## Wednesday, 24 March 2010 18:15–19:15

**POSTER SESSION**

# Tumour biology and immunology

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### Perioperative assessment of the kinetics of circulating tumour cells in patients with operable breast cancer based on cytokeratin-19 mRNA detection

M. Daskalakis[1], I. Askoxylakis[1], D. Mavroudis[2], E. Sanidas[1], V. Georgoulias[2], J. Mlissas[1], D.D. Tsiftsis[1]. [1]*University General Hospital of Heraklion, Surgical Oncology, Heraklion, Greece;* [2]*University General Hospital of Heraklion, Medical Oncology, Heraklion, Greece*

**Background:** The studies on circulating tumor cells (CTCs) provide new insight into the biology of metastasis. The aim of this study was to evaluate the effect of surgery on the kinetics of CTCs in patients with surgically resectable breast cancer.

**Materials and Methods:** The detection of CK-19 mRNA-positive CTCs by real-time reverse transcriptase polymerase chain reaction in the blood was analysed in 104 stage 0−IIIA breast cancer patients at four different times: prior to surgery, upon completion, 24 hours after surgery, and 15 days after surgery. Furthermore, a late sample was assessed prior to initiation of adjuvant chemotherapy in a subgroup of 53 patients. As negative controls, peripheral blood was obtained from 50 female patients undergoing excision of benign lesions and from 11 female patients receiving surgery for early stage colorectal cancer.

**Results:** No significant difference was noted between samples of breast cancer patients at different time intervals with respect to the median CK-19 mRNA-positive cells. The overall percentage of breast cancer patients who were CK-19 mRNA-negative before surgery and turned positive at any time point postoperatively was 14.9%. There was no significant correlation between CK-19 mRNA-positivity and classical prognostic factors. A significant increase in CK-19 mRNA-positivity (32.1%) was observed in the late sample of the subgroup of 53 patients after a median of 54 days.

**Conclusions:** CTCs identified perioperatively based on CK-19 mRNA detection in patients undergoing surgery for early breast cancer are independent of the surgical procedure. There is no clear correlation to indicate which patients are more likely to have detectable CTCs. Although in the perioperative period CTCs are detected in a low proportion of patients, the detection rate may increase over time and with longer follow-up.

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### The role of suppressors of cytokine signaling in human breast cancer

W. Sasi[1], W.G. Jiang[2], A.K. Sharma[1], K. Mokbel[1]. [1]*St George's Hospital and Medical School, Breast and Endocrine Surgery, London, United Kingdom;* [2]*Cardiff University, Surgery, Cardiff, United Kingdom*

**Background:** Suppressors of cytokine signaling (SOCS) are important negative feedback regulators of the JAK/STAT signaling pathway, and have been recently investigated for their role in the development of different cancers. In this study, we examined the expression of SOCS1−7 genes in normal and breast cancer tissue and correlated this with several clinico-pathological and prognostic factors.

**Materials and Methods:** SOCS1−7 mRNA extraction and reverse transcription were performed on fresh frozen breast cancer tissue samples (n = 127)and normal background breast tissue (n = 31). Transcript levels of expression were determined using real-time PCR and analysed against TNM stage, tumour grade, and clinical outcome over a 10 year follow-up period.

**Results:** SOCS 1, 4, 5, 6 and 7 expression decreased with increased TNM stage (TNM1 vs. TNM3 p = 0.039, TNM1 vs. TNM4 p = 0.016, TNM1 vs. TNM3 p = 0.012, and TNM1 vs. TNM3 p = 0.044 respectively). SOCS 2 and 3 expression decreased with increased Nottingham Prognostic Index (NPI) (NPI1 vs. NPI3 p = 0.033, and NPI2 vs. NPI3 p = 0.041 respectively). SOCS7 expression decreased with higher tumour grade (Grade 3 vs. Grade 2 p = 0.037). After a median follow-up period of 10 years, we foundhigher levels of SOCS1, 2, and 7 expression among those patients who remained disease-free compared to those who developed local recurrence (p = 0.0073, p = 0.021, and p = 0.039 respectively). Similarly, we found higher levels of SOCS 2, 4, and 7 expression in those who remained disease-free compared to those who developed distant recurrence (p = 0.022, p = 0.024, and p = 0.033 respectively). Patients who remained disease-free had higher levels of SOCS 1 and 3 expression compared to those who died from breast cancer (p = 0.02, and p = 0.033 respectively). The disease free survival (DFS) and overall survival (OS) curves showed that higher levels of SOCS 1, 3 and 7 were significant predictors of better DFS (p = 0.015, p = 0.024, and p = 0.03 respectively) and OS (p = 0.005, p = 0.013, and p = 0.035 respectively). Higher levels of SOCS 4 were significant in predicting better OS (p = 0.007) but not DFS.

**Conclusions:** Higher mRNA expression levels of SOCS 1, 3, 4, and 7 are significantly associated with earlier tumour stage and better clinical outcome in human breast cancer.

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### Prediction of lymph node involvement in breast cancer from primary tumour tissue using gene expression profiling

A. Smeets[1], A. Daemen[2], I. Van den Bempt[3], O. Gevaert[4], H. Wildiers[1], R. Drijkoningen[5], P. Van Hummelen[6], R. Paridaens[7], C. Sotiriou[8], M.R. Christiaens[1]. [1]*University Hospitals Leuven, Multidisciplinary Breast Centre, Leuven, Belgium;* [2]*Catholic University Leuven, Electrical Engineering, Leuven, Belgium;* [3]*University Hospitals Leuven, Department of Pathology, Leuven, Belgium;* [4]*University Hospitals Leuven, Department of Electrical Engineering, Leuven, Belgium;* [5]*Virga Jesse Ziekenhuis Hasselt, Department of Pathology, Leuven, Belgium;* [6]*Catholic University Leuven, VIB MicroArray Facility, Leuven, Belgium;* [7]*Catholic University Leuven, Multidisciplinary Breast Centre, Leuven, Belgium;* [8]*Jules Bordet Institute, Translational Research Unit, Brussels, Belgium*

Lymph node involvement is the most important prognostic factor in breast cancer, but yet little is known about the underlying molecular mechanisms. Whether a "lymphatic metastasis signature" can be defined for breast cancer is currently unclear. Here, to identify a molecular signature associated with nodal metastasis, gene expression analysis was performed on a very homogeneous group of primary breast tumors (postmenopausal, ER+, HER2-, grade 3 invasive ductal cancer).

The datasets considered in this study are two own datasets (prospective dataset containing 48 node negative and 48 node positive samples; independent dataset containing 20 samples) and 5 publicly available datasets on breast cancer for which the lymph node status was provided (106 samples). For our datasets, RNA was isolated and hybridized to the Affymetrix Human U133 Plus 2.0 microarray chip. All datasets were preprocessed with MAS 5.0. We used the annotation provided by Dai for the conversion of probes to genes. The 5000 most varying genes were included. Finally, our prospective dataset was standardized per sample across all genes. The other datasets were first reduced to the subset of genes included in our final model before standardization. A model was built by weighted Least-Squares Support Vector Machines at a significance level for gene inclusion of 0.05. The Spearman correlation coefficient was used to identify genes that gradually increase or decrease with changing lymph node ratio (number of positive lymph nodes divided by total number of lymph nodes). A 10-fold cross-validation strategy was followed to train a model on our 96 patients. The model that has been validated on the internal and the external datasets contained those genes that were selected in at least half of the cross-validation iterations at the significance level 0.05 (241 genes). The area under the ROC curve for the internal dataset is 0.647 and 0.651 for the external datasets. The model includes a high number of apoptosis related (26) and zinc ion binding (43) genes. Pathway analysis using the Molecular Signatures Database revealed multiple relevant gene sets i.e. BRCA, BAF57, Van 't Veer.

In conclusion, our model provides evidence that lymph node involvement in breast cancer is not a random process. Whether the model is a general predictor for lymph node involvement will be evaluated in a next step.

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### Strategy to augment the efficacy of immunotherapy for refractory breast cancer: a pilot clinical study of adoptive cell therapy combined with trastuzumab

N. Seki[1], U. Toh[2], T. Fujii[2], S. Nakagawa[2], H. Otsuka[2], K. Shirouzu[2], H. Yamana[2]. [1]*Kurume University, Research Center For Innovative Cancer Therapy, Kurume, Japan;* [2]*Kurume University, Department of Surgery, Kurume, Japan*

**Background:** Cancer targeting mAb Trastuzumab as a single agent has activity in metastatic breast cancer; however, the mechanism of action for this clinical activity is not fully understood. Whereas interruption of HER family member signaling occurs, trastuzumab also could interact with host immune-cells via its Fc domain. Based on these data, a clinical trial was performed to test whether trastuzumab, when combined to adoptive cell therapy in patients with HER2+ tumor, can increase the therapeutic efficacy, and be safely given.

**Materials and Methods:** 14 patients with recurrent breast cancer (HER2+ vs. HER2−: 7pts vs. 7pts) who had failed the conventional chemo-radiotherapy were enrolled. Autologous-tumor cell stimulated lymphocytes