(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.

Results: Between 1994 and 2005 breast cancer incidence increased significantly among women 40 years or older with oestrogen positive tumours. However, the incidence of oestrogen negative tumours seems to be constant. The increase in breast cancer incidence has solely arisen among oestrogen positive tumours in both pre-menopausal and post-menopausal women. Hence, the relative amount of ER positive tumours rose from 70.1% to 81.5%.
Conclusion: The finding that the rapid increase in breast cancer incidence has solely occurred within ER positive cancers suggests that it may be related to changes in life style and environmental factors rather than a genetic or biological change of the disease itself.

Poster presentations (Wed, 26 Sep, 14:00–17:00)
Breast cancer – pre-clinical science

2006 POSTER

The potential role of bone derived cells in the development of breast cancer metastases

A.P. Molloy[1], R.M. Dwyer[2], M.J. Kerin[1]. [1]*University College Hospital, Dept of Surgery, Galway, Ireland;* [2]*Department of Surgery and Regenerative Medicine Institute, National University of Ireland, Galway, Ireland*

Despite advances in treatment, over 80% of patients with advanced breast cancer will develop bone metastases for which there is no cure. Thought to involve a complex cascade of cell-cell interactions, the mechanisms of bone metastases are still largely unknown. Bone is a very dynamic environment with continuous cell turnover, which may play an important role in directing the homing and engraftment of circulating breast cancer cells. Mesenchymal Stem Cells (MSCs) and osteoblasts are two subpopulations of cells that exist within bone. MSCs have the potential to differentiate into a range of cell types, and when cultured under appropriate conditions will develop into osteoblasts.
The aim of this project was to investigate the potential role of bone derived MSCs and osteoblasts (NHOst) in directing breast cancer cell migration, and to identify factors mediating their interactions.
Primary culture of MSCs, NHOsts and breast cancer cell lines (MDA-MB-231 and BT-474) was performed. Breast cancer cell migration in response to MSCs and NHOsts was measured using Transwell™ inserts. Media containing β-Glycerophosphate, ascorbic acid and dexamethasone was used to induce MSC differentiation into osteoblasts. MCP-1 and VEGF were quantified using ChemiArray™ and ELISA™ at various stages of differentiation. The potential role of MCP-1 in breast cancer cell migration was investigated using a monoclonal antibody to the chemokine.
There was a significant increase in migration of both breast cancer cell lines in response to factors secreted by NHOsts (5–10 fold increase) and MSCs (6–10 fold increase). MSCs were shown to secrete a range of chemokines including IL-6 & 8, TIMP 1 & 2 and MCP-1. Levels of MCP-1 secreted by differentiating MSCs increased from 319 pg/ml (Day 3) to 12,280 pg/ml (Day 21), while VEGF increased from 100 pg/ml (Day 3) to 1040 pg/ml (Day 21). MSC differentiation into osteoblasts was confirmed by the presence of calcium deposits following Von Kossa staining. A monoclonal antibody to MCP-1 resulted in inhibition of MDA-MB-231 (20% reduction) and BT-474 (30% reduction) migration in response to NHOst cells, confirming a role for this chemokine in the migratory effects seen.
Bone derived MSCs and osteoblasts secrete varying levels of chemokines throughout differentiation that play a potentially important role in mediating breast cancer cell migration. Further investigation of the specific mode of action of these chemokines may provide novel therapeutic targets for treatment of advanced breast cancer.

2007 POSTER

Recommendations for the prevention of aromatase inhibitor-associated bone loss in women with breast cancer

M.S. Aapro[1], P. Hadji[2], A. Brufsky[3], M. Tubiana-Hulin[4], T. Guise[5], J.J. Body[6]. [1]*Doyen IMO Clinique De Genolier, Institut Multidisciplinaire d'Oncology, Genolier, Switzerland;* [2]*Phillips-University of Marburg, Dept. of Endocrinology Reproductive Medicine, Marburg, Germany;* [3]*University of Pittsburgh, Dept. of Hematology/Oncology, Pittsburgh, USA;* [4]*Centre Rene Huguenin, Dept. of Oncology, St. Cloud, France;* [5]*University of Virginia, Dept. of Internal Medicine, Charlottesville, USA;* [6]*Institut Jules Bordet, Universite Libre de Bruxelles, Brussels, Belgium*

Background: Patients with breast cancer are at increased risk for fracture compared with healthy age-matched women; however, there is currently no guidance for identification of women at high fracture risk. It is well established that osteoporotic women (T-score ⩽2.5) require bisphosphonate therapy to increase bone mineral density (BMD) and reduce the risk of fracture, yet this threshold appears inadequate for averting fractures in breast cancer patients, particularly those receiving aromatase inhibitor (AI) therapy.
Material and Methods: We performed a literature review to identify factors that contribute to the increased risk of fracture observed in women with breast cancer. Using an evidence-based medicine approach, we selected risk factors that can be used to determine when to initiate bisphosphonate treatment and to identify the appropriate bisphosphonate for AI-associated bone loss treatment.
Results: With the exception of AI treatment, risk factors for fracture were chosen based on their validation in large populations of postmenopausal women. Risk factors for fracture in patients with breast cancer were AI therapy, T-score ⩽1.5, age >65, family history of hip fracture, personal fragility fracture history after age 50, or oral corticosteroid use of >6 months. Additional risk factors were identified for which guidance could not be provided because available data were insufficient: chemotherapy, radiotherapy, low body mass index, low weight, family fracture history, and smoking. Available data clearly suggest that combined risk factors contribute to fracture risk independent of BMD; therefore, BMD measurement should not be the sole criterion to assess fracture risk in this patient population. Randomized clinical trials support zoledronic acid 4 mg every 6 months for prevention of AI-associated bone loss when a patient is identified to be at risk, and data with other bisphosphonates are emerging.
Conclusions: Our guidance for the treatment and prevention of AI-associated bone loss is as follows: In addition to calcium and vitamin D, any patient initiating AI therapy with a T-score ⩽2.0 should receive zoledronic acid 4 mg twice per year. In addition, any patient receiving AI therapy with any 2 of the following risk factors, T-score ⩽1.5, age >65 years, family history of hip fracture, personal history of fragility fracture after age 50, or oral corticosteroid use of >6 months, should receive zoledronic acid as preventative therapy.

2008 POSTER

The E-ZO-FAST trial: Zoledronic acid (ZA) effectively inhibits aromatase inhibitor associated bone loss (AIBL) in postmenopausal women (PMW) with early breast cancer (EBC) receiving adjuvant Letrozole (Let)

N. Schenk[1], A. Lombart[2], A. Frassoladti[3], P. Neven[4], G. Jerusalem[5], I. Deleu[6], J. Mebis[7], M. Maerevoet[8], J. Miller[9], R. Dias[10]. [1]*Novartis, Global Medical Affairs, Newtown PA, USA;* [2]*Institute Oncologica, Servicio de Oncologia, Valcenia, Spain;* [3]*Azienda Policinico Hospital, Divisione di Oncologia Medica, Modena, Italy;* [4]*UZ Gasthuisberg, Dienst Oncologi, Leuven, Belgium;* [5]*CHU Sart Tilman, Service d Oncologie, Liege, Belgium;* [6]*AZ Maria Middelares, Service d Oncologie, St Niklaas, Belgium;* [7]*Virga Jesse Ziekenhuis, Service d Oncologie, Hasselt, Belgium;* [8]*Clinique St. Pierre, Service d Oncologie, Ottignies, Belgium;* [9]*Novartis, Biostatistics, Florham Park, USA;* [10]*Novartis, Global Medical Affairs, Florham Park, USA*

Background: Letrozole is safe and effective in the treatment of receptor positive EBC in PMW. But like other aromatase inhibitors (AIs), long-term letrozole is associated with loss of bone mineral density (BMD) and a higher incidence of fractures. This multicenter open-label randomized study evaluates an immediate or delayed strategy of bone protection therapy with ZA in preventing AIBL in PMW with EBC who are receiving adjuvant Let therapy.
Material and Methods: 522 PMW with stage I-IIIa ER+ and/or PR+ EBC starting Let (2.5 mg qd x 5 yrs) were randomized to immediate ZA (4 mg IV infusion q 6 mos) vs delayed ZA in 66 centers in South America, Europe, the Middle East, Korea, and South Africa and received Let. The delayed group received ZA when either post-baseline T-score decreases to less than −2.0 SD or if a non-trauma fracture occurs. The primary endpoint is the percent change in lumbar spine (LS) BMD with key secondary endpoints of percent change in total hip (TH) BMD and safety.
Results: Patient enrollment started 9 April 2004 and ended 11 August 2005. The median age on both arms was 58 (range 40–81). Baseline characteristics were similar between arms. Patients were stratified by prior adjuvant chemotherapy, menopausal status, and baseine BMD T-Score. The 12 mos LS BMD revealed that the upfront ZA group shows a mean increase of 2.7%, the delayed group had a mean decrease of 2.7%, resulting in a significant difference of 5.4% between groups ($p > 0.0001$). For the 12 mos TH BMD, upfront arm results were a mean increase of 1.7%; the delayed arm had a mean decrease of 1.6%. This is a significant difference of 3.3% between groups ($p < 0.0001$). The median duration of Let therapy was similar in both arms (18.5 vs 18.6 months). Patients on the immediate arm had received a median of 3 dose of ZA at the time of this analysis. Only 35 patients on the delayed arm had started ZA. At month 12, the most common adverse event was arthralgia, as expected. The incidence was similar in both arms (35.7% vs 38.9%). Seven patients had a clinical fracture (2 in the immediate group and 5 in the delayed group).