Materials and Methods: For this study, we used 4 Head and Neck (H & N) cancer cell lines representative of various localizations: CAL 33 and CAL 27 from base of the tongue, Fadu from the pharynx and SQ20B from the larynx.
Results: Here, using a polyamine-coupled fluorescent probe, we show that the PTS is active in all head and neck cancer cell lines regardless the tumor localization. In these models, flow cytometry demonstrated that the PTS incorporates quickly, massively and specifically the probe into cancer cells. Confocal microscopy observations revealed that the spermine probe accumulates into the cell nuclei, the site of action of F14512 which is a potent topoisomerase II inhibitor. Considering this property, we evaluated the potential of the F14512 (Pierre Fabre laboratories, France) in these H & N cancer cell lines. F14512 contains a PTS-recognized spermine side chain attached to an epipodophyllotoxin moiety targeting topoisomerase II. We found that F14512 presents a much higher cytotoxicity than etoposide in the 4 cell lines. Competition assays showed that this effect is dependent of the PTS activity and confirmed the targeted action of F14512 against cells with active PTS.
Conclusion: The high efficiency of F14512 in the head and neck cancer cell lines is reported here for the first time and may be of interest for the future development of this novel drug candidate, currently in phase 1 clinical trial in leukemia. Studies are in progress, using fresh tumor biopsies from patients with head and neck cancer, to analyze the PTS status of the tumors using the specific spermine-containing fluorescent probe and to evaluate the activity of F14512.

PP 49

Prognostic value of GLUT1 and MCT4 expression in adeno- and squamous cell non-small cell lung cancer

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Background: Hypoxia leads to changes in tumor cell metabolism such as increased glycolysis. Markers related to hypoxia and glycolysis could be prognostic indicators in non-small cell lung cancer (NSCLC). In this study, glucose transporter 1 (GLUT1) and monocarboxylate transporter 4 (MCT4) expression were correlated with survival in stage I, II and resectable stage IIIA NSCLC.
Materials and Methods: GLUT1 and MCT4 expression were determined in 91 NSCLC fresh frozen biopsies using immunohistochemical techniques and a computerized image analysis system. Markers were analyzed for adenocarcinomas (n = 41) and squamous cell carcinomas (n = 35) separately. Eighty-five patients were retrospectively evaluated for relapse and survival.
Results: Squamous cell carcinomas demonstrated higher GLUT1 expression, relative to adenocarcinomas. Also, in squamous cell carcinomas, GLUT1 and MCT4 expression increased with increasing distance from the vasculature, whereas in adenocarcinomas upregulation of MCT4 was already found at closer distance from vessels. In adenocarcinomas, high GLUT1 expression correlated with a poor differentiation grade and positive lymph nodes at diagnosis. High GLUT1 plus high MCT4 expression was associated with a poor disease-specific survival in adenocarcinomas ($p = 0.032$).
Conclusion: A different tumor cell metabolism was found for adenocarcinomas and squamous cell carcinomas. Adenocarcinomas may use aerobic glycolysis as a primary energy source, whereas the metabolism of squamous cell carcinomas seems to rely on mitochondrial oxidation with anaerobic glycolysis in case of limited availability of oxygen. High GLUT1 plus high MCT4 expression indicated an aggressive tumor behavior in adenocarcinomas. This subgroup of tumors may benefit from new treatment approaches, such as MCT4 inhibitors.

PP 104

Stroma production within the primary tumor correlates with poor survival for stage I−II colon cancer patients

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Background: Recent models on metastatic invasion focus on the tumor-"host" interface, in particular the role of the stromal tissue. There is a strong emphasis that CAF's (cancer-associated fibroblasts) are important promotors for tumor growth and progression. We anticipate that changes in the proportion of stroma in the primary tumor reflect progression. The intra-tumor stroma percentage has previously been reported by our group as a strong independent prognostic parameter. CRC patients with a high stroma percentage within the primary tumor have a poorer prognosis. Validation of this parameter has been tested in a cohort of patients from the VICTOR trial (Vioxx in colorectal cancer therapy: definition of optimal regime as anticancer intervention involving selective COX-2 inhibitors).
Materials and Methods: Tissue samples from 710 patients participating in the VICTOR trial were analyzed for their stroma percentage using conventional microscopy. Each sample was analyzed by two individual observers in a blinded manner. Tissue samples consisted of 5 μm Haematoxylin and Eosin (H & E) stained sections from the most invasive part of the primary tumor. Stroma-high (>50% stroma) and stroma-low (⩽50% stroma) groups were evaluated with respect to survival time.
Results: OS and DFS were lower in the stroma-high population (OS $p < 0.0001$, HR = 1.96; DFS $p < 0.0001$, HR = 2.15). Within the total patient population the five year OS was 69.0% versus 83.4% and DFS 58.6% versus 77.3% for stroma-high versus stroma-low patients. For patients with stage II CRC, OS and DFS were also lower for the stroma-high group (OS $p = 0.034$, H = 1.95; DFS $p = 0.005$, HR = 2.04). The 5 year OS for this group was 79.8% versus 89.1% and for DFS 71.1% versus 83.3% for stroma-high versus stroma-low patients. Within the stage III CRC group, 5 year OS of 61.7% versus 76.1% was observed and for DFS 50.2% versus 69.4% (OS $p = 0.019$, HR =1.61; DFS $p < 0.0001$, HR = 1.86) for stroma-high versus stroma-low patients. Results of the Quasar II with randomized treatment with Bevacizumab are currently under evaluation but will be presented at the conference.
Conclusion: This study validates the intra-tumor stroma ratio as an independent prognostic factor of CRC in an independent patient series. Patients with a high intra-tumor stroma percentage have a poorer prognosis. This parameter could be a valuable addition to current high-risk parameters such as TNM-status and MSI status used in routine pathology reporting.

PP 20

Methylation profile and chemoradioresistance in rectal cancer

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Background: Although neoadjuvant chemoradiotherapy (NCRT) in rectal cancer represents the gold standard for clinical practice, more than one third of patients do not respond. Epigenetic aberrations, such as DNA methylation, have been shown to play a role in rectal cancer progression and prognosis. The present study aimed to analyze the potential of specific gene hypermethylation in predicting resistance or sensitivity to NCRT in order to optimize therapeutic strategies.
Materials and Methods: Fifty candidates for NCRT were recruited, and pretreatment paraffin-embedded biopsies from all cases were analyzed by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA). A probemix containing 26 probes was used to detect the methylation status of promoter regions of 24 different tumor suppressor genes. Methylation status was analyzed in relation to pathologic response evaluated by tumor regression grade (TRG), according to Dworak criteria.
Results: Frequent high methylation was observed for six sites (ESR1, CDH13, CDKN2B, RARB, IGSF4, APC), but no correlation with TRG was found. Conversely, interesting results emerged for CHFR and BRCA2 gene methylation. In particular, low levels of CHFR and high levels of BRCA2 methylation, which characterized about 25% of the entire study population, were indicative of clinical response in 75% of cases. The inverse profile, which included another 25% of the population, was associated with clinical resistance in 91% of cases.
Conclusion: The results from the present study suggest that quantitative epigenetic classification of rectal cancer by MS-MLPA could be useful in predicting radiochemosensitivity or resistance. In particular, methylation status of CHFR and BRCA2 proved indicative of sensitivity or resistance to NCRT in about 50% of the overall population. Further studies are ongoing to confirm these preliminary findings.

PP 57

Interaction of 4-demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) with melanoma melanin metabolism and cell death

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Background: DM-CHOC-PEN is a polychlorinated pyridine cholesteryl carbonate, which is in Phase I clinical trials in patients with advanced cancer − IND 68,876. DM-CHOC-PEN is an active and stable member of a large series of carbonates with improved activity in intracranially (IC) implanted human xenograft models − U251 and D54 glioma and MX-1 breast cancer (CCP, 64, 829, 2009). B-16 melanoma was evaluated in vitro and in vivo for sensitivity to DM-CHOC-PEN and a novel drug impact on DOPA oxidase − a potential tumor marker, is reported here.
Materials and Methods: B-16 melanoma cells were cultured using RPMI media with 5% FBS and pen/strep @ 37ºC in a CO2 incubator. Drugs were