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POSTER

# Estrogen receptor negative breast cancers express estrogen receptor mRNA

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**Background:** Approximately one third of primary human breast cancers do not express the estrogen receptor gene alpha ( $\alpha$ ) and are classified as ER-negative. Compared to ER-positive breast cancers, these cancers have a worse prognosis and limited treatment options. The ER-negative status of breast cancers is ascertained by ER protein assays. Very few studies have assayed ER $\alpha$  gene expression in this aggressive form of breast cancer. In this study, we have assayed ER mRNA levels in a large cohort of archival primary human breast carcinomas to further elucidate the mechanisms leading to the ER-negative phenotype.

**Materials and Methods:** We examined the relationship between ER $\alpha$  mRNA expression using quantitative real time PCR and ER protein status as obtained by cytosolic assays in 250 archival primary human breast tumors. High quality RNA was extracted from 250 primary tumors that have been cryopreserved for up to nine years. RNA quality was verified by gel electrophoresis and visualization of ribosomal bands, by OD260/280 ratios, and by amplification of housekeeping genes. Quantitative real time PCR using the Light Cycler system was used to determine ER mRNA concentrations for all tumors.

**Results:** All of the 200 ER-negative tumors expressed ER $\alpha$  mRNA at levels that significantly overlapped those of the 50 ER-positive tumors. The mean ER mRNA concentrations for the ER-positive and ER-negative tumors were  $1.14 \times 10^3$  fmol/ $\mu$ g RNA and  $1.27 \times 10^3$   $\mu$ g RNA respectively. The lowest and highest ER mRNA concentrations were similar and the mean ER mRNA values did not differ significantly between the two breast cancer groups ( $p > 0.50$ ). Quantitative PCR of housekeeping gene h-PBGD in the ER-positive and ER-negative tumors showed similar starting RNA quantities and qualities in the two groups. This was further demonstrated on agarose gel electrophoresis.

**Conclusions:** Thus, the lack of ER $\alpha$  protein expression in ER-negative breast cancers is not due to a lack of ER $\alpha$  gene expression but is due to post-transcriptional mechanisms. Increasing evidence links ligand-activated ER $\alpha$  dependent gene transcription with ER proteolysis. The presence of ER $\alpha$  gene expression in ER-negative breast cancers may explain why some of these cancers respond to tamoxifen. Our data raise the possibility that ER-negative breast cancers are not estrogen independent for growth.

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# Mammography screening pathology, a new challenge for the histopathologist – a two year review on the first mammography screening project in Germany

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**Purpose:** The aim of this investigation was to critically review the role of the histopathologist in a mammography screening project.

**Methods and Materials:** The first model mammography screening project in Germany has been running in Bremen for the last two years. All women between 50 and 70 years of age have been offered mammography and any suspect findings were investigated by core biopsy. These cases were reviewed in the Pathology Dept. of the University Muenster and discussed at a weekly multidisciplinary meeting. Lesions were classified using the 5 point B classification scale as recommended by the European Union.

**Results:** In the first two years of the screening project, a total of 22,000 women had mammography. Suspicious areas were detected and core biopsies were obtained from 401 of these women.

46% were classified B5 (malignant) 46% of these cases were classified B1,2 (regular breast tissue, benign) and 8% of these cases were classified B3 and B4 (atypia probably benign, suspicious of malignancy). In 90% of all cases there was agreement amongst the reviewing histopathologists, but in 10% there was no interobserver consensus. These disagreements centered on flat epithelial atypia, atypically hyperplasia and papillary lesions.

**Conclusions:** The consensus amongst experienced breast histopathologists is satisfactory in clearcut cases, but it should be improved in less well classified lesions. These lesions are more likely to be seen in a screening situation and in core biopsies. In this area it is certainly advisable to cooperate closely with the clinician and radiologist.

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# The complex between urokinase plasminogen activator (uPA) and its type-1 inhibitor (PAI-1) denotes poor disease outcome in 576 node-negative breast cancer patients

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**Background:** Members of the plasminogen activation system, e.g. urokinase-type plasminogen activator (uPA) and its main inhibitor (PAI-1), are involved in tumor growth and dissemination. Both uPA and PAI-1 are established prognostic factors in breast cancer. In this study we investigated whether the complex of uPA with PAI-1 is also associated with the natural course of the disease.

**Material and Methods:** The levels of uPA, PAI-1 and uPA:PAI-1 complex were measured by our in house ELISAs in tumor tissue of 576 patients (median follow up = 61 months) with node-negative invasive breast cancer. Patients did not receive adjuvant systemic therapy. Univariate and multivariate survival analyses with well-known clinicopathological factors were performed.

**Results:** uPA:PAI-1 complex levels were associated with adverse histological grade and inversely associated with estrogen and progesterone receptor status. In univariate survival analyses, increasing levels of uPA:PAI-1 complex were associated with a reduced relapse-free survival (RFS) and overall survival (OS,  $P < 0.001$  for both). In multivariate analyses, uPA:PAI-1 complex level was an independent prognostic factor for OS ( $P = 0.039$ ), but not for RFS ( $P = 0.240$ ). However, when uPA and PAI-1 were not included in this analysis, uPA:PAI-1 complex reached statistical significance for both RFS and OS ( $P = 0.029$  and  $P = 0.007$ , respectively).

**Conclusions:** uPA:PAI-1 complex levels have a strong prognostic value in univariate analysis of RFS and OS in breast cancer. This prognostic value persisted in multivariate analysis with established clinicopathological factors. When uPA and PAI-1 are included in the multivariate analysis, uPA:PAI-1 complex has still independent prognostic value for OS, suggesting an interaction of the complex with progression of metastasis or with therapy.

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# Predictive value of activated tyrosine kinase (Tyr1248) in patients with HER2-overexpressing metastatic breast cancer (MBC) treated with trastuzumab + chemotherapy regimens

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**Background:** The activation of HER2 receptor through the autophosphorylation of its tyrosine residues, mainly the Tyr1248, has been regarded as one of the possible key points at which *de novo* resistance to single agent trastuzumab (H) could occur. Results from our previous experience with H given as single agent to pts with MBC did not show any statistically significant imbalance in the clinical outcome according to the phosphorylated status of HER2 (pHER2). The aim of this study was to investigate the role of pHER2 when H is given in combination with chemotherapy (CT).

**Patients and Methods:** Fifty eight pts received H with paclitaxel (38 pts), docetaxel (10 pts), vinorelbine (8 pts), cisplatin (1 pt) and capecitabine (1 pt) within a compassionate use program of H in Belgium. Their medical files were reviewed and the archival samples were centralised in order to assess the status of HER2 by FISH (Path-Vysion probe, Vysis, Illinois, USA), or by IHC (moAb CB-11, Novocastra, Newcastle, UK), and HER2 phosphorylation in the Tyr1248 (clone PN2A, Neomarker, CA, USA).

**Results:** Median age of the study population was 51 years (29–76), ECOG PS 1 (0–3). Twenty two of 58 pts (40%) had received adjuvant hormoneotherapy (HT) and 38 (65.5%) adjuvant CT. Median number of metastatic sites was 2 (range 1–4) and 40 pts (69%) had visceral involvement. Median number of lines for advanced disease was 1 for HT and 2 for CT. Median duration of H-CT treatment was 8.1 months (0.5–35.2 months). Ten pts had a complete response (17.2%), and 20 a partial response (34.5%), for an overall response rate of 51.7%. Stable disease was documented in 17 pts (29.3%), progressive disease in 10 pts (17.2%), while in one patient response to treatment was not assessable. Actuarial median time to progression was 6.1 months, actuarial median survival time was not reached, with a survival rate of 75% of pts at 13 months. Fifty four pts fulfilled the criteria to enter the analysis (evaluable for response, amplification/overexpression of HER2, available pHER2). A statistically significant difference ( $p = 0.03$ ) in response rate between pts with pHER2+

(8/9 pts, 88.9%) and pHER2- (21/45 pts, 46.7%) has been observed, while no significant difference in the rate of clinical benefit was found ( $p=0.24$ ).

**Conclusions:** A comprehensive analysis of the patterns of expression of HER2 downstream effectors, both in pts treated with single agent H and with H-CT, is currently ongoing.

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### Peritumoral lymph vascular density correlates with lymph node metastases in invasive lobular carcinoma of the breast

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**Background:** The most significant prognostic factor in breast cancer is the status of the axillary lymph nodes.

The presence of metastases in axillary lymph nodes may depend on the ability of the tumor to induce lymphangiogenesis. Vascular endothelial growth factor C (VEGF-C) activating vascular endothelial growth factor receptor 3 (VEGFR-3) is the major pathway for the tumor to induce lymphangiogenesis and facilitate tumor cell to disseminate into lymph vessels.

The aim of the study was to evaluate the role of lymphangiogenesis in lymphatic dissemination in invasive lobular carcinoma (ILC).

**Methods:** Paraffin-embedded tissue for immunohistochemical staining was obtained from 95 patients with ILC who underwent sentinel node biopsy at the Breast Surgery Unit of the Helsinki University Hospital. The tissue sections were stained with LYVE -1, VEGFR-3 and VEGF-C antibodies.

Two observers evaluated and interpreted the results of the immunohistochemical staining without knowledge of the clinical data of each patient. Estimating the percentage of tumor cells staining negative, weak positive or strong positive assessed VEGF-C staining. Tumors staining weakly positive more than 20% or strong positive more than 10% were regarded as positive.

Lymph vessel density (LVD) was assessed in three vascular hotspots by scanning the tumor at low power using a Chalkley array graticule.

**Results:** The LYVE-1 positive vessels were located almost completely peritumorally. The mean LVD was 3.7 (range 0–7.3). This mean was used as a cutoff point to create two groups of low density vessel count and high density vessel count. A high LVD showed a positive correlation with the presence of lymph node metastases ( $P=0.01$ ), table 1.

Table 1. Relationship between the lymph node status and tumor characteristics and VEGF-C staining and Chalkley count.

Factor	VEGFC Negative	VEGFC Positive	P	LVD low	LVD high	P
Lymph node metastasis			0.693			0.01
Negative	36 (61)	23 (39)		37 (63)	22 (37)	
Positive	17 (57)	13 (43)		9 (33)	18 (67)	
Tumor size			0.488			0.622
≤2 cm	43 (61)	27 (39)		36 (52)	33 (48)	
>2 cm	10 (53)	9 (47)		10 (59)	7 (42)	
Grade			0.514			0.865
I	22 (56)	17 (44)		20 (54)	17 (46)	
II–III	31 (63)	18 (37)		24 (52)	22 (48)	
LVD			0.908			
low	26 (62)	16 (38)				
high	24 (63)	14 (37)				

VEGF-C staining was negative in 53 (60%) patients and positive in 36 (40%) patients.

Despite extensive testing, immunostaining by the anti-VEGFR-3 antibody resulted in unreliable staining of the vasculature and high background staining of tumoral and stromal areas.

**Conclusion:** There is a significant correlation between peritumoral LVD and the presence of lymph node metastases in ILC.

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### Prognostic factors in breast cancer are highly influenced by the type of end-points used for relapse or survival

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**Background:** The reliability and clinical usefulness of prognostic factors is based on data collected in survival studies. Several definitions of end-

points and competing events are used, but explicit definitions are provided in less than half of published studies.

**Methods:** We have studied the impact of various definitions of end-points and competing events on estimated 10-year survival in a cohort of 457 patients with primary operable breast cancer. The end-points and competing events that we used in our analyses were ipsilateral locoregional recurrent disease, contralateral breast cancer, distant metastasis, second primary malignancy, death related to breast cancer and death not related to breast cancer.

**Results:** The estimated survival probabilities were significantly influenced by the definition of end-points and competing events. The magnitude of this influence depended on the patient-, tumor-, and treatment characteristics of the studied population. During the median follow-up period of 10 years 30% of patients died. Thirty-six percent of all deaths were not related to breast cancer. Ten percent of deaths were observed in young patients (<50 year) as compared to 67% of deaths in the elderly (≥70 year). The estimated 10-year relapse rate varied between 30% and 47% depending on the definition of relapse. The magnitude of the relative risk ratio between a prognostic favourable and unfavourable feature was also influenced by the definition of survival and/or relapse. The relative risk for survival depending on tumour size (≤20 mm vs. >20 mm) was 2.57 when disease specific death was the only end-point used as endpoint, but only 1.48 when all described events were used as end-points.

**Conclusions:** Estimation of both survival and validity and clinical usefulness of prognostic factors depend on the definition of end-points and competing events. When prognostic factors in primary breast cancer are evaluated for survival the most appropriate definition is disease free interval with relapse defined as either locoregional recurrence or distant metastases. Non breast cancer related deaths must be censored, all other events ignored.

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### Effect of HER2 and p53 expression on response to postoperative adjuvant therapy with tegafur-uracil (UFT) and tamoxifen in women with breast cancer

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**Background:** Meta-analysis of randomized controlled trials has shown that UFT, an oral fluoropyrimidine derivative, has a reduction in the annual odds of recurrence (RAOR) of 21% after resection in women with Stage I–IIA breast cancer and that concomitant treatment with tamoxifen (TAM) is particularly effective (RAOR=26%). We studied the effect of HER2 and p53 expression on inhibition of recurrence by TAM monotherapy or a combination of UFT and TAM. Resected specimens obtained from patients registered in randomized controlled studies were used.

**Methods:** Among women registered in randomized controlled studies comparing TAM with UFT plus TAM, given as postoperative adjuvant therapy (third ACETBC trial, all women had estrogen-receptor positive tumors), those who were premenopausal and were axillary lymph node positive were studied. The expression of HER2 and p53 in tumor tissue was studied immunohistochemically with the use of anti-HER2 antibody (DAKO) and anti-p53 antibody (NOVO). Three pathologists evaluated the results in a blinded method.

**Results:** Tumor specimens from 192 subjects (TAM monotherapy, 97; UFT plus TAM, 95) were assessable. In the TAM monotherapy group, the rate of recurrence-free survival was significantly poorer in subjects with HER2-positive tumors than in those with HER2-negative tumors (log-rank test,  $p=0.0485$ ). Similarly, the rate of recurrence-free survival was poorer in subjects with p53-positive tumors than in those with p53-negative tumors (log-rank test,  $p=0.014$ ). In subjects given UFT plus TAM, there was no relation between the rate of recurrence-free survival and HER2 or p53 expression.

**Conclusion:** Our results suggest that the response to TAM monotherapy depends on expression of HER2 and p53, whereas that to UFT plus TAM does not depend on expression HER2 or p53.