen in 6 patients (24%) and 10 patients (40%) respectively. ER status changed from positive to negative in 1 patient (4%). PR status changed from positive to negative in 4 patients (16%) and from negative to positive in one patient (4%). One patient (4%) changed from HER2 negative to HER2 positive after NC.

**Conclusions:** Change in 1 patient (4%) from HER2 negative to HER2 positive lead to change in adjuvant treatment who would have otherwise not received trastuzumab. Though Q scores changed in 24% and 40% for ER and PR respectively no change was observed with regards to hormonal adjuvant treatment. A study with a bigger cohort might address this issue. We suggest that ER/PR/HER2 status should routinely be checked in both core biopsy sample and also resection specimen.

## 1304

## POSTER

**Immunocytochemical analysis of mitochondrial protein UCP4 and its correlation with apoptotic and predictive markers in breast cancer**

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**Introduction:** In recent years, mitochondria have been recognized as regulators of cell death via apoptosis and necrosis, as well as modifier events via mitochondrial DNA (mtDNA) alterations providing a possible proliferative advantage to the tumor cells, in addition to their essential role for cell survival. The crucial role of mitochondria in apoptosis is reinforced by the observation that mitochondria contribute to program cell death signaling via the production of reactive oxygen species (ROS). Uncoupling proteins (UCPs) are a family of mitochondrial transporter proteins that are implicated to the maintenance of the basal metabolic rate. UCP4, a member of UCPs family, participates in apoptosis because of its early phylogenetic occurrence. Activation of the mitochondrial pathway of apoptosis is regulated by members of bcl family of proteins.

**Purpose:** The objective of the present study was to detect the correlation of UCP4 with other apoptotic and prognostic markers in patients with breast carcinoma.

**Material and Methods:** Ninety (90) imprint smears from surgically resected breast cancers were studied immunocytochemically with the use of monoclonal antibodies to estrogen receptors ER, to apoptotic markers bcl-2, and P53, to proliferative marker ki67, as well with the use of polyclonal antibody to UCP4. Immunocytochemical staining was performed by using Alkaline Phosphatase Anti Alkaline Phosphatase (APAAP) method.

**Results:** UCP4 positive cytoplasmic expression was detected in 41 (45.5%) of breast cancer smears, bcl2 in 36 (40%) and positive nuclear expression of P53 in 52 (57.7%), ER in 19 (21.1%) and ki67 in 59 (65.5%) of cancer breast cases. Increased UCP4 expression was significantly associated with P53, ki67 overexpression, with low expression of ER and downregulation of bcl2 in histologically poor tumor differentiation and presence of lymph node metastasis.

**Conclusions:** Dysregulation of normal cell death mechanisms plays an important role in the pathogenesis, progression of breast cancer and in responses of tumors to therapeutic intervention. Taken together the results of our study suggest that UCP4 as an uncoupling protein of mitochondrial membrane is implicated in the apoptosis pathway and associated with high grade breast tumors, indicating oxidative mechanism at the mitochondrial level in the tumor cells.

## 1305

## POSTER

**PSMB7 is associated with anthracycline resistance and is a prognostic biomarker in breast cancer**

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**Background:** Chemotherapy is the most important systemic therapy of breast cancer in receptor negative patients. However, to date individual markers failed to correctly predict resistance against anticancer agents. We used gene expression patterns attributable to chemotherapy-resistant cells to detect potential new biomarkers related to anthracycline resistance. One of the genes, PSMB7 was selected for further functional studies and clinical validation.

**Materials and Methods:** We contrasted the expression profiles of four pairs of different human tumor cell lines of gastric, pancreatic, colon and breast origin and of their counterparts resistant to the topoisomerase inhibitors daunorubicin or doxorubicin. Top 5 genes related to chemoresistance identified by Prediction Analysis of Microarrays (PAM) were also validated by immunohistochemistry. RNA interference was used to silence one of the top genes, PSMB7, which had an elevated expression in the resistant cell lines. After silencing, doxorubicin treatment was performed, and cell vitality was measured using a Casy automated cell counter system. Finally, microarray gene expression of GEO raw microarray samples with available progression free survival data was downloaded. The arrays were MAS5 normalized, and the expression of the PSMB7 gene was used to group the samples in two group (below or above of the average expression). In these, Kaplan-Meier survival plot was generated using Winstat.

**Results:** Immunohistochemistry verified the differential expression of the top discriminating genes (ABCB1, TOP2A, TOP2B, MKI67 and PSMB7). We achieved a silencing of 86% of PSMB7 using an RNAi oligo at the 548. nucleotide in the 5<sup>th</sup> exon. After doxorubicin treatment 30.60%±31.2% of the resistant cell lines survived. The silencing of PSMB7 in the resistant cell lines decreased survival to 5.87%±10.2%, which had a statistical significance of p=0.0346. We downloaded 1079 microarray samples with available clinical follow-up from GEO. In these, 442 patients had a PSMB7 expression higher than the average (2800). The comparison of the survival of the two groups consisting of patients having high and low PSMB7 expression resulted in high significance (p=0.006).

**Conclusion:** Our findings suggest a role of the proteosome in the development of anthracycline resistance. High PSMB7 expression is an unfavorable prognostic marker in breast cancer.

## 1306

## POSTER

**Increased systemic microRNAs in breast cancer correlate with tumour microRNA profile and clinicopathologic characteristics**

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**Background:** Mic(ro)RNAs have come to the fore of translational research due to their ability to control gene expression and protein translation. Dysregulated miRNA expression in breast cancer is well documented: oncogenic miRNAs have been shown to differentiate breast tumour from normal tissue (*miR-21*, *miR-10b*, *miR-155*) and other more specific miRNAs have been found to correlate with clinicopathologic parameters such as hormone receptor status. Given their small size, miRNAs are remarkably stable, rendering their potential detection in the circulation of breast cancer patients diagnostically and prognostically informative.

This study aimed to determine whether miRNAs were detectable in the circulation of breast cancer patients, to assess the relationship between breast tumour and circulating miRNA profiles, and to evaluate their potential as biomarkers for disease detection and monitoring.

**Materials & Methods:** Refined RNA extraction techniques were optimized for blood, serum and plasma samples. MiRNA was extracted from blood and a panel of 7 oncogenic miRNAs was quantified in 130 reverse

transcribed blood miRNA samples by RQ-PCR. Advanced QBase Plus software and SPSS were used for biostatistical analysis of the data.

**Results:** Whole blood samples were preferable to serum and plasma for greater detection and relative quantification of circulating miRNAs. Of 7 breast cancer specific miRNAs investigated, across 130 whole blood samples (106 preoperative samples from breast cancer patients and 24 from healthy age matched female controls), the levels of two miRNAs were found to be significantly higher in blood from breast cancer patients compared to controls ( $p < 0.001$ ). Increased systemic miRNA levels in breast cancer patients are also reflected in breast tumours. We also report significant associations between circulating miRNAs and the clinicopathologic variables ER and HER2/neu receptor status, and nodal status.

**Conclusion:** This study is the first to demonstrate that miRNAs are detectable and in the circulation of breast cancer patients, compared to controls. The circulating miRNA expression profile correlates with the tumour miRNA expression profile and with various biopathologic parameters of breast cancer. In this, lies the expectation that circulating miRNAs will be clinically useful as a novel minimally invasive tool to aid in the early diagnosis and monitoring of breast cancer.

1307

POSTER

# CXCL12/SDF1 expression by breast cancers is an independent prognostic marker of progression

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**Purpose:** The cytokine CXCL12 is synthesized by metastasis target tissues and has been shown to attract tumor cells that express the receptor, CXCR4. However, epigenetic silencing of CXCL12 has recently been reported to increase the metastatic potential of breast cancer cells and the reintroduction of the cytokine gene into MDA-MB-231 breast carcinoma cells decreases the number of metastases formed *in vivo*. We therefore wished to know whether CXCL12 expression correlates with relapse free and overall survival in human breast cancer patients.

**Experimental Design:** CXCL12 and CXCR4 expression was analyzed in one hundred archival breast cancer samples by immunohistochemistry and in two breast cancer microarray datasets of 408 cases. Data were analyzed by uni- and multivariate COX regression analysis.

**Results:** CXCL12 and CXCR4 are expressed by epithelial tumor cells and by stromal and endothelial cells. Microarray gene expression analysis and immunohistochemistry revealed that CXCL12 but not CXCR4 expression significantly correlates with disease free and overall survival in estrogen receptor positive and negative cancers. Expression of the estrogen receptor  $\alpha$  and CXCL12 does not correlate.

**Conclusions:** CXCL12 is a strong, independent prognostic marker. We propose that saturation of the receptor through autocrine CXCL12 production reduces chemotaxis towards CXCL12 releasing metastasis target tissues.

1308

POSTER

# Evaluating the prognostic role of serum extracellular domain (ECD) of HER-2/neu (s-HER2) in patients (PTS) with metastatic (M) breast cancer (BC): results of an observational study

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**Background:** BC is the leading cause of cancer-related death affecting women worldwide. Since the last decades, many efforts have been made to identify different subsets of BCs on the basis of molecular markers that might help in defining the prognosis. HER2/neu belongs to a family of 4 transmembrane tyrosine kinase (TK) cell membrane receptors mediating cell growth, differentiation and survival, its overexpression occurring in about 20%-30% of BCs and being associated with a more aggressive tumour behaviour and a poor prognosis. The ECD can be cleaved from the cellular surface and measured in the peripheral blood. A potential prognostic and predictive role of elevated s-HER-2 levels has been previously reported.

**Material and Methods:** we prospectively measured the s-HER2 levels of consecutive MBC pts [ELISA immunometric test (cut-off 13 ng/ml)], in order to investigate the possible correlation with the clinical situation. The assessment was done at the first visit (bs-HER2) and then every 3 months. All pts gave their written informed consent.

**Results:** 46 consecutive MBC pts have been enrolled (median age 63 ys (35-85) so far; ER+ 30 pts (65%); PgR+ 21 pts (46%); IHC evaluation of HER2 was available in 37 pts [16 pts (35%) positive (IHC3+ or IHC2+ FISH+), 21 pts (46%) negative, 9 pts (19%) unknown]. All pts were treated with standard hormone (8 pts) and/or chemotherapy (28 pts), including antracyclines and taxanes. Trastuzumab was given as needed (13 pts).

A total of 325 samples have been tested (mean = 7/pt). Elevated bs-HER2 were found in 27 (59%) pts, of which 12 HER2 positive and 12 HER2 negative; median ECD values were significantly higher in pts with HER2 positive ( $P < 0.0001$ ).

A significant correlation was found between elevated bs-HER2 and number of M sites ( $p < 0.05$ ), as well as between high ECD levels and visceral/nodal disease ( $p < 0.05$ ). Noteworthy, a significant correlation has been found between the increase or the decrease of ECD values and PD or non-PD respectively ( $p = 0.0001$ ). Moreover, with regard to OS, a statistically significant difference, regardless of HER2 status, has been observed between pts with elevated and lower ECD levels (log-rank test  $p = 0.003$ ).

**Conclusions:** Our data seem to suggest that serum HER2 levels might be used as a "real-time" non invasive marker, allowing repeated evaluations of HER2 status during the whole disease history, with possible future therapeutic implications.

1309

POSTER

# Invasive lobular carcinoma: Preliminary study of the efficacy of a blood based gene expression test for early detection of breast cancer

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**Background:** Mammography has limited efficacy for the detection of breast cancer of lobular origin. Invasive lobular carcinoma is particularly difficult to detect since this cancer type shows diffuse infiltration and often presents on mammography as a subtle distortion rather a solid mass. In addition, this cancer type is rarely associated with microcalcifications which are characteristic signs of malignancies. Due to the later detection of lobular cancers the likelihood of surviving lobular cancer is less than that seen for ductal cancer.

We have developed a blood based gene expression test for the detection of breast cancer using samples from women with ductal carcinoma due to the higher prevalence of this cancer type. We have previously reported detection of ductal carcinoma with accuracies ranging from 75-82% [1-3]. Here we report the findings from a preliminary study using lobular cancers and healthy controls (without mammographic findings) with a blood based gene expression signature developed from ductal breast cancer.

**Materials and Methods:** A 96-assay format gene expression signature developed from samples from 294 women was used to establish a PLS classifier. The resultant signature was used with blood samples from women with lobular cancer and healthy controls from a European/US cohort. Whole blood was collected in PAXgene<sup>TM</sup> tubes and shipped on dry ice to a central laboratory. RNA extraction was performed using PAXgene<sup>TM</sup> Blood RNA kit according to the manufacturer's instructions.

**Results:** Of the 14 lobular cases, 11 were correctly classified as cancer. Of the 14 healthy controls, 11 of 14 were correctly classified. These results are consistent with the accuracy of the test for ductal tumours.

	Mean age (y)	Lesion diameter (mm)	Correctly predicted
Stage 1 N = 10	65	8-20	8/10
Stage 2 N = 4	69	15-50	3/4
Healthy controls	51	NA	11/14

**Conclusions:** The present study provides an indication that lobular cancers are detected by the blood based gene expression test with implication that lobular and ductal cancers elicit a common response in whole blood which is recognised by the test.

The study suggests the test has a potential for detecting a form of cancer which is difficult to detect with normal mammographic procedures. Additional cases of lobular cancer are required to support these preliminary findings.

## References

[1] Sharma P et al. (2005) Breast Cancer Res. 7 (5): R 634-44.