(AuTL) was administrated into recurrent tumor site biweekly, and additionally trastuzumab (2 mg/kg) was infused systemically every week in the 7 HER2+ patients. Patients continued on therapy until disease progression.

Furthermore, to assess the mechanism of trastuzumab-effects in the therapy, dendritic cells (DC) from peripheral monocytes of several healthy donors were generated in IL-4/GM-CSF in vitro, and fed with trastuzumab-treated/-untreated HER2+ tumor cells or tumor cell lysate. These antigen-loaded DCs were examined in the phenotype, cytokine productions, and the ability to induce HER2 specific T cells in vitro.

Results: In the clinical trial, one patient of PR was observed in the HER2+ group, which showed marked regression in the injected field of metastatic lymph node, but none of PR in the HER2−. SD/PD was 3/3 or 3/4 pts in the HER2+ or HER2− group, respectively. The carcinomatous pleural effusion was disappeared and/or well controlled in 6 pts (HER2+ vs. HER2−; 4 vs. 2 pts), and the tumor marker proteins (CEA, CA15−3, TPA) were decreased significantly in 5 pts (HER2+ vs. HER2−; 4 vs. 1 pts). Adverse effects were tolerable in all the patients.

In DC experiments in vitro, trastuzumab-opsonized antigen-loaded DC showed significant enhancement of the ability to induce CD8+ T cells specific for HER2-peptides with the higher production of IL-12p70.

Conclusions: Adoptive cell therapy combined with trastuzumab is a well-tolerated regimen. Our preliminary data suggest that this strategy may benefit heavily pretreated HER2+ metastatic breast cancer patients. It might be in part due to the involvement of mAb in the ability of DC cross-presentation followed by the enhancement of antitumor cellular immunity.

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BRCA1 mutation is strongly associated with a triple negative phenotype in breast cancer patients

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Introduction: Our aim was to compare the differences in clinical presentation and tumor pathology features among breast cancer patients with BRCA1 and BRCA2 mutations and non-BRCA carriers.

Material and Methods: Tumor pathologic features (histology, hormone receptor and Her2 expression) and clinical characteristics (age and clinical stage at diagnosis, gender, bilaterality, and BRCA mutation status) were examined in 188 breast cancer patients who underwent BRCA germline genetic testing between 2002 and 2008 through a retrospective review of our hereditary cancer database.

Results: Of the 188 patients, 129 (69%) were non-BRCA carriers, 28 (15%) were BRCA1, and 31 were BRCA2 (16%). Age at diagnosis was similar among the three groups (40, 42 and 46 respectively, non-statistical different). Among male breast cancer patients (7), none was a BRCA1 carrier and three were BRCA2 carriers. Bilaterality was more frequent in BRCA1 and BRCA2 carriers compared to non-carriers (25%, 24%, and 14%, respectively, non-statistically different). Triple-negative breast cancer (estrogen receptor, progesterone receptor, and HER-2/neu negative) was diagnosed in 85% (18/21) of the BRCA1 carriers, 21% (4/19) of the BRCA2 patients, and 29% (27/93) of the non-BRCA patients ($p < 0.01$). We did not observe any patient with a BRCA1 mutation and HER2 overexpression ($p < 0.01$ compared with BRCA-2 and non-BRCA carriers), while HER2 overexpression was similar between BRCA2 (15%) and non-BRCA carriers (23%) ($p = 0.47$).

Conclusions: Breast cancer in BRCA1 mutation carriers is more frequently triple negative while patients with BRCA2 mutations have a similar clinical and pathologic phenotype than non-BRCA patients. These differences may have therapeutic implications.

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Ductal carcinoma of the breast with morphologic and inmunohistochemical features like columnar cells

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Background: Columnar cell lesions (CCL) of the breast are detected with increasing frecuency in routine practice. The frequent coexistence of CCL with low grade DCIS in the same breast and overlapping morphologic features with ADH and DCIS provides evidence for CCL being a candidate precursor in the progression to low grade DCIS and invasive carcinoma. This hypothesis has been supported by the similar cytologic appearance of cells within atypical cystic lobules and low grade DCIS of the same specimens or cells within CCL and cells comprinsing coexisting DCIS or tubular carcinoma.

Material and Methods: In 500 cases of breast cancer in our routine practice, we identified four cases of ductal carcinoma with morphologic features like columnar cells. We assessed inmunohistochemistry (IHC) studies for estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), HER-2/neu, cytokeratin 19 (CK 19), cytokeratin 5/6 (CK5/6), cytokeratin 34bE-12 (CK34bE-12), Bcl-2, cyclin D-1 (CD1), Ki67 MIB1. Results of hormonal receptors were scored by H-Score previously described. HER-2/neu were scored positive with score 3. Ki67MIB1 was scored by percent of the positive tumor cells.

Results: The characteristics of the patients and IHC are shown in the table. Microscopically the tumors showed irregular ducts lined with one or two layers columnar cells with prominent apical cytoplasmic snouts and intraluminal secretion. Nuclear/cytoplasmic ratio was increased. Nuclei round to ovoid, hyperchromatic nuclei with inconspicuous nucleoli. In other areas there were solid pattern and complex architecture with micropapillae, fronds, arcades, rigid bridges. We observed in three cases in some areas intraluminal calcifications.

Gender/Age	Tumor size (cm)	Axilla status	Symptom duration	ER	PR	AR	HER2neu	CK19	CK5/6	CK34β E 12	CD1	Bcl2	Ki-67
Female/94	3×3	1 node +	1 year	200+	180+	+	−	+	−	+	+	+	30%
Female/47	2.5×2	-	2 years	200+	170+	+	−	+	−	+	+	+	20%
Female/80	2×2	-	2 years	150+	100+	+	−	+	−	+	+	+	20%
Female/43	1.5×1	3 nodes +	1 year	200+	170+	+	−	+	−	+	+	+	30%

Conclusion: The morphologic and inmunohistochemical features of these carcinomas are similar with columnar cells. These carcinomas could be the malignant form of the CCL. We need further studies for categorize these tumors.

Wednesday, 24 March 2010 18:15–19:15

POSTER SESSION

Molecular biology, markers

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The prognostic value of angiogenesis genes polymorphisms in women with infiltrating ductal breast carcinoma

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Background: Angiogenesis is an important step in the development of infiltrating ductal carcinoma which is the most common histologic type of breast cancer. Polymorphisms in genes encoding angiogenic factors or their receptors are known to predispose to breast cancer [Schneider et al., 2008; Clar et al., 2009]. The aim of our study was to investigate the association of functional polymorphisms in the *VEGF-2578C*/A (rs699947), *FGFR2A*/G (rs1219648), *TGFB1–509C>T* (rs1800469) and *IL10–592C>A* (rs1800872) genes with infiltrating ductal breast carcinoma risk, progression and response to neoadjuvant chemotherapy.

Material and Methods: Two hundred sixteen patients with operable primary infiltrating ductal breast carcinoma ($T_{1-4}N_{0-2}M_0$; age from 20 to 77 years) who received two-four cycles of neoadjuvant chemotherapy in the Tomsk Cancer Research Institute were included in the present study. The healthy women ($n = 286$; age from 30 to 75 years) from Western Siberian region were used as the control group. DNA was extracted from peripheral blood and the genotypes were analyzed using PCR-restriction fragment length polymorphism protocols.

Results: The frequencies of VEGF-2578A/A, FGFR2G/G and IL10–592A/A variants were significantly higher in the patient group when compared with controls (OR = 2.3, $p = 0.002$; OR = 2.3, $p = 0.002$ and OR = 3.2, $p = 0.008$ respectively). Significantly lower frequencies of FGFR2A/A and

TGFB1–509TT genotypes were showed in the patient group in comparison to the controls ($p = 0.008$ and $p = 0.03$ respectively). We have observed an increased frequency in the VEGF-2578A/A genotype among women with positive regional lymph node metastases compared to patients with negative regional lymph node metastases ($p = 0.02$). A significant difference was found between the luminal B and luminal A subtype tumor of patients carrying the VEGF-2578C/C genotype ($p = 0.04$). In addition, patients with the FGFR2A/A genotype exhibited a non-statistically significant better response to neoadjuvant chemotherapy ($p = 0.06$). There was also trend for association between FGFR2G/G genotype and worse response to neoadjuvant chemotherapy in infiltrating ductal breast carcinoma patients ($p = 0.08$).

Conclusions: These findings indicate that genetic variants in VEGF-2578A/A, FGFR2G/G and IL10–592A/A are associated with infiltrating ductal breast carcinoma risk. Polymorphism in VEGF gene may serve as molecular marker related with regional metastasis and molecular subtype of tumor. The polymorphic variants of FGFR2 gene may be a potential prognostic factor for response to neoadjuvant chemotherapy in infiltrating ductal breast carcinoma patients.

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In patients with early breast cancer, populations of immature, cytokine producing plasmacytoid dendritic cells (PDC) decrease in tumour draining lymph nodes and express TLR9

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Background: Plasmacytoid dendritic cells (PDC) represent a distinct subset of dendritic cells (DC) capable of producing large amounts of type-1 interferons after stimulation of toll-like receptors (TLR)-7 and 9. In breast cancer draining LN, PDC secrete cytokines such as IL-10 and IL-12 with the potential to polarise local T cell responses. Despite being frequently identified within the tumour microenvironment, PDC remain poorly characterised in human cancer.

Materials and Methods: Plasmacytoid DC and myeloid DC (MDC) were identified using flow cytometry in cell suspensions of control LN and good and poor prognosis breast tumour draining axillary LN (TDLN) as defined by the Nottingham Prognostic Index (NPI). Immunohistochemistry and immunofluorescent microscopy was performed on frozen Control LN and TDLN sections to localise PDC and determine IL-10 and IL-12 expression. Immunofluorescence microscopy of magnetically sorted PDC populations was undertaken to determine TLR-9 expression.

Results: PDC constituted a prominent immature population in all LN studied. In both control and TDLN the maturation status of PDC and MDC subset populations was similar. When compared to poor prognosis LN, PDC proportions decreased in LN draining good prognosis breast cancer ($p < 0.05$). Immunohistochemistry identified CD123+ BDCA-2+ PDC within the cortex and sinus system of control and tumour draining LN. In LN containing metastatic breast cancer, CD123+ BDCA-2+ PDC were found within and at the cancer periphery. Immunofluorescence microscopy localised BDCA-2^+ PDC co-expressing IL-10 or IL-12 to the T cell areas of control and tumour draining LN. TLR-9 expression was identified on PDC sorted from control and tumour draining LN.

Conclusions: PDC were found in close proximity to malignant cells in metastatic LN as well as T cells in control LN and TDLN. The identification of PDC in the sinus system of control LN and TDLN suggests that they can gain access to tissue afferent lymphatics. The expression of IL-10 and IL-12 by PDC in the T cell areas of control LN and TDLN confirms that PDC are able to produce polarising cytokines. In patients with breast cancer, the migration and cytokine secretion of PDC populations may play a pivotal role in anti-tumour responses. The expression of TLR-9 by PDC also makes them a target for therapeutic intervention.

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Relation between methylation promoters gene and estrogen receptor (ERS1) and her2/neu status in breast cancer patients

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The CpG island metylator phenotype is associated with distinct clinicopathological caracteristics as Estrogen Receptor (ESR1) positive and amplification HER2 in breast cancer.

Objective: To investigate the relation between DNA promoter methylation and the prognostic clinico-pathological features of breast cancer, including diagnosis and treatment response and to evaluate epigenetics differences in tumor-related genes to ESR1 and HER2/neu status in primary breast cancer.

Material and Methods: We quantified methylation levels of promoter of 5 genes (ERS1, RAR-β, 14-3-3 sigma, APC, E-Cadherin) which are to confer growth advantage to cells in 107 women with breast cancer and 108 control subjects. Real Time QMS-PCR SYBR green (methylation-specific PCR) was used to analyze the hypermethylation. Tumours were classified as phenotype basal, luminal A, Luminal B and phenotype HER2+.

Results: Ours analyses revealed low or absent methylation *ESR1*and *14-3-3*σ in healthy controls and significant differences between breast cancer patients (pts) and healthy controls in relative serum levels of methylated gene promoters *ESR1* ($p = 0.0112$) and *14-3-3*s ($p = 0.0047$). Presence of methylated *ESR1* in serum of breast cancer patients was associated with ER-negative phenotype ($p = 0.0179$). Of the available cases, 60 pts (56%) were Luminal A, 10 pts (9.3%) Luminal B, 13 pts (12%) Basal like and 9 pts (8.4%) HER2+. We observed that methylated ERS1 was preferably associated with phenotype Basal Like and worse interval progression free and survival global though $p > 0.05$ and the amplification HER2+ was correlation with significant more frequent methylation gene ($p < 0.05$). Thet hypermethylation of normal ERS1 and 14-3-3σ combined differentiated between breast cancer patients and healthy controls ($p = 0.0001$) with a sensitivity of 81% (95% CI: 72−88%) and specificity of 88% (95% CI: 78−94%).

Conclusions: This study identifies the presence of variations in global levels of methylation promoters genes in healthy controls and breast cancer with different phenotype classes and shows that these differences have clinical significance. These showed that frequent methylation had a strong association with molecular phenotype of breast cancer and perhaps in the future can explain therapy resistance related to RE and HER2/neu status in breast cancer patients.

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Novel germline mutations in BRCA2 gene among breast and breast-ovarian cancer families from Poland

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Background: The aim of our study was an assessment of the spectrum of *BRCA2* gene mutations and their frequency in women and men with familial breast cancer and ovarian cancer, in whom no mutations were found in *BRCA1* gene.

Material and Methods: 105 probands were selected (97 women and 8 men) and treated in the Oncology Center in Warsaw and the Oncology Center − Branch in Cracow in the years 1998–2008 and remain in care of the Genetic Counselling Clinic, Oncology Center in Warsaw. The patients were aged 17−67 years; (median age 46 years). The presence of molecular changes was examined in DNA isolated from peripheral blood lymphocytes. Germline mutations in 27 exons of the *BRCA2* gene were screened by "touchdown" PCR amplification, DHPLC and sequencing. Missense mutations were classified by multiple-sequences alignments of orthologous BRCA2 protein sequences with T-Coffee software.

Results: Thirty-nine molecular variants were identified in the study group, including eight changes determined for the first time (five pathogenic