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; $P = 0.001$),