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Adjuvant endocrine therapy in pre- and postmenopausal women with primary breast cancer. A 25-year report of the Copenhagen Breast Cancer Trials.

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Background: In the beginning of the 1970 Tamoxifen (TAM) - a new antioestrogen - was shown to be safe and effective in advanced disease and the response was correlated to the content of estrogen receptor protein (ER) in the tumor cells. The effect of diethylstilboestrol (DES) was also related to the ER status of the disease. The objectives of the studies were to evaluate the efficacy and safety of adjuvant endocrine therapy and to correlate the effect to the ER status of the tumor.

Material and methods: 360 consequetive patients with stage I-III breast cancer were entered from 1975 to 1978 into two controlled double-blind studies. Premeonpausal patients were randomised to receive either placebo (PL) or Tamoxifen 30mg daily for two years. Postmenopausal patients received either PL, TAM or DES 3mg daily for two years. Postoperative radiotherapy were administered to all. The tumor cells were in most patients analysed for content of estrogen receptor protein.

Results: Updated results after 25 years of observation will be presented and compared to the results of an extensive analysis after 10 years of oberservation.

Breast cancer genetics and biology

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Molecular analysis of eighteen most recurrent mutations in the BRCA1 gen in 59 Chilean breast cancer families

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BRCA1 accounts for nearly all families with multiple cases of early onset breast and/or ovarian cancer and about 45% of families with breast cancer only. Although to date more than 1.237 distinct mutations, polymorphism and variants have been described, several mutations have been found to be recurrent in the gene. We have analyzed 59 Chilean breast/ovarian cancer families (Table 1) for the eighteen most recurrent mutations in the BRCA1 gene.

Table 1. Chilean breast cancer families at high risk for breast cancer predisposing mutations

Case category for selection	Mean age at diagnosis of individual (years)[b]	Number of families (% total families)
Multiple-case families (* 3[a])	51.10	29 (49.1%)
Multiple-case families (2[a])	50.00	14 (23.7%)
Early onset (≤ 40 years) breast cancer	34.33	7 (11.9%)
Bilateral breast cancer	47.67	5 (8.5%)
Male and ovarian cancer	74.30	1 (1.7%)
Breast cancer and ovarian cancer	47.00	4 (5.1%)
Total	49.45	59 (100%)

a: Number of breast cancer cases per family, including first-degree, second-degree, and distant relatives. b: Mean age at onset of all individuals in the family affected with breast and/or ovarian cancer (whether sampled or not).

The analysis of the five exons and two introns where these mutations are located was made using mismatch PCR assay, ASO, restriction analysis, allele specific PCR assay and sequentiation techniques. Two BRCA1 recurrent mutations (185delAG and 300T→ G) and one variant of uncertain significance (3867 G→ A) were found in four of our families. Also, a new mutation (4185delCAAG) and one polymorphism previously described (3232 A→ G) were found in other two families (Table 2). The 185delAG was found in a 3.38% of the families and each of the others were present only in one of the families of this cohort. Therefore these mutations are not especially recurrent in the Chilean population. The variant of uncertain significance and the polymorphism detected could represent a founder effect of Spanish origin.

Abstract 403 – Table 2. Germline BRCA1 mutations

Family	Female breast cancer	Average Age (years)	Ovarian Cancer	Average Age (years)	Male breast cancer	Cancer at other sites[b]	BRCA1 mutation	Exon	Effect
F4	2	50	–	–	0	Prost, Ut	185delAG	2	Ter codon 39
F46	3	56	3	43,3	0	Pan, Test, Co, Melan	185delAG	2	Ter codon 39
F13	4	40.25	–	–	0	Prost, St, Kid, Lu, Bo	300T→ G	5	Cys to Gly
F14	3	41.5	–	–	0	Ut	3867G→ A	11	Glu to Lys
F21	3	47	–	–	0	–	4185del4	11	Ter codon 1364
F25	3	45.6	–	–	0	Ut, Pan, St	3232 A/G	11	Polymorphism

F: Family; Ov: Ovarian cancer, Prost: Prostate cancer, Ut: Uterus cancer, Co: Colon cancer, Pan: Pancreatic cancer, Melan: Melanoma, Test: Testicular cancer, St: Stomach cancer, Kid: Kidney cancer, Lu: Lung cancer, Bo: Bone Cancer.

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G-protein coupled receptors and anti-apoptotic signalling by estrogen independent of estrogen receptor

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Introduction: In breast cancer, steroids in particular estrogen, functioning through its receptors (ER) contribute to tumour progression by regulating the transcription of target genes. Recent studies however suggest that estrogen may mediate some of cell survival properties through an ER-independent mechanism involving mitogen-activated protein kinase (MAPK) pathway.

Methods: The ER-positive MCF-7 and the ER-negative SKBR3 human breast cancer cell lines were incubated in the presence and absence of EGF and estrogen with or without G-protein coupled receptor (GPCR) inhibitor, pertussis toxin and EGF receptor inhibitor, AG 1478. Phospho-c-raf, phospho-Erk, phospho-cdc2 and survivin expression were detected using western blotting techniques.

Results: In MCF-7 and SKBR3 human breast cancer cells, we have found that EGF and estrogen stimulation rapidly increased the phosphorylation of c-raf, Erk and subsequently an increase in cdc2 phosphorylation and an up-regulation of survivin expression. A further increase was noted in the presence of EGF in combination of estrogen. Moreover, estrogen induced a translocation of phospho-cdc-2 from the cytosol to the nucleus indicating activation of the phospho-cdc-2/survivin complex. The effects of EGF on phosphorylation of c-raf, Erk, cdc2 and survivin were attenuated by AG1478. The effects of estrogen on phosphorylation of c-raf, Erk, cdc2 and survivin were attenuated by pertussis toxin and to the lesser extent by AG1478.

Conclusion: These observations implicate estrogen in the MAPK cell survival mechanism and survivin anti-apoptotic pathway. Elucidation of ER-independent estrogen survival pathways may in part explain clinical observations of steroid therapy resistance in breast cancer.

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Modeling the effect of age in T1-T2 breast cancer

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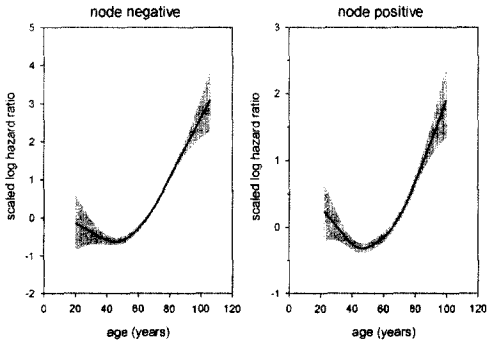
Purpose: 1) To determine the functional form of age (i.e. determine the relationship between age and survival or mortality without making rigid assumptions), while adjusting for the effect of other known variables; 2) to search for a parsimonious algebric representation of that functional form.

Material and Methods: Women from the US Surveillance, Epidemiology, and End Results Program, with histologically confirmed pT1-2 pN0-1 M0 breast carcinoma diagnosed in 1988-1997. The martingale residuals obtained from a multivariate proportional hazards (PH) model in node-negative (N0) cases were analyzed by a Poisson regression procedure applied to the age covariate. Non-linearity of age on the log hazard ratio

was assessed. The age covariate was then iteratively fitted with parametric functions until the linearity condition of the PH model was satisfied. Finally, the parametric function of age found in N0 was verified in a PH model applied to node-positive (N+) cases.

Results: The analysis by martingale residuals was performed on 58,139 N0 cases. Age was significantly non-linear in PH models. The graph of functional form showed a U-shape of the effect of age on mortality (Fig1). An appropriate transform was obtained with the function: Age + |Age-50|$^{1.5}$. Modeling based on 25,665 N+ cases found a similar U-shape functional form. The transform applied to a PH model based on N+ cases improved the model, but the linearity condition of PH was satisfied only by using Age+|Age-50|$^{1.8}$.



Discussion: The U-shape functional form indicate an abnormal age pattern in which younger patients experienced the same mortality as much older patients, e.g. patients aged 20 had the same relative mortality risk as patients aged 60-65. The modeling suggests that the age pattern has two components: a linear log hazard ratio which represents the normal aging process, and a non-linear component which might represent the disease related age process. The non-linear component is more intense the farther away from the peri-menopause period as expressed by the absolute difference |Age-50|, and more intense in N+ than in N0 as expressed by the larger 1.8 exponent. We hypothesize that efficacy of cancer treatment might be detected by a change in the non-linear component. The modeling approach might represent in that case a powerful measurement of treatment effects.

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Genomic DNA amplification of decoy receptor 3 (DcR3) correlates with lymphatic invasion and lymphnode metastasis in breast cancer.

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Background: Decoy receptor 3 (DcR3) shows inhibitory effect to Fas-mediated apoptosis (Nature 1998; 396 (6712): 699-703). We have reported positive relationship between DcR3 mRNA expression and the gene amplification in breast cancer tissues (The 23rd Annual San Antonio Breast Cancer Symposium; abstract#380), suggesting that breast cancer, in some part, express DcR3 under the gene amplification to evade the apoptotic mechanism. In the present study, we examined the relationship between DcR3 genomic amplification and clinicopathologic factors to clarify its effect(s) in human breast cancer.

Materials & Methods: One hundred patients who underwent operations for primary breast cancer at Niigata University Hospital between 1996 and 2000 were selected for the present study. Genomic DNA of 100 breast cancer tissues and 14 normal breast tissues was extracted respectively from paraffin embedded sections of surgical specimens by microdissection under light microscope. Real-time quantitative PCR was performed to measure genomic amplification of DcR3 by standardizing with b-globin gene. The results were expressed as DcR3/b-globin ratio (D/b), and compared with clinicopathologic factors, disease free survival (DFS) and overall survival (OS) of patients. D/b of both cancer tissues and normal tissues were also compared, and genomic amplification in cancer tissue was defined as D/b > 1.55; greater than mean + 2SD of normal breast samples. Statistical analysis was performed by Mann-Whitney U-test and Breslow- Graham-Wilcoxon test, and the statistical significance was defined as $P < 0.05$.

Results: D/b was significantly higher in cancer tissues compared to normal tissues ($p<0.0001$). In cancer tissue, D/b was significantly higher in the lymphatic invasion positive group compared to negative group ($p=0.0056$), and was also significantly higher in the lymphnode metastasis positive group compared to negative group ($p=0.0396$). There was no significant association between D/b and other clinicopathologic factors, such as age, tumor size, venous invasion or hormone receptor status. The DFS was significantly lower in the genomic amplification positive group compared to negative group ($p=0.0397$), however, the OS showed no statistical difference with or without genomic amplification.

Conclusion: These results suggest that DcR3 gene amplification in breast cancer might be involved in both lymphatic invasion and lymphnode metastasis of cancer cells, and might decrease DFS.

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Abnormalities of erbB oncogenes in locally advanced breast cancer

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Background: The *erbB* family of protooncogenes (*erbB-1, erbB-2, erbB-3, erbB-4*) and receptors encoded by them play an important role in normal cell growth and in neoplastic transformation. Literature data indicate that some abnormalities of *erbB* oncogene family (amplification, deletion) have special importance in breast cancer development, correlate with tumor aggressiveness and with worse clinical outcome. Therefore, these abnormalities may be potentially useful for determining prognosis and for optimizing breast cancer treatment.

Aim of the study: This study was designed to determine gene dosages of *erbB* oncogene family in breast cancer. The relationship of these abnormalities with (CA)n dinucleotides polymorphism and with loss of heterozygosity (LOH) in *erbB1* was examined. Molecular parameters were analyzed in relation to clinical and pathological features of the tumors and to chemotherapy response.

Matherials and methods: Study subjects included 32 chemotherapy naive patients (pts) with primary inoperable locally advanced breast cancer (any T,N_2, any N,T_4). All pts were managed with induction chemotherapy. Tumor (incisional or core needle biopsy) and blood samples were taken prior to treatment and frozen immediately for further analysis. Chemotherapy regimens included ET (docetaxel 100 mg/m^2, epirubicin 90 mg/m^2; 6 pts), FEC (5-Fu 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2; 8 pts) and FAC (5-Fu 500 mg/m^2, doxorubicin 50 mg/m^2, cyclophosphamide 500 mg/m^2; 18 pts). Tumor measurement was performed after each cycle and at the completion of induction chemotherapy. Double differential PCR (ddPCR) was used for detection of *erbB* oncogene family abnormalities (gene amplification/deletion). Microsatellite polymorphism of *erbB-1* was examined by PCR with fluorescently labeled primers, followed by capillary electrophoresis and quantitative analysis of PCR product with GeneScan system, using automated sequencer ABI PRISM 310.

Results: Amplifications of *erbB-1, erbB-2, erbB-3, erbB-4* (defined as AGCN value >1.6) were detected in 5.9%, 26.5%, 2.9% and 2.9% of examined cases, respectively. Deletions, defined as AGCN value <0.4 occurred only in *erbB-1* and was found in 26.5% of all cases. There was a polymorphic simple sequence repeat region of 12-20 CA repeats detected in the first intron of *erbB-1*. Homozygotes comprised 31% of the examined group. The majority of the homozygous pts revealed 14/14 CA repeat combination. LOH (most frequently affecting shorter allele) was determined in breast cancer heterozygotes and occurred in 50% of cases. Correlation between these findings and clinical outcomes in extended group of 50 pts will be presented at the meeting.

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New sequence variants, recurrent BRCA1/BRCA2 mutations and new aberrations in BRCA1 promoter region in breast and ovarian cancer cases from Upper Silesia in Poland.

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Purpose: Germline mutations within BRCA1 and BRCA2 are responsible for a significant fraction of hereditary breast and ovarian cancer cases. BRCA