of these tumours, PDEC are usually excluded from clinical trials employing the mTOR inhibitor RAD001 and actually their activity in PDEC is tested only in cell lines.
Objective: We conducted a retrospective analyses of PDEC in a mono-institutional series revised according to WHO classification and validated with immunohistochemistry (IHC) for endocrine markers. We also aimed to testing if mTOR is expressed in the human PDEC.
Methods: Between 1984 until 2007, 640 NETs referred to our Institution for diagnosis, treatment and follow-up, of whom 36 (5.6%) were diagnosed as PDEC (excluding SCLC). The staining for mTOR was optimised employing slides of normal kidney as positive control. To ensure antibody specificity, consecutive sections were incubated in the absence of primary antibody. The immunoreactivity was evaluated on a semiquantitative scale considering the extent (score: 0−4) and the intensity (score: 0−3) of staining. The product was used to obtain an immunostaining score (total score 0−12).
Results: At diagnosis gender distribution was 19 males and 17 females and median age 59 years (range, 17−75). The primary site was: pancreas 12 (34%), colon 6 (19%), lung 6 (19%), unknown 5 (14%), small bowel 4 (11%), others 3 (10%) in particular 30 pts (83%) had Stage IV disease while 6 (17%) underwent surgery. The overall survival was 18 months (range, 4−61+). mTOR expression is maintained at similar levels in 80% of samples, with no relationship with tumour origin, function, proliferation rate valued through MIB-1.
Conclusions: In our series PDEC are more frequent and have a longer survival than in the literature. Our biological findings demonstrate expression of mTOR in human PDEC and support an extended analysis in order to understand the role of mTOR and the real activity of RAD001 in PDEC.
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1324 POSTER

Detection of circulating tumour cells in locally advanced colorectal cancer: preliminary data

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Background: Circulating tumor cell (CTC) number at baseline and follow-up has been recently shown as an independent prognostic factor in metastatic colorectal cancer. We seek to investigate the prognostic and predictive role of CTCs in rectal cancer patients undergoing neo-adjuvant chemo-radiotherapy (CX-RT) before curative surgery.
Patients and Methods: In a prospective single-institution study, patients with cT3−4 or N+ rectal cancer staged by transrectal ultrasound and/or pelvic MRI and chest-abdomen CT scan, are submitted to capecitabine (825 mg/mq, orally, twice daily continuous) with concomitant radiotherapy (50.4 Gy/fractions to the primary tumor and perirectal nodes), followed by two cycles of capecitabine (1250 mg/mq, orally, tid 14/21 days). Primary endpoint is evaluation of CTCs at baseline (t0), after neoadjuvant therapy and before surgery (t1), after surgery (t2), and at 6-month follow-up (t3) and its correlation with survival parameters. CTCs counts with immunomagnetic separation in 7.5 ml peripheral blood were performed at the time-points mentioned above (CellSearch System, Veridex Inc).
Results: Twenty-six patients (16 male; 10 female; median age: 63±13 yrs; range: 44−83 years) underwent t0 sampling, 8 pts completed CX-RT and therefore underwent t1 and t2 sampling. At baseline (t0) three patients presented 1 CTC (12%), one 2 CTCs (3.5%), one 27 CTCs (3.5%) while in twenty-one (81%) no CTCs were detected. At t1 and t2 none of the eight pts analyzed showed CTCs. No significant correlation between uTNM at baseline and number of CTCs was found; in addition, among uN0 patients only one resulted to have CTCs.
Conclusions: A CTCs count $\geqslant$1 is found in 15% of our patients, but the sample is too small for statistical analysis. However, furtherdata will allow to determine prognostic and predictive significance of CTCs during treatment in this setting.

1325 POSTER

Regulation of Tissue factor (TF) in colorectal cancer: association with KRAS

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Background: Tumor progression in colorectal cancer is determined by genes such as KRAS, BRAF and p53, but the responsible effector pathways involved are incompletely understood. Tissue factor (TF), a glycoprotein involved in hemostasis, is considered a key driver in cancer-related thrombosis and regulates targets such as prothrombin (T) via protease-activated receptor (PAR). *In vitro* studies in colorectal cancer cell lines have shown that mutated KRAS enhances TF expression and concomitant p53 mutations lead to further TF increase. Moreover serum TF level and activity were correlated with KRAS status of colorectal tumors in an animal model. However whether this mechanism is relevant in patients with colorectal cancer is not known.
Material and Methods: Expression of TF, PAR-1 and T were determined with Affymetrix gene (U133A) chip analysis on a microarray database of colorectal cancer genotyped for KRAS, BRAF and p53. Serum and plasma samples will be prospectively collected in patients with liver-confined metastatic colorectal cancer treated within protocols. Expression analysis was correlated to the underlying mutation of the tumor sample using a modified t-test.
Results: Data on 165 primary colorectal cancer (n: 96 wildtype (wt)-KRAS; n = 52 mutated (mut)-KRAS; n = 17 mut-BRAF) was available. Mut-KRAS was significantly correlated with increase in Tissue factor ($p = 0.027$) and decrease in PAR-1 ($p = 0.037$); prothrombin expression was borderline increased ($p = 0.054$). The information on p53 mutation was only available in 104 primary tumors (34 wt-p53; 70 mut-p53). No increase in TF was observed in patients with mut-p53/wt-KRAS (n = 46); increase in TF was observed when both mut-p53 and mut-KRAS (n = 24) were jointly present but corresponded to the level of TF with k-ras mutation alone.
Conclusion: The correlation of TF and PAR-1 with mutated KRAS may be a new effector pathway of KRAS in humans which could serve as new diagnostic test as well as a new target for future drug development. Correlative studies as well as serum analysis of TF are ongoing to better characterize this association.

1326 POSTER

Immune-regulatory (FoxP3+)-T-cell tumor infiltration status is predictive of benefit from chemo-immunotherapy with gemcitabine, oxaliplatin, 5-FU/FA plus GM-CSF and aldesleukine (GOLFIG) in metastatic colon cancer patients

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Background: GOLFIG is a novel chemo-immunotherapy regimen, combining gemcitabine, oxaliplatin, 5-FU/FA with immunoadjuvant GM-CSF and aldesleukine, which resulted safe and very active in colon cancer patients. Anti-tumor activity and immunity feedback to the treatment resulted strictly correlated. The best outcome was observed in patients showing autoimmunity signs, rise in central-memory-T cells, and decline in peripheral and tumor infiltrating immuno-regulatory T (T_{reg}) cells. On these bases, we investigated a possible correlation between T_{reg} tumor infiltration at diagnosis and clinical outcome of these patients.
Methods: An immunohistochemistry study was carried out to quantify the infiltration of T_{reg} ($FoxP3^+$) lymphocytes in tumor samples of 41 colon cancer patients who received FOLFOX-4 chemotherapy or GOLFIG chemo-immunotherapy as enrolled in the ongoing phase III GOLFIG-2 trial. T_{reg} tumor infiltration score (range 0 to 5) was then correlated with survival (OS) and time to progression (TTP).
Results: A higher T_{reg} tumor infiltration score (score 3−5) was associated to a longer OS and TTP in the whole patient population (high vs low score; TTP = 18 vs 9.4 months; $P = 0.002$; OS = 55.7 vs 28.9 months; $P = 0.001$),

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