## Saturday, 23 March 2002 9:00–10:30

PROFFERED PAPERS

# Molecular markers II

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### Progression and prognosis in early breast cancer; can breast cancer treatments be individualized ?

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Breast cancer is heterogeneous with a spectrum of clinical outcomes. Patients with node-negative breast cancer have a 70 - 80% chance of being cured of the disease with local therapy only. With the available prognostic markers we cannot identify who are the patients who will develop metastatic disease therefore we are currently offering chemotherapy to the majority of them. Improved prediction of the likelihood of metastases will allow the sparing of chemotherapy from women who do not need it and intensify it in those who will benefit from it.

Using immunohistochemistry we analyzed the expression of phenotypic markers of progression: angiogenesis, adhesion and invasion in archival tissue of node-negative patients treated only with local therapy (median follow-up 170 months). The following markers were studied: angiogenesis, Her2-neu, p53, p21, E-cadherin, nm23H1, vimentin, uPA, matrix metalloproteinase 2 (MMP-2). Univariate and multivariate analysis for disease-free survival was performed. The multivariate analysis also included tumor size, patient age and nuclear grade.

In multivariate analysis nm23H1 (P=0.035), MMP-2 (P=0.006), p53 (P=0.005) and E-cadherin (P=0.001) were significant. Her2-neu and uPA were not prognostic in either univariate or multivariate analysis. The other factors were significant or showed a trend for significance in univariate analysis, but were not significant in the multivariate analysis.

These data indicate that significant markers for outcome in node-negative breast cancer are p53 which is at the crossroads of many cellular functions and markers of adhesion (E-cadherin) and invasion (nm23H1, MMP-2). A combination of these biomarkers may allow the individualizing of systemic therapy. The implications of these finding for breast cancer progression and predicting outcome will be discussed.

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### Detection method and stage in BRCA1/2 mutation carriers

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**Background:** It is hypothesized that in BRCA mutation carriers, mammography (MG) is an insufficient screening method because the tumors tend to be of high grade and faster tumorgrowth, developing at a young age when dense breast tissue hampers mammographic detection.

**Aim:** Evaluation of screening results in mutation carriers.

**Patients and Methods:** Retrospective analysis of breast cancers (BC) detected in women that were afterwards identified as BRCA1/2 mutation carriers, who were followed because of a positive family history or a first BC. The screening consisted of monthly breast self examination (BSE), clinical breast examination (CBE) every six months and annual MG.

**Results:** Twenty-nine BCs were found in 25 BRCA1- and 3 BRCA2 carriers; fifteen first BCs and fourteen contralateral BCs (Table). Two BCs were found by MRI, these small tumors were localized by ultrasound. Age at negative MG was 43.2 years SD 9.0 and at positive MG 44.9 years SD 9.4.

| Characteristics | BSE N = 18 62% | CBE N = 3 10% | MG N = 6 21% | Total 29 100% |
|---|---|---|---|---|
| age | 43.2 yr ± 9.5 | 48 yr ± 13.6 | 42.3 yr ± 8.3 | 43.9 yr ± 9.7 |
| MG negat. | 8/16 50% | 3/3 100% | – | 13/27 48% |
| pTis | – | – | 1/6 17% | 1/29 3% |
| tumor size | 19 mm ± 7.9 | 28.7 mm ± 11.9 | 12.8 mm ± 8.6 | 18.5 mm ± 9.4 |
| pN0 | 15/18 83% | 1/3 33% | 4/6 67% | 21/29 72% |

**Conclusion:** Seventy two percent of breast cancers in mutation carriers was first detected by BSE or CBE. Only 21% was found with MG. Although tumor size was smallest in MG + MRI detected BCs, the difference was not of statistical significance.

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### Trawling the peripheral circulation for disseminating breast cancer cells and micrometastatic lymph node deposits using 3 new molecular markers: UROC28, CLSP and BCSG1

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**Background:** UROC28, CLSP (Calmodulin-like skin protein) and BCSG1 (Breast cancer specific gene) are three recently described genes, which have been shown, in different studies, to be overexpressed in invasive breast cancer. The aim of this study is to evaluate the suitability of these genes as potential markers in the detection of disseminating cancer cells and micrometastatic lymph node deposits.

**Methods:** Multiple aliquotted 20 ml samples of fresh blood were spiked with varying numbers of the MDA231 breast cancer cell line, ranging from 106 down to 101 cells. From this larger 20 ml volume, 1ml of blood was subjected to RNA extraction, reverse-transcription and conversion to cDNA. This cDNA underwent PCR with the primers for the genes in question at the optimum temperatures for the individual primers. The PCR products were viewed by electrophoresis on a 2% agarose gel.

**Results:** We were able to detect as few as 15 cancer cells per ml of blood using all three markers individually. UROC28 was not detected in blood from healthy volunteers, but was present in MDA cancer cells and in lymph nodes with micrometastatic deposits. CLSP was less sensitive as a marker for disseminating cancer cells because of its potential presence in blood samples, however it too was noted to be overexpressed in MDA cells and in lymph nodes with micrometastatic deposits. BCSG1 is more sensitive than both the above as it is undetectable in normal breast tissue and is highly expressed in metastatic breast cancer. These markers were able to differentiate between nodes with proven metastatic deposits and those that were histologically and molecularly free from cancer. Our results further indicate that, although individually these genes are capable markers for metastatic disease in breast cancer, their sensitivity and specificity are greatly enhanced when used in combination. This shows the enormous potential of using combined molecular markers to detect disseminating cells and micrometastatic deposits in breast cancer.

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### hTERT expression in human breast cancer and adjacent non-cancerous breast tissue: correlation with tumour stage

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**Introduction:** Telomerase is a ribonucleoprotein responsible for synthesising the repetitive nucleotide sequence that makes up telomeres at the end of chromosomes. Without telomerase activity each round of cellular division results in shortening of the telomeres. This can lead to chromosomal instability and cell senescence, whereas continued telomerase activity allows the possibility of cellular immortality. Telomerase is active in 85–90% of human cancers but is undetectable in most normal somatic cells. Three components have been identified, of which hTERT (human telomerase reverse transcriptase) is the one which appears to confer enzyme activity.

**Aims:** To investigate the expression of hTERT in human breast cancer and adjacent non-cancerous tissue (ANCT) using quantitative RT-PCR, and to correlate levels of expression with tumour stage.

**Materials and Methods:** Samples of breast tumour and macroscopically normal breast tissue 1cm from the tumour were taken from 43 patients undergoing breast cancer surgery. Total cellular RNA was extracted using the RNeasy (Qiagen) kit. Quantitative RT-PCR was performed using the ABI PRISM 7700 Sequence Detector (Perkin-Elmer Applied Biosystems) with primers against hTERT.

**Results:** For ANCT the median copy number for hTERT mRNA was 141 copies per $\mu$g of RNA (range 0.880–2 $\times$ $10^5$). Of 43 specimens 86% had less than 1000 copies of hTERT RNA per micro g of total RNA.

For breast cancer tissue the median copy number for hTERT mRNA was 2.71 $\times$ $10^6$ (range 1.02 $\times$ $10^2$ to 6.87 $\times$ $10^7$). All 43 specimens expressed more than 100 copies of hTERT mRNA and 88% expressed more than 1000 copies per $\mu$g of RNA. Statistical analysis using a two-way analysis of variance showed this difference to be highly significant ($p<0.0001$). No correlation was found with levels of hTERT mRNA and nodal status, tumour size, ER status or presence of lymphovascular invasion.

**Conclusions:** These results show that there is a significant difference

in the expression of hTERT mRNA between cancerous and non-cancerous breast tissue. There is potentially a use for hTERT as a diagnostic marker for breast malignancy, particularly using a cut-off point for significant levels of expression. However there is no correlation with tumour stage, suggesting that post-transcriptional modification of hTERT mRNA may be altering the amount of active enzyme that is produced, since telomerase enzyme activity has been shown to correlate with tumour size and nodal status.

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### cDNA microarray gene expression profiles as a potential prognostic and predictive tool for an improved management of breast cancer

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**Aim:** The purpose of this study is to correlate gene expression patterns generated from cDNA microarrays with clinico-pathological characteristics and clinical outcome in breast cancer.

**Methods:** RNAs from a randomly selected group of breast cancer patients with known clinical outcome were analyzed using a 7600 gene cDNA microarray constructed at the National Cancer Institute. Permutation-based multiple comparisons procedures were used to identify individual genes differentially expressed between groups defined by the standard clinicopathologic variables ER status, grade, menopausal status, nodal status, and tumor size. Hierarchical clustering and principal components methods were applied for unsupervised analyses of expression patterns. Cox proportional hazards regression with adjustment for standard clinical and pathological variables was used to identify genes significantly associated with survival.

**Results:** Gene expression patterns were found to be strongly associated with ER status and moderately associated with grade, but not strongly associated with menopausal status, nodal status, or tumor size. Cluster analyses suggested 2 or 3 clusters, and these appeared related to, but not completely explained by ER status. Sets of genes significantly ($p < 0.001$) associated with relapse free survival and breast cancer-specific survival after adjustment for standard clinico pathological variables were identified. These genes were involved in a variety of molecular pathways, including differentiation (CRIP2), immune (CCBP2), and stress response (HSPA5).

**Conclusions:** ER phenotype is associated with distinct gene expression signatures. Furthermore, our results suggest some candidate genes potentially associated with relapse free survival and breast cancer-specific survival.

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### The prevalence of the 4G4G genotype polymorphism of the plasminogen aktivator inhibitor (pai-1) 4G5G gene, in patients with breast cancer

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Plasmatic PAI activity/concentrations and tumor tissue concentrations are elevated in patients with breast and other kind of cancers whereas this increase correlates with poor prognosis of patients. The 4G/5G deletion/insertion polymorphism is in the promoter region of the PAI *1 gene whereas the 4G allele is associated with increased gene transcription in cell lines in vitro and with increased PAI-1 concentrations in carriers. The prevalence of 4G allele in breast cancer patients and healthy women was investigated and compared with the plasmatic PAI activity.

Blood samples were drawn from 48 nonconsecutive and unselected women with a first diagnosis of breast cancer the morning (8oo * 10oo) prior to surgery. Another 48 healthy women served as controls. Investigations of the 4G/5G polymorphism were performed at the university of Munster using PCR and PAI activity was estimated with a chromogenic uPA dependent test (DADE * Behring, Liederbach, Germany).

Breast cancer patients were significantly older than the controls (58.5 $\pm$ 10.2 y vs. 50.8 $\pm$ 13.2 y; $p < 0.05$). The prevalence of the 4G4G * allele in breast cancer patients (43.8%) was significantly higher as compared to the controls (14.6%; $p < 0.001$). For PAI activity a cut-off level of 3.8 U/ml was used (Thromb. Haemost 1999). The mean PAI activity was 4.8 $\pm$ 2.1 U/ml and significantly higher as compared to the controls (3,4 $\pm$ 1.3 U/ml; $p < 0.001$). In breast cancer patients the prevalence of the 4G/4G-allele was associated with an elevated PAI activity being present in 67% of carriers.

This is the first study that investigated the 4G/5G deletion/insertion polymorphism of the PAI * 1 gene in breast cancer patients. These preliminary results show a surprisingly high prevalence of the 4G4G allele in breast cancer patients. These results are hypothesis generating with respect to a contribution to the fibrinolytic/proteolytic potential of breast cancer cells that is an important prerequisite for successful tumor invasion and metastasis. Further studies including other gynecologic cancer types are on their way

## Saturday, 23 March 2002 9:00–10:30

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# Epidemiology and prevention

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### Breast cancer in women treated with supradiaphragmatic radiation therapy for hodgkin's disease: the Mayo Clinic experience

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**Objective:** To evaluate overall risk, contributing risk factors, detection, pathology, and management of breast cancer (BC) in women previously treated with supradiaphragmatic radiation therapy (SDRT) for Hodgkin's disease (HD).

**Methods:** Medical records of 2,202 women seen at the Mayo Clinic (MC) for HD between 1950-93 were reviewed. The records of 653 women treated with SDRT at MC were abstracted and follow-up (FU) questionnaires mailed.

**Results:** Patient Characteristics: Median age of 653 patients (pts) at SDRT was 31.9 years (y) (range (r) 2.6-86.5 y), median FU was 8.3 y (r 0-47.9 y). Thirty pts developed 34 BC, their median age at SDRT was 22.7 y (r 13.2-51.5 y). Median interval between SDRT and BC was 19.9 y (r 0.7-42.3 y). Median age at diagnosis of BC was 44.4 y (r 27.5-70.8 y). BC Risk: Standard morbidity ratio (SMR) was 3.0 (09% CI 2.0-4.3) ($p<0.001$). A significant increase in SMR was seen after 15 y of FU and continued through 30 y of FU. SMR was inversely related to age at SDRT up to age 30. For pts > age 30 at SDRT the SMR was 1.2 (95% CI 0.5-2.2) vs. SMR 8.8 (95% CI 5.4-13.4) for pts < age 30 ($p<0.0001$). A family history (FH) of BC, and splenectomy significantly increased BC risk by univariate analysis ($p=0.002$, $p=0.0134$ respectively) and multivariate analysis ($p=0.009$, $p=0.023$ respectively). The impact of FH of BC and the impact of splenectomy were greatest in pts 30 y or older ($p=0.0055$, $p=0.021$ respectively). BC Characteristics: Mode of detection in 32 BC: 15 by self-exam, 13 by mammogram, and 14 by clinical exam. Location (65% upper outer quadrant) and histology were similar to those in general population. Stages (St) of 30 BC: 7-St 0, 11-St I, 9-St II, 3-St III. All 34 BC were treated with modified radical mastectomy.

**Conclusion:** The risk of BC is increased in women treated with SDRT for HD before age 30 and in women who have undergone splenectomy. Patients and physicians should be aware of this risk.

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### Clinical presentation, treatment and prognosis of tubular carcinomas of the breast: a population-based study

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**Background:** Tubular carcinoma of the breast is a rare, well-differentiated histologic subtype of invasive carcinoma, known for its favorable prognosis. Our objective was to evaluate clinical and pathological features of these tumors, assess long-term outcome and clinical management of these patients (pts), particularly the need of axillary lymph node dissection.

**Methods:** This study includes all pts with primary tubular carcinoma of the breast (n=50), ie < 1% of 5392 breast cancer cases recorded at the regional population-based cancer registry between 1980 and 1999. We studied patients' characteristics, method of discovery, tumor size, surgical margins, axillary node involvement, hormonal receptors, nuclear grade, type of surgery and use of adjuvant therapy. Survival was calculated using Kaplan-Meier method from the date of initial diagnosis and factors modifying prognosis were determined by the Cox model.