however, those patients with high tumor infiltration of FoxP3$^+$-T cells who received GOLFIG regimen showed the most favorable outcome (high vs low score; TTP = 20.8 vs 11.6 months; $P = 0.04$; OS = 68.1 vs 41 months; $P = 0.04$). A COX regression model demonstrated in these patients that a high T_{reg} tumor infiltration score is an independent variable of long survival and prolonged TTP.
Conclusion: Our results suggest that GOLFIG chemoimmunotherapy is highly effective in colon carcinoma patients with high FoxP3$^+$ infiltration score and that T_{reg}-tumor infiltration score may be a favorable prognostic marker in colon cancer patients.

1327 POSTER

Real-time risk evaluation of metastasis using circulating tumor cells

A. de Albuquerque[1], S. Kaul[2], N. Fersis[3], D. Ernst[4], A. Teubner[5], G. Breier[6]. [1]*Faculty of Medicine Carl Gustav Carus at University of Dresden, Department of Diagnostic at Klinikum Chemnitz gGmbH, Chemnitz, Germany;* [2]*Klinikum Chemnitz gGmbH, Department of Diagnostic, Chemnitz, Germany;* [3]*Klinikum Chemnitz gGmbH, Department of Gynecology and Obstetrics, Chemnitz, Germany;* [4]*Klinikum Chemnitz gGmbH, Department of Surgery, Chemnitz, Germany;* [5]*Klinikum Chemnitz gGmbH, Department of Internal Medicine II, Chemnitz, Germany;* [6]*Faculty of Medicine Carl Gustav Carus at University of Dresden, Department of Pathology, Dresden, Germany*

Background: From all the techniques available for detection of circulating tumor cells, the immunomagnetic separation is the most advanced one. In this experimental work we present a variance for this technique by the introduction of 2 antibodies (BM7 and VU1D9) for tumor cell selection and the use of a multimarker panel for identification of circulating tumor cells in peripheral blood of patients with adenocarcinomas.
Methods: Samples from patients were divided in native probes and matched calibrator probes containing 2 and 10 adenocarcinoma cells. The high affinity antibodies BM7 (MUC1) and VU1D9 (EpCAM) were used for immunomagnetic tumor cell enrichment from two 5 mL probes of peripheral EDTA-blood of metastatic breast cancer patients and local advanced or/and metastatic gastrointestinal cancer patients. Separated cells were lysed and used for mRNA isolation and c-DNA synthesis. A real-time quantitative RT-PCR approach using MESA FAST SYBR Assay (Eurogentec®) and FAM-labeled TaqMan probes and primers (Roche AG®) for the epithelial markers cytokeratin 19 and 20 (CK19 and CK20), mammaglobin 1 (MG1), carcinoembryonic antigen-related cell adhesion molecule 5 (CEA), epithelial cell adhesion molecule (EpCAM), aldehyde dehydrogenase 1 family, member A1n (ALDH1), baculoviral IAP repeat-containing 5 (Sur), HER-2, immunosupressive CD276 (B7-H3), chemokine receptor 4 (CXCR4), hypoxia inducible factor (HIF-1alpha), metastasis associated in colon cancer (MACC) and transketolase-like 1 (TKTL1) were used for tumor cell identification.
Results: Sensitivity of the marker panel was validated in calibration tests with 2 cells and 10 cells and specificity was confirmed by examination of blood from healthy donors. Positivity rate of local advanced and/or metastatic gastrointestinal cancer patients was 73.5%, while 67.7% of metastatic breast cancer patients showed multimarker positivity. The marker with the highest expression level in metastatic breast cancer patients was CK19 followed by EpCAM. In local advanced and/or metastatic gastrointestinal cancer patients the most frequent identified genes were EpCAM, Survivin and CEA.
Conclusion: The optimized surrogate marker panel from the networks of apoptosis, invasion, angiogenesis and stem cell phenotype should improve early detection of metastasis as well as monitoring of therapy response and selection of tailored therapy regimes. Circulating tumor cells expressing the newly introduced markers Sur, TKTL1 and HIF-1alpha are clearly associated with aggressive tumor behaviour and poor clinical outcome.

1328 POSTER

Oral leukoplakia – identification of possible biomarkers trough mass spectrometry

D. Pereira[1], D.R. Camisasca[1], L.R. Gonçalves[1], R. Santana[1], R.C. Lindenblatt[2], F.C.S. Nogueira[3], C.H.S. Garcia[3], S.Q.C. Lourenço[2], R.B. Zingali[4], G. Alves[5]. [1]*Istituto Nacional de Cancer (INCA), Servico de Hematologia, Rio de Janeiro, Brazil;* [2]*Universidade Federal Fluminense, Programa de Pós-Graduação em Patologia, Niterói, Brazil;* [3]*Universidade Federal do Rio de Janeiro, Laboratório de Biologia de Sistemas, Niterói, Brazil;* [4]*Universidade Federal do Rio de Janeiro, Unidade de Proteômica e Espectrometria de Massas, Rio de Janeiro, Brazil;* [5]*Instituto Nacional de Câncer, Serviço de Hematologia, Rio de Janeiro, Brazil*

Background: The principal oral and oropharyngeal lesions which may be precursor lesions for cancer are white patches (leukoplakia) and red patches (erythroplasia) or mixed red and white lesions. Leukoplakias are the most common of them. This study aimed to compare by 2-D gels electrophoresis a pool of saliva from oral leukoplakia patients and a control group to determine possible protein biomarkers of the disease.
Methods: Leukoplakia group was composed of 10 patients (4 males and 6 females, ±73.7 years old) with histopathologic confirmation of the diagnosis. Patients were selected in the Stomatology Service from Odontoclínica Central do Exército (OCEx, RJ – Brasil). All of them were asked to spit their saliva for 5 minutes, at least 1 hour after last meal. Whole saliva control samples (10 non-smoking adults) were collected. Protease inhibitor (PMSF 1 mM) and 1 mM of EDTA were added to sample, they were submitted to centrifugation (14,000 g, 15 minutes) and stored at −80ºC. Bio-Rad DC-Protein Assay determined protein concentration. Proteins were precipitated with cold acetone, and 1 mg of total salivary protein were separated using two-dimensional (2-D) gel electrophoresis over a pH range between 3−10 L (18 cm). Spot demarcation and matching was performed through ImageMaster 5.0. Protein identification was made through electrospray ionization-tandem mass spectrometry (MALDI-TOF-TOF). Obtained data were searched against the NCBI non-redundant protein databases using Mascot software.
Results A mean of 226 spots were identified in the 3 leukoplakia 2-D gels, and a mean of 262.3 spots in control group. Five spots were found to be up regulated in leukoplakia. Apoliprotein A1 and cystatin-1 were the most significative proteins identified among them. Keratin type 1 and lysozyme C were only found in leukoplakia.
Conclusions Differences in salivary protein profile in 2-D gel electrophoresis from control and oral leukoplakia subjects can be observed. Validation of this data on a new set of individuals would reinforce their role as biomarkers for oral leukoplakias.

1329 POSTER

A sequential use of the Risk of Malignancy Index and Ovarian HistoScanning for the differential diagnosis of adnexal masses

E. Vaes[1], R. Manchanda[2], R. Nir[3], D. Nir[3], H. Bleiberg[4], A. Robert[1], U. Menon[2]. [1]*Université Catholique de Louvain, EPID, Brussels, Belgium;* [2]*UCL, EGA Institute for Women's Health, London, United Kingdom;* [3]*AMD, HistoScanning, Waterloo, Belgium;* [4]*Jules Bordet Institute, Brussels, Belgium*

Purpose: Ovarian cancer has a high case-fatality rate. Outcomes can be improved by appropriate surgery by gynecological oncologists. This is dependent on accurate preoperative diagnosis of adnexal masses. The Risk of Malignancy Index (RMI) which combines menopausal status, serum CA125 and ovarian ultrasound is widely used for this purpose. This paper evaluates the use of RMI in combination with Ovarian HistoScanning, a novel computerized technique to interpret ultrasound data.
Patients and Methods: Three current versions of RMI were assessed in 199 women enrolled in a prospective HistoScanning study. Ultrasound scores were obtained by blinded analysis of archived images by 2 experienced, independent sonographers. HistoScanning was modeled as a second line test for RMI between a lower cut-off (LC) and an upper cut-off (UC), using different thresholds (HSCU) for a positive HistoScanning result. The cut-offs (LC, UC, HSCU) that maximized the Youden index were determined in a training set (70% of patients) and validated in a testing set (30% of patients).
Results: There was no significant difference in the AUC between the 3 RMI indices. The best performing RMI at the clinical cut-off of 250, RMI_2, had a sensitivity of 81.6% (95%CI = [73.9; 89.3]) and specificity of 80.8% (95%CI = [73.0; 88.6]). Combining HistoScanning with RMI_3 on the ensemble of data with optimized cut-offs (LC, UC, HSCU) = (105, 2100, 20) resulted in a significantly higher sensitivity and specificity of 88.8% (95%CI = [82.6; 95.0]) and 93.9% (95%CI = [89.2; 98.6]) respectively.
Conclusion: Ovarian HistoScanning improves diagnostic accuracy of RMI and this is achievable without the use of additional expertise dependent and time consuming imaging.

1330 POSTER

A universal assay for detection of oncogenic fusion transcripts by oligo microarray analysis

R.I. Skotheim[1], M. Eken[1], G. Thomassen[1], G.E. Lind[1], R.A. Lothe[1]. [1]*Norwegian Radium Hospital, Department of Cancer Prevention, Oslo, Norway*

Background: The ability to detect oncogenic fusion transcripts is important both to cancer research and in clinical diagnostic settings. However, the available methodologies to detect such fusions all have their distinct shortcomings. We have recently published a novel oligonucleotide microarray strategy whereby one can screen for all known oncogenic fusion transcripts in a single experiment (Skotheim *et al.*, Mol. Cancer, 2009). Here, we

provided proof-of-principle data identifying fusion genes from a set of 6 leukaemia and prostate cancer samples. The aim is now to establish this as a robust tool that can be used to test for an extended set of known and candidate fusion genes in both a research and clinical diagnostic setting.
Materials and Methods: A combination of measurements of chimeric transcript junctions with exon-wise measurements of individual fusion partners is used. We have now gone through various literature and database sources, and our database of fusion genes now contains 559 previously reported fusion genes. A second-generation of the fusion gene microarray has been designed where about 500,000 oligos are used to interrogate each sample (NimbleGen HD2 technology, 3-plex microarrays).
Results and Conclusions: The new version of the fusion gene microarray have so far been successfully picking up known fusion genes from leukaemia cell lines, and we are currently moving into analyses of diagnostic cancer samples from leukaemia and sarcomas, as well as cell lines of various origins in search for known fusion genes in new cancer types. The method bears promise of an important complement to currently used diagnostic and research tools for the detection of fusion genes in neoplastic diseases.

1331 POSTER

Identifying the primary site using gene expression profiling in patients with carcinoma of an unknown primary (CUP): a feasibility study from the GEFCAPI

M. Gross-Goupil[1], Y. Loriot[1], T. Lesimple[2], Y. Merrouche[3], E. Blot[4], C. Massard[1], M.C. Mathieu[1], K. Fizazi[1]. *[1]Institut Gustave Roussy, Villejuif, France; [2]Centre Eugène Marquis, Rennes, France; [3]Institut de cancérologie de la Loire, Saint Etienne, France; [4]Centre Henri-Becquerel, Rouen, France*

Background: CUP are an heterogeneous family of neoplasms with a dismal prognosis, with empiric chemotherapy as the recommended treatment. The aim of this study was to evaluate the feasibility of a 500-mRNA microarray to identify the tissue of origin in patients with CUP.
Patients and Methods: Diagnostic biopsy formalin-fixed, paraffin-embedded (FFPE) specimens from 22 patients with CUP were prospectively collected. Gene expression profiling was performed using oligonucleotide microarray that contains 495 genes selected as highly differentially expressed between 49 tumor types (CupPrint®).
Results: The assay was successfully performed on specimens from 18 of the 22 patients (82%). It could not be performed because of a low RNA preservation in the remaining 4 cases. The median age was 57 years (range: 29−70 years). The median delay from tissue shipping to receipt of CupPrint® result was 11 days (range: 1−26 days). The most common tissues of origin identified were lung cancer (22%) and colorectal cancer (17%). Of note, a primary cancer which would not be adequately treated by an empiric chemotherapy regimen currently recommended in CUP (like cisplatin-gemcitabine or carboplatin-paclitaxel) was identified in about half patients: kidney cancer (1), hepatocarcinoma (1), colorectal cancer (3), head and neck cancer (2) and cholangiocarcinoma (1).
Conclusion: Gene expression profiling of FFPE biopsy specimens from patients with CUP is feasible in a reasonable delay, making it feasible in clinical practice. A phase III randomized trial is planned to compare therapy based on gene expression-suspected primary cancer versus empiric chemotherapy.

1332 POSTER

The distribution of recurrence scores in Europe and Middle East (EME) compared with the US

G. Palmer[1], J. Vaughn[1], D.J. Schneider[1], B. Haack[1]. *[1]Genomic Health Inc., Medical Affairs, Redwood City CA, USA*

Background: The Recurrence Score (RS), derived from the quantitative RT-PCR analysis of 21 individual genes, quantifies the likelihood of distant recurrence in hormonal-treated patients with estrogen receptor (ER)-positive breast cancer. We examined whether there are differences in the RS distribution between EME and the US.
Material and Methods: 2,676 tumor specimens successfully examined in the Genomic Health laboratory from January 2004 through April 2009 that were submitted by physicians from EME were included in the analyses. Quantitative expression of 16 individual cancer related genes was measured by the pre-specified 21 gene Recurrence Score assay (Oncotype DX®) on a scale from 0 to 15 (relative to reference genes), where a one unit increment is associated with a 2-fold change in expression. RS is calculated from a published equation (Paik et al, NEJM 2004) using the quantitative expression of five proliferation-related genes (CCNB1, Ki-67, MYBL2, STK15 and Survivin), four ER-related genes (ER, PR, Bcl2 and SCUBE2), two HER2-related genes (HER2 and GRB7), two invasion-related genes (CTSL2 and STMY3), and three single genes, BAG1, CD68 and GSTM1. Based on the observed distribution of expression among the tumors for each individual gene and group, we determined the range in RSs for low, intermediate and high risk patients and compared these results to those submitted by physicians in the US.
Results: The distribution of the results from EME and the US were consistent. The table shows the distribution of RSs that may be observed for low, intermediate or high risk patients between these two regions.

	EMEA (n = 2676)	US (>90,000)
RS 0−17	51%	52%
RS 18−30	37%	35%
RS >31	12%	13%

Conclusions: The distribution of the RSs in EME is similar to that in the US even though there are differences in practice pattern management of early stage breast cancer. The 21 gene Recurrence Score assay (Oncotype DX®) Oncotype DX breast cancer assay consistently identifies >50% of patients who have a RS <18. This finding is consistent with the results from the validation (Paik et al., NEJM 2004) and confirmatory studies (Habel, BC Res. 2006; Dowsett et al., SABCS 2008). Patients with a RS < 18 have been shown to have minimal, if any, chemotherapy benefit, so this assay has potential clinical utility in EME as it does in the US (Paik et al., JCO, 2006; Albain et al., SABCS 2007).

1333 POSTER

Aberrant expression of ZDHHC14 gene in human tongue squamous cell carcinoma

T. Onda[1], N. Yamamoto[1], T. Yakushiji[1], R. Takagi[1], I. Kamiyama[1], T. Uchiyama[1], N. Takano[1], T. Shibahara[1]. *[1]Tokyo Dental College, Oral and Maxillofacial Surgery, Chiba, Japan*

Background: Molecular alterations in a number of oncogenes and tumor suppressor genes associated with metastasis of cancer could be important clues to predicting and suppressing metastasis. The aim of this study was to identify differentially expressed gene(s) among lymph node-positive (pN(+)) cases and lymph node-negative (pN(−)) cases in tongue squamous cell carcinoma (TSCC).
Patients and Methods: In this study, genetic aberrations and gene expression profiles were examined in 20 cases of primary TSCCs, paired normal oral tissues, 6 TSCC-derived cell lines, and 2 normal oral keratinocytes (NOKs). Whole genome profiling using the Affymetrix 10K SNP Mapping Array was performed on 3 pN(+) cases, 2 pN(−) cases of TSCCs and correspondence to normal tissues. In addition, we also examined mRNA expression level of the candidate gene product identified.
Results: We found that DNA copy number abnormality of chromosome 6q region is associated with metastasis of TSCCs. *ZDHHC14* is on 6q25.3, a region gained in pN(+) cases of TSCCs when compared with pN(−) cases. Quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) showed that *ZDHHC14* was over expressed in all TSCC derived-cell lines when compared with primary cultured NOKs at the mRNA level. Similar to TSCC-derived cell lines, high frequencies of *ZDHHC14* up-regulation were evident in mRNA levels of primary tumors (n = 8/20, 40%). This up-regulation also is closely associated with lymph node status (p = 0.019).
Conclusions: These results suggest that *ZDHHC14* expression may be correlated with lymph node metastasis and offer clues to the planning of new treatments such as early detection, prevention, and therapy for TSCC metastasis.

1334 POSTER

Spleen tyrosine kinase as a novel candidate tumour suppressor gene for human oral squamous cell carcinoma

S. Ogane[1], T. Onda[2], N. Takano[2], T. Yajima[2], T. Uchiyama[2], T. Shibahara[2]. *[1]Kameda Medical Center, Department of Oral and Maxillofacial Surgery, Chiba, Japan; [2]Tokyo Dental College, Department of Oral and Maxillofacial Surgery, Chiba, Japan*

Background: Spleen tyrosine kinase (Syk) is a non-receptor type of protein-tyrosine kinase that is widely expressed in several epithelial cells. We analyzed the mutational and methylation status of the *spleen tyrosine kinase* (*Syk*) gene and both mRNA and protein levels in primary oral squamous cell carcinoma (OSCC) and OSCC-derived cell lines and examined the function of the *Syk* gene in OSCC-derived cell lines in vitro.
Material and Methods: The seven human OSCCs-derived cell lines used in this study were Ca9−22, Ho-1-N-1, HSC-2, Ho-1-u-1, HSC-4, KON and KOSC-2. Primary cultured normal oral keratinocytes (NOKs) were used as a normal control. Tumors with patient-matched normal oral tissues (when