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

added to the cells in a growth phase and after 16 hrs removed. Adult C57BL mice in groups of 5−6 female mice with measurable SC growing B-16 melanoma nodules were dosed IP daily (150–200 mg/kg) for 5 days with DM-CHOC-PEN and monitored daily until death or moribund and sacrificed. Temozolamide (TMZ) was used as control.
Results: In vitro, DM-CHOC-PEN had an IC50 of 0.5 μg/mL vs. B-16 melanoma cells. Floating heavily melanotic cells that formed were separated, analyzed for DM-CHOC-PEN and found to contain 125% more drug than did the adhered amelanotic cells. For the in vivo studies, T-C for mice bearing B-16 melanoma treated with DM-CHOC-PEN vs. controls was 60−82%; thus supporting the in vitro observations. For TMZ, the T-C was 78%.
Conclusion: Electronic modeling studies support DM-CHOC-PEN's ability to act as a pyridinium co-factor in the transfer of electrons from DOPA to the intermediary metabolism pool. Previously, we reported that dacarbazine inhibited DOPA oxidase and melanin formation in melanoma pts, resulting in amelanotic melanomas; often considered a more aggressive variant (Pigment Cell 2, 327–338, 1976). Although tyrosine-DOPA transport/metabolism is not a target for DM-CHOC-PEN (its MOA is considered to be via alkylation/adduct formation with N7-guanine), the accumulation of intracellular melanin does influence/interfere with cellular metabolism. In the current Phase I study, pts with melanoma lesions will be biopsied for DM-CHOC-PEN content and tumor tissue DOPA oxidase activity/melanin content when possible. Early results from the Phase I trial will be included with this presentation; no toxicity has been observed to date. A possible role of DOPA oxidase in drug selection to treat melanoma will be discussed. Supported by NCI/SBIR grants − CA85021 and CA132257.

PP 73

Prognostic relevance of constitutive expression of γ-H2AX in triple negative breast cancers

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Background: Constitutive expression of γ-H2AX, a key indicator of double stranded DNA breaks, is found in a number of cancers but not in normal tissues. The DNA damage repair (DDR) pathway may be disrupted in these cancers, resulting in a higher risk of unrepaired lesions even in the absence of DNA damage inducing therapy such as radiotherapy. Data from recent studies indicate that the endogenous expression of DNA damage response factors may also be associated with damaged telomeres. Here, we quantified constitutive γ-H2AX expression in a large number of breast cancer cell lines (n = 54) and in a cohort of human breast cancers (n = 122) enriched for triple negative tumors.
Materials and Methods: Formalin fixed paraffin embedded breast cancer cell lines and tumors were immunohistochemically analyzed for expression of γ-H2AX and its downstream factor 53BP1.
Results: Expression of γ-H2AX was assessed in 54 different breast cell lines, embedded in triplicate in a cell line microarray and correlated with estrogen receptor (ER), progesterone receptor (PR) and HER2 receptor status, mutation status and breast cancer subtype. We found that triple-negative cell lines, BRCA1 mutated cell lines and basal-like tumors exhibited the most γ-H2AX foci. A borderline significant association with p53 status was found. Next, a tissue microarray of a cohort of 122 node-negative, non-adjuvantly treated breast cancer patients was stained for γ-H2AX. No direct correlation with triple negativity was found in these patients. However, in the triple negative breast cancer patients a high number of γ-H2AX foci had a significantly worse prognosis ($p = 0.006$ for triple negative (n = 42) vs. $p = 0.417$ for ER, PR or HER2 positive (n = 54) patients). A similar association with survival was found for 53BP1 and γ-H2AX and 53BP1 combined.
Conclusion: Enhanced endogenous γ-H2AX and 53BP1 expression, indicative of malfunctioning DNA repair, reveals a subset of patients with triple negative breast tumors that have a significantly poorer prognosis. We are currently assessing whether this specific subset of patients exhibits defects in the DDR pathway, e.g. BRCA1 and/or p53 mutations, or have damaged telomeres, which would explain the prognostic association.

PP 31

INT70/09 Phase II study of Pazopanib (PZP) monotherapy for patients (pts) with relapsed/refractory urothelial cancer (UC): updated results of a proof-of-concept trial

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Background: Discouraging results have been reported in relapsed/refractory UC with the use of salvage therapies (Rx). In 2nd line setting, median PFS and OS approximate 3 and 6 months (mos), respectively. On 10/2010 we reported preliminary, yet encouraging, results of a phase II trial with PZP, a multitargeted drug with distinct anti-angiogenic activity (ESMO 2010, LBA#23). An update of the trial is presented.
Materials and Methods: Eligibility included histologically confirmed UC failing ⩾1 CDDP-based Rx for metastatic disease. PZP 800 mg once daily until disease progression or unacceptable toxicity was planned. Both CT scan and PET/CT scan were set at baseline and q4weeks thereafter. RECIST v.1.1 response-rate (RR) was the primary endpoint. Circulating VEGF, VEGFR1−2, KIT, HGF, IL4−8−12, OPN, TIMP-1 as well as SNPs of 13 candidate genes and circulating tumor cell analysis were planned.
Results: 36 of a total planned of 41 pts were enrolled from 02/10 to 03/11 (28 males, 8 females). Median age was 64 yrs (42−79). 13 pts (36%) had UC of the upper urinary tract and 23 had a bladder primary tumor. 33 pts had multiple disease sites (median 3, range 1−5). Median number of prior cytotoxic agents was 3 (2−8), of prior Rx lines was 2 (1−4). 30 pts (83%) had visceral metastases (hepatic in 17 pts). Median ECOG PS was 1 (0−2). 4 pts (11%) had a confirmed RECIST-defined partial response (PR), 26 had a stable disease (83% clinical benefit). 19 pts (53%) had a clear necrotic evolution of multiple metastases and/or a decreased SUV at PET consistent with PR. Of the 34/36 pts having 2 mos minimum follow up, median PFS and OS were 3 mos (1−11) and 6 mos (2−11), respectively. 5/36 (14%) had a very long-term PFS (> 10 mos). G3 hypertension occurred in 2 pts, G1−2 asthenia in 13, diarrhoea in 5, anemia and hand-foot syndrome in 3 pts each. No discontinuations/dose reductions were needed.
Conclusion: This is the first report of a consistent activity and potential efficacy of a targeted agent in UC. Though the PR-rate by RECIST is low, half of pts had a densitometric/metabolic response, the majority of pts had a clinical benefit and PFS-rate is promising (approaching pure 2nd line results). Results of biomarker analysis will be available in Sept 2011 and may help to personalize treatment and to corroborate new response criteria to angiogenesis inhibitors. This proof-of-concept trial is part of a multi-targeting platform aimed at elucidating the role of microenvironment in UC.

PP 32

Pilot study of cisplatin, 5-fluorouracil and a taxane (TPF) in patients (pts) with advanced squamous-cell carcinoma (SCC) of the penis: results from a single-institution series

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Background: Few data indicate poor to moderate activity of chemotherapy in advanced penile SCC, and no definitive acquisition is available concerning timing for integrated surgery. Pts with metastatic bilateral or pelvic nodes show an overall survival (OS) of 15% and less than 10%, respectively. We evaluated TPF in either neoadjuvant (NA), adjuvant (A) or metastatic (M) setting in a single-center pilot trial.
Materials and Methods: 3−4 courses of paclitaxel 120 mg/m2 d1 or docetaxel 75 mg/m2 d1 + cisplatin 75 mg/m2 d1 + 5-FU 750 mg/m2 96 hrs continuous infusion from d1, q3wks were provided. Primary endpoint (EP) was progression-free survival (PFS). Safety profile, response rate (RR) and OS were the secondary EPs. Immunostaining for p53, p16, p63, EGFR, HER2/neu and mutational analysis of TP53 were planned on available tissue.
Results: From 7/2004 to 03/2011, 46 consecutive pts were treated, 40/46 evaluable for response and outcome. 8 pts underwent paclitaxel-PF and 32 docetaxel-PF. Grade ⩾ 3 hematologic toxicity was observed in 4 pts, grade ⩾ 3 renal and neurotoxicity occurred in 1 pt each. Median PFS and OS in the whole series were 6 (1−73) and 9.5 mos (1−73) respectively. Positive p53 staining significantly associated with better OS and PFS at univariate analysis (Log-Rank test $p = 0.0421$ and $p = 0.0483$, respectively). Adjuvant setting: 17 pts (4 bilateral pN+ and 11 pelvic pN+) underwent adjuvant TPF. Median PFS and OS were 10 mos (1−73) and 13 mos (1−73). 10 pts (59%) were alive with 17 mos (1−73) of median follow-up (f-u). Neoadjuvant setting: 16 pts with cN2/3 SCC (9 cN3) were treated, either at diagnosis (11) or following recurrence after prior lymphadenectomy (5). Median PFS was 4 mos (1−46). 3 pts achieved a complete response (CR) and 6 pts achieved a partial response (PR, RR = 62%). OS was 5 mos (3−46). 11/16 pts underwent surgery that was radical in 9 (82%). 3 pathologic-CR (27%) have been achieved. 8 pts (50%) were alive with a median f-u of 9 mos (3−46). Metastatic setting: 7 pts were treated. 2 had a PR and 1 a SD that lasted a median of 5 mos (3−8), and all died of disease. Median PFS and OS were 2 mos (1−8) and 5 mos (2−12).
Conclusion: Perioperative TPF was effective in advanced penile SCC, either in A or NA setting. It deserves further investigation including earlier stages (probably all cN+). For the first time a prognostic value of p53 has been reported in penile SCC. Mature results on the predictive role of biomarkers will be available in Sept 2011.