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

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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

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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

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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

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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