cases were interviewed concerning their educational, occupational, marital, parenthood, smoking, and social insurance status. Data were analyzed in relation with gender, age at diagnosis, stage of disease, and follow up duration.
Results: All 65 cases (M/F: 50/15) were >18 years of age (median 23, 18−40) at the time of study. Median age at diagnosis was 9 years (2−19). Median follow up time was 16 years (4−26). 12/65 cases (19%) had stage I; 29 (46%), II; 18 (29%), III; and 4 (6%), IV disease. 38/65 (59%) cases had a profession, 27/65 (41%) did not. 34/65 (52%) cases were working at a job. 58% of females didn't work compared to 31% of the male patients didn't ($p = 0.08$). 36/65 (56%) patients were non-smokers, 8 (12%) ex-smokers, and 21 (32%) smokers (3/15 females, 18/50 males; $p = 0.06$). 3 females and 11 males (14/65; 21.5%) were married; 6/14 (43%) (M/F = 3/3) had offsprings. 54/65 (83%) cases had any kind of social insurance; all females had social insurance compared to 74% of the males ($p = 0.02$). There was no significant difference in employment, smoking, marital status, and having social insurance between the cases according to age at diagnosis (<10 or >10 years), and follow up time (<15 or >15 years), and having university education; also between gender and marital status. There was no significant difference between early (I-II) or advanced stages (III-IV) and having higher education, employment, smoking, marital status or having social insurance ($p > 0.05$).
Conclusions: That educational status of our patients was not inferior than the normal population is satisfying. All patients should be advised not to smoke. In this series HD survivors did not have important disadvantages in social life. Patients should be encouraged to continue education, work, marry and so have a better quality of life.

Breast Cancer

Oral presentations (Mon, 24 Sep, 10.45−12.15)
Breast cancer – preclinical

2000 ORAL

Quantification of free circulating tumor DNA in plasma as a diagnostic marker for breast cancer

M. Ferreira[1], R. Catarino[1], A. Sousa[2], H. Rodrigues[3], R. Medeiros[1].
[1]Portuguese Institute of Oncology, Molecular Oncology & Virology Unit, Porto, Portugal; [2]Portuguese Institute of Oncology, Surgical Oncology Department, Porto, Portugal; [3]Portuguese Institute of Oncology, Medical Oncology Department, Porto, Portugal

Background: Breast cancer is the leading cause of cancer death in women worldwide. There is a need to develop new approaches that may facilitate earlier diagnosis and more effective treatments. Increased knowledge of molecular pathogenesis of breast cancer offers a basis for the use of molecular markers in biologic fluids for early detection, as well as identification of higher-risk individuals.
The purpose of our study was to determine whether the amounts of circulating DNA could discriminate between breast cancer patients and healthy individuals by using real-time PCR based DNA quantification methodology and determine the kinetics of circulating plasma DNA in surgically treated patients.
Material and Methods: Our standard protocol for quantification of cell free plasma DNA involved 175 consecutive patients with breast cancer and 80 healthy controls. The quantification was perfomed by real-time PCR amplification of the human telomerase reverse transcriptase gene (hTERT).
Results: We found increased levels of circulating DNA in breast cancer patients compared to control individuals (105.2 vs 77.06 ng/ml, $p < 0.001$). We also found statistically significant differences in circulating DNA amounts in patients before and after breast surgery (105.2 vs 59.0 ng/ml, $p = 0.001$). Increased plasma cell free DNA concentration was a strong risk factor for breast cancer, conferring an increased risk for the development of this disease (OR, 12.32; 95% CI, 2.09−52.28; $p < 0.001$). High levels of plasma DNA were also correlated with a decrease in patients' overall survival ($p = 0.043$). There were no association between clinicopathological parameters and concentrations of cell free circulating DNA.
Conclusions: Diagnostic assays based on blood sample analysis are becoming an area of study with growing interest, mainly because of the simplicity of sampling and the future potential of automation of the technical methods for clinical applicability. In conclusion, cell-free DNA is significantly increased in plasma of breast cancer patients, which is associated with an increased risk for the development of this disease and decrease of patient's survival. Therefore, quantification of circulating DNA by real-time PCR may be a good and simple tool for early detection of breast cancer with potential to clinical applicability together with other current methods used for monitoring the disease.

Plasma DNA concentration as a risk factor for breast cancer

		Patients (n = 175) N (%)	Controls (n = 80) N (%)	OR*	95% CI*	P*
High [fcDNA]	Yes	99 (56.6)	73 (91.2)	8.01	3.49−18.38	<0.001
	No	76 (43.4)	7 (8.8)			
Very high [fcDNA]	Yes	133 (76.0)	78 (97.5)	12.32	2.90−52.28	<0.001
	No	42 (24.0)	2 (2.5)			

*For High [fcDNA], $P < 0.001$, OR = 6.48 and 95% CI: 2.76−15.20; For Very high [fcDNA], $P = 0.003$, OR = 9.30 and 95% CI: 2.14−40.35, using logistic regression analysis adjusted by age.

2001 ORAL

Circulating tumor cells (CTCs) in peripheral blood of primary breast cancer patients – Results from the translational research program of the German SUCCESS-Trial

B. Rack[1], C. Schindlbeck[1], S. Hofmann[1], A. Schneeweiss[2], M. Rezai[3], M.W. Beckmann[4], K. Pantel[5], A. Schneider[6], W. Janni[1], H. Sommer[1].
[1]University of Munich, Department of Gynecology and Obstetrics, München, Germany; [2]University of Heidelberg, Department of Gynecology and Obstetrics, Heidelberg, Germany; [3]Luisenkrankenhaus, Breastcenter, Duesseldorf, Germany; [4]University of Erlangen, Department of Gynecology and Obstetrics, Erlangen, Germany; [5]University Medical Center Hamburg-Eppendorf, Institute for Tumor Biology, Hamburg, Germany; [6]Charité Campus Benjamin Franklin, Department of Gynecology and Obstetrics, Berlin, Germany

Background: In metastatic breast cancer, the presence of CTCs has been shown to be associated with bad prognosis and their persistence predicted lack of treatment efficacy. Only limited data, however, has been published in the adjuvant setting. We evaluated the role of CTCs in peripheral blood at primary diagnosis and during adjuvant chemotherapy, endocrine and bisphosphonate treatment within the SUCCESS-trial (n = 3658 pts.)
Methods: We analyzed methods of 23 ml of peripheral blood from 1767 N+ and high risk N− primary breast cancer patients before systemic treatment. 852 of these patients have undergone follow-up blood sampling after completion of chemotherapy. The presence of CTCs was assessed with the CellSearch System (Veridex, Warren, USA). Briefly, after immunomagnetic enrichment with an anti-Epcam-antibody, cells were labeled with anticytokeratin (8, 18, 19) and anti-CD45 antibodies to distinguish epithelial cells and leukocytes.
Results: 10% of pts with a blood sampling before systemic treatment (n = 170) showed >1CTC before the start of systemic treatment (mean 13, range 2–827). While we found 2 CTCs in 5% of patients, 3% had 3−5 CTCs and 1% 6−10 and >10 CTCs each. The presence of CTCs did not correlate with tumor size ($p = 0.07$), grading ($p = 0.30$), hormonal status ($p = 0.54$) or Her2-Status of the primary tumor ($p = 0.26$). However, we observed a significant correlation with the presence of lymph node metastases ($p = 0.015$). None of 24 healthy individuals showed more than 1 CTC.
Among those 852 patients with follow-up blood sampling after the completion of cytostatic treatment, 11% were CTC positive before starting systemic treatment (mean 7, range 2–166), while 7% of patients presented with >1CTC after completion of chemotherapy (mean 6, range 2−84). Of those, initially CTC positive, 10% remained positive (n = 9) and 90% had a negative CTC test after chemotherapy (n = 82). Of those initially CTC negative, 93% remained negative (n = 711), whereas 7% returned with a positive CTC test (n = 50) ($p = 0.24$).
Conclusions: The SUCCESS-trial is the first trial to perform the detection of CTCs in a large number of primary breast cancer patients with this highly standardized and easily applicable approach. If the observed persistence of CTCs after completion of adjuvant chemotherapy is prognostically relevant, will have be further evaluated with longer follow-up.

2002 ORAL

Low SIAH2 expression in breast cancer is associated with resistance to endocrine therapy

M.P.H.M. Jansen[1], L.C.J. Dorssers[2], K. Ritstier[1], J.A. Foekens[1], I.L. van Staveren[1], J. Helleman[1], M.P. Look[1], A.M. Sieuwerts[1], J.G.M. Klijn[1], P.M.J.J. Berns[1]. *[1]Erasmus MC, Medical Oncology, Rotterdam, The Netherlands; [2]Erasmus MC, Pathology, Rotterdam, The Netherlands*

Background: Low expression levels of Seven-in-Absentia Homolog 2 (SIAH2) were observed in our microarray and quantitative real-time PCR

(qRT-PCR) analysis of hormone receptor positive (HR+) tumors from patients with advanced breast cancer resistant to first-line tamoxifen therapy. The aim of this study was (a) to correlate SIAH2 expression with disease outcome including patients treated with other therapy strategies and (b) to determine the role of SIAH2 in endocrine therapy resistance using in vitro cell line models.
Materials and Methods: In 1321 retrospectively collected primary breast tumor specimens SIAH2 levels were measured with qRT-PCR and related with disease outcome in different patient subsets. Human breast cancer cell lines ZR-75−1, EGFR transfected ZR-75−1 (ZR/HERc), and MCF7 were treated with estrogen (E2), epidermal growth factor (EGF) and ICI164.384 (a selective estrogen receptor modulator). ZR/HERc is resistant to ICI whereas ZR-75−1 and MCF7 are sensitive. Furthermore, SIAH2 expression was down regulated in MCF7 with siRNAs and subsequently treated with ICI. SIAH2 levels were determined with qRT-PCR and western blotting. Cell number counts were determined as a measure of therapy resistance.
Results: Low SIAH2 levels in tumors from lymph node positive patients with HR+ tumors associated significantly with a worse disease free survival (DFS) after adjuvant tamoxifen therapy (N = 145; HR = 0.76; P = 0.003) or chemotherapy (N = 231; HR = 0.77; P = 0.003). Multivariate analysis of SIAH2, as continuous variable, showed an independent and significant association with DFS (N = 145; HR = 0.80; P = 0.048) in the adjuvant tamoxifen setting and with progression-free survival in the advanced tamoxifen setting (N = 298; HR = 0.81; P = 0.010).
Our cell line studies confirmed the regulation of SIAH2 expression by the estrogen receptor since it was induced by E2 and repressed by ICI. Interestingly, EGF treatment of ZR/HERc decreased SIAH2 levels. Mock silenced MCF7 remained sensitive to ICI and had significant less cell counts after 96hrs ICI treatment compared to ICI untreated cells (23% decrease; P < 0.001; N = 3). In contrast, SIAH2 silencing resulted in a modest decrease in cell number after 96hrs ICI treatment (2%; P = 0.57), indicating that SIAH2 is involved in therapy resistance.
Conclusions: Low SIAH2 levels in breast tumors are associated with resistance to endocrine therapy in adjuvant as well as in advanced setting and in vitro studies demonstrated ICI resistance after SIAH2 gene silencing.

2003 ORAL

Gefitinib enhances response to chemotherapy in triple-negative Breast Cancer (BrCa)

B. Corkery[1], N. O' Donovan[2], M. Clynes[2], J. Crown[1]. *[1]St. Vincent's University Hospital, Dept. of Oncology, Dublin, Ireland; [2]National Institute for Cellular Biotechnology, Toxicology, Dublin, Ireland*

Background: Triple-negative BrCa lacks expression of hormone receptors and HER-2 but frequently expresses EGFR. Triple-negative BrCa is associated with early relapse and poor survival. There is currently no specific targeted therapy for triple-negative BrCa. The aim of this study is to assess the potential role of EGFR inhibition in the treatment of triple negative BrCa.
Methods: EGFR expression and downstream signalling was examined in triple-negative BrCa cell lines grown in the presence and absence of serum (BT20, HCC1937, and MDA-MB-231), by western blot. IC50 assays were determined using the acid phosphatase assay. Three EGFR inhibitors, gefitinib (G) and erlotinib (T), which are small-molecule tyrosine kinase inhibitors, and cetuximab (E) which is a monoclonal antibody against EGFR, and chemotherapy (CRx) drugs docetaxel (D), carboplatin (P) and doxorubicin (A) were tested. The controls were HER2+ BrCa cell lines, BT474 and SKBR3 which express low levels of EGFR.

HCC1937	% inhibition single agent	% inhibition combination
G (5 μM)	21.7±7.6	-
P (2.5 μM)	17.7±3.4	39.3±4.1
P (5.0 μM)	37.7±8.8	52.4±5.8
P (10.0 μM)	52.0±10.2	62.5±6.9
D (0.75 nM)	25.9±5.3	51.6±5.7
D (1.5 nM)	52.8±3.4	70.0±2.5
D (3.0 nM)	68.8±0.6	77.7±1.8
A (8.75 nM)	25.9±10.9	50.3±11.0
A (17.5 nM)	41.5±9.9	61.7±8.1
A (35 nM)	53.0±8.1	74.6±7.6

Results: The three triple-negative cell lines express high levels of EGFR. EGFR and downstream signalling molecules, Akt and MAPK, were constitutively phosphorylated in the serum-free medium, that is, in the absence of exogenous ligand. IC50 values for G and T were significantly higher in the triple-negative than in the HER2+ cell lines. E did not cause significant inhibition in any cell line (max inhibition 20% at 100 μg/ml E). IC50 values for G were lower than for T in the triple-negative cell lines (IC50s for HCC1937: G – 8.4±1.5 μM; T – 26.2±9.3 μM). Combined EGFR inhibition with CRx was tested in HCC1937 cells. G combined with P, D or A for 5 days showed an additive effect on inhibition of proliferation (Table). Alternate scheduling of the drugs did not significantly influence response.
Conclusions: Our results suggest that EGFR signalling is constitutively activated in triple-negative BrCa cells. Although they are not as sensitive to EGFR inhibition as HER2+ BrCa cells, the addition of gefitinib appears to enhance response to CRx in triple negative BrCa cells.

2004 ORAL

Novel breast cancer susceptibility loci identified in west Swedish families and candidate gene analysis

A. Bergman[1], Y. Mori[1], J. Wernersson[1], J. Berggren[1], T. Martinsson[1], S. Nilsson[2], A. Wallgren[3], J. Wahlstrom[1], P. Karlsson[3], M. Nordling[1]. *[1]Biomedicine, Clinical Genetics, Göteborg, Sweden; [2]Chalmers, Mathematics and Statistics, Göteborg, Sweden; [3]Clinical Sciences, Oncology, Göteborg, Sweden*

Background: The two major breast cancer susceptibility genes BRCA1 and BRCA2 were identified more than ten years ago. Depending on population studied, mutations in these genes are responsible for a varying percentage of familial breast cancer. However, the increase in risk of developing breast cancer cannot be explained by mutations in BRCA1/2 in a majority of familial cases. In this study, we attempted to identify chromosome regions harboring cancer predisposing genes and subsequently analyze selected candidate genes.
Methodology: One large family and 13 small to medium-sized families with multiple cases of breast cancer were analyzed by genome-wide linkage analysis. In order to reduce genetic heterogeneity all families were selected within a relatively isolated geographic region (western Sweden). The genome scan was performed by genotype analysis of 10,000 SNP markers on microarrays (Affymetrix). Candidate genes SAFB1, SAFB2, TP53, XRCC1, CYP17, ERCC2 were analyzed by direct DNA sequencing in patient germline DNA.
Results: The strongest evidence of linkage (HLOD 2.34) was obtained on chromosome region 10q23.32-q25.3. A further two regions were identified, with HLOD scores above 2.10 on 12q14-q21 and 19p13.3-q12. The large family in the study exceeded LOD 1.5 in three regions: 10q23.32-q25.3, 19q13.12-q13.32, and 17p13. Mutation analysis of SAFB1 and SAFB2 revealed three silent polymorphisms in coding sequence and further two in intronic sequence. Breast cancer associated low risk alleles of TP53, XRCC1, CYP17 and ERCC2 were present in various numbers in affected women.
Conclusion: Our results indicate that one or more of the suggested regions may harbor genes that are involved in the development of breast cancer. Possible polygenic effect due to multiple, incompletely penetrant susceptibility genes may explain why multiple regions were identified. Fine mapping of identified chromosome regions is warranted in order to narrow down the candidate regions as well as the analyses of further candidate genes.

2005 ORAL

Breast cancer incidence in relation to oestrogen hormone receptor status in Denmark 1994–2005

J. Bigaard[1], C. Stahlberg[2], M. Dûring[3], M. Ewertz[4], N. Kroman[5]. *[1]Copenhagen University Hospital, Breast and endokrine surgery, Copenhagen, Denmark; [2]Odense University Hospital, Pathology department, Odense, Denmark; [3]Rigshospitalet, Danish Breast Cancer Cooperative Group, Copenhagen, Denmark; [4]Aalborg University Hospital, Oncology, Aalborg, Denmark; [5]Copenahgen University Hospital, Breast and endokrine surgery, Copenhagen, Denmark*

Objective: Breast cancer is the most common cancer among women in Denmark. Within the last 40 years the incidence has been increasing 2−3% per year. The rise in incidence has not been investigated in relation to oestrogen hormone receptor status (ER) on larger population-based material. We investigated the increase in breast cancer within age groups of premenopausal and postmenopausal women in a 12 year period selected due to the stringent use of immune histochemistry for ER definition.
Material: Register data was obtained from the Danish Breast Cancer Group database, which contains close to all Danish women registered with a histologically verified diagnose of invasive breast cancer, between 1 January 1994 and 31 December 2005. Oestrogen receptor (ER) status was defined as positive if more than 10% of the tumour cells were positive using uniform immune histochemistry technique. In all, 36,482 women were included.