and clinical parameters (such as bone metastases, pain and quality of life), circulating bone remodeling (skeletal) parameters (alkaline phosphatase, C- and N-terminal fragments of type I collagen, osteocalcin, Vitamin D), inflammatory (IL-8 and TNF-alpha) and metabolic parameters (BMI, serum cholesterol and triglycerides).
Materials and Methods: From April 2010 to August 2010, we enrolled 33 metastatic cancer patients with tumors at different sites (M/F: 16/17, mean age 66 years): 17 patients with bone metastases, 16 with metastases not involving bone. Comparison between groups (controls vs cancer patients and cancer patients with vs without bone metastases) was performed by two-sided Student's t test. Correlation between OPN/SPARC and the other variables was performed by Spearman's correlation analysis.
Results: OPN and SPARC in cancer patients were significantly higher compared to controls but did not differ between patients with or without bone metastases. OPN showed a positive significant correlation with C and N terminal fragments of type I collagen ($r = 0.390$ and $r = 0.410$, $p = 0.024$ for both), IL-8 ($r = 0.390$, $p = 0.034$) and a negative significant correlation with quality of life ($r = -0.400$, $p = 0.025$) and BMI ($r = -0.300$, $p = 0.046$). SPARC showed a positive significant correlation with BMI ($r = 0,360$, $p = 0.049$). Moreover, patients with 3 month survival (613.7 ± 229.2 ng/ml versus 195.8 ± 165 ng/ml, $p < 0.001$).
Conclusion: The results of the present study show that high OPN levels are associated with poor survival in advanced cancer patients. Further studies are warranted to assess the role of OPN and SPARC to both monitor the effects of antineoplastic regimens and to assess them as potential targets of new treatment strategies.

PP 78

Role of mucin3 gene in intrinsic resistance to oxaliplatin

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Background: Although representing a milestone in the treatment of colorectal cancer (CRC) little is known about the mechanisms responsible for intrinsic resistance to oxaliplatin (OXA). We previously identified a set of genes whose expression was associated with intrinsic resistance to OXA in a panel of 14 CRC human cell lines. The aim of this work was to validate these findings in order to typify genes that could play a role as predictive markers of response to OXA-based treatment in CRC patients.
Materials and Methods: Candidate genes selected in a previous microarray analysis were validated by qRTPCR with specific Taqman® Assays in 14 CRC cell lines comparing resistant ($R = IC50 > 1\ \mu M$) versus sensitive ($S = IC50 < 1\ \mu M$) groups. MTT assay was used to establish OXA, SN38, cisplatin and 5-fluorouracil sensitivity profiles. Genes showing statistically significant differences between both OXA sensitivity groups (U-Mann–Whitney) were considered as positively validated. MUC3A gene methylation status was analyzed by bisulphite treatment and PCR and correlated with mRNA expression results.
Results: Expression levels of 6 (MUC3, ABHD3, ERN1, JARID2, MPP6, TNFSF13) out of 16 genes were analysed by qRTPCR. Only Mucin3 (MUC3A and B) was positively validated so that as compared with S cells, R cells expressed very low levels of MUC3 A and B (MUC3A mean 4.89 ± 2.91 and $0.97 \pm 1.11$for S and R cells respectively, $p = 0.01$; MUC3B mean 8.52 ± 5.49 and 1.47 ± 1.76 $p = 0.008$). These data correlated with that obtained from the microarray (Rho-Spearman: $R=0.7$ $p = 0.006$). This feature was OXA-specific since we did not observe cross-resistance with other drugs. We only observed a slightly correlation with gene methylation status in those cells with the lowest and highest values of MUC3A expression. In a small sample ($N = 22$) of paraffin-embebbed tumors from CRC patients treated with first line OXA-based chemotherapy MUC3A and B overexpression was associated with response to treatment (86.7% of responders had expression levels >33% percentile; Fisher's $p = 0.12$; OR 3.75).
Conclusion: MUC3 A and B gene down-regulation is associated with intrinsic resistance to OXA both in vitro and in CRC patients. We could not demonstrate that regulation of MUC3A expression was dependent on methylation status. Although MUC3A and B are potential predictive markers of OXA response in CRC patients, functional studies are being conducted in order to elucidate the molecular mechanisms linking them with OXA intrinsic resistance and their potential role in CRC treatment selection.

PP 90

Glioma cell motility is modulated by CXCL12/CXCR4

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Background: Gliomas are brain tumours that account for more than 50% of the tumors that arise within the central nervous system. They are higly proliferative, angiogenic and locally very invasive. Despite the considerable advances in the knowledge of the mechanisms underlying the genetics, biology and clinical behavior, GBM pathogenesis is not completely understood and until now, there is not a therapeutic strategy to reduce the invasive and proliferative ability of the glioma cells. One of the therapeutic targets currently studied is the chemokine receptors. CXCR4 is the cell-surface receptor of CXCL12 also named stromal-derived stromal factor 1 and was associated to the tumorigenesis process in breast, prostate, kidney and brain. In order to understand the role of CXCL12/CXCR4 in gliomas we studied the survival and motility of glioma cells treated with AMD3100, an antagonist of the CXCR4.
Materials and Methods: We used the U-118 glioma cell line. The assays were performed in the presence and/or absence of CXCL12, and AMD3100. CXCR4 expression in glioma cells was evaluated by western blot and immunofluorescence. Cell survival was evaluated by flow cytometry. Cell migration study was performed using the scratch assay. Cytoskeleton organization was analysed using phalloidin.
Results: Our results showed that CXCR4 is expressed in the U-118 cell line. AMD3100, the CXCR4 antagonist, induces disruption of the cytoskeleton and a significant reduction of the migration and invasion ability of the glioma cells. The study of cell survival demonstrated that the inhibition of CXCR4 activity induces a decrease in cell survival.
Conclusion: Taking these results altogether it is possible to conclude that the activation of CXCR4 by CXCL12 promoted proliferation, survival, and migration of U-118 glioma cells, confirming that CXCR4/CXCL12 signaling pathway may contribute for the growth and invasive characteristics of GBM. In addition, it was demonstrated that the inhibition of the CXCR4/CXCL12 pathway by AMD3100, significantly inhibited the survival and motility of the glioma cells, indicating that the effect of AMD3100 should be confirmed in vivo in order to evaluate the potential of CXCR4 as a therapeutic target in the GBM treatment.

PP 74

A subtype representing activated ATM signalling in PTEN-null tumours

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Background: PTEN is frequently lost in cancer cells through genetic mutation or epigenetic silencing. Loss of PTEN function has been widely reported to cause up-regulation of the PI3K/AKT signalling pathway resulting in increased cell growth, proliferation and survival. More recently it has been reported that PTEN null cells demonstrate genomic instability through increased ROS and oxidative stress induced DNA damage. The aim of this study was to identify a biomarker for PTEN status in human cancers.
Materials and Methods: A metagene representing ATM activation was generated from public cell line data of AT fibroblasts treated with gamma-irradiation. This was used to perform hierarchical clustering analysis of a public DNA microarray profiling dataset with known PTEN IHC status. The metagene was validated in PTEN wildtype and null cancer cell lines.
Results: We found that PTEN null cells have elevated levels of ROS and furthermore activation of the DNA damage signalling kinase, ATM. In agreement with this, the ATM metagene signature correlated with PTEN mutation in breast cancer tumours. Scoring of PTEN wildtype and null cancer cell lines from various tissues using the metagene correlated with ATM activation and sensitivity to inhibition of ATM. Furthermore we show that inhibition of ATM caused DNA damage, cell cycle arrest and apoptosis in PTEN deficient cells suggesting a novel therapeutic strategy.
Conclusion: These observations suggest that ATM may represent a therapeutic target in PTEN deficient tumours and furthermore ATM activation may also be an important biomarker of PTEN mutation or loss.

PP 94

Polyamine Transport System (PTS) activity and hijacking in cancer cells: new option in Head and Neck tumors treatment with the polyamine-containing drug candidate F14512

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Background: The Polyamine Transport System, although not clearly identified at the molecular level in eukaryotic organisms, was found over activated in many types of cancer cells, such as leukemia, prostate, melanoma and NSCLC. Polyamines are implicated in many biological functions, and the need for polyamines in tumor cells, conveyed by the PTS, is crucial. New therapeutic strategies consist to use this transport system to deliver a cytotoxic agent specifically into cancer cells. Head and neck cancer remains the 6th common cancer with a very poor survival rate indicating the crucial need for new targeted strategies.

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