this strategy, we intend to enhance the efficiency of cancer therapies, and cover up possible resistance to individual treatments. Studies are in progress to assess the in vitro efficacy of these combined treatments. In parallel, we perform preclinical trials to investigate the importance of these mechanisms for tumor formation and metastasis in vivo. The efficacy of these treatments is first investigated in chicken metastasis assays. Besides, we have generated transgenic mice over-expressing Met in a temporally and spatially regulated manner. Met-overexpressing cells also express the luciferase, for non-invasive monitoring of primary tumours and metastasis. These mice are precious tools to better understand Met-triggered tumorigenesis and validate the efficiency of combined treatment to un-favour cell survival in Met-triggered cancers.

Altogether, by targetting Met activity and its downstream survival signals, we will elucidate the efficacy of novel combined anticancer therapies.

291 Poster

A standardized approach to animal study data collection, analysis and management practices expands possibilities for the sharing of results

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The lack of data digitization and a standardized approach to data collection, analysis, content, format and storage conventions makes animal study (AS) data access and cross-study comparisons impractical within and across research organizations. Current methods of AS data collection, analysis and management for animal studies in oncology are highly variable and primarily manually-based, using combinations of paper notebooks and electronic spreadsheets. As a consequence, data cannot be accessed and shared effectively because: 1) data recorded in lab notebooks remain un-indexed and inaccessible once collected; 2) spreadsheets often contain only primary measurement data and are stored in data silos, 3) AS conditions, methods and model details are recorded inconsistently, in insufficient detail, and reside in multiple locations and formats. The unintended consequence is a systematic, unintentional, and yet preventable waste of data, information, organizational knowledge, time and resources. Continued improvements in in vivo modeling result in an ever-increasing amount of information which is becoming increasingly difficult to manage. A growing number of scientists are finding that commercial AS management software applications provide a standardized approach to managing this information which reflects industry-wide best practices. These software systems also improve process efficiency, data integrity and security, and data accessibility as well as increase the transparency of study data and processes, facilitate personnel oversight, IACUC compliance, and inter-study comparability. Standardization of data into a single, digital form would enable the creation of a centralized, web-based study repository for the voluntary sharing and pooling of AS data from many labs, which would give investigators, journals, and institutions the opportunity to publish their both negative and positive non-proprietary results. By having the details on historical studies available, researchers could obviate needless repetition of studies, reduce the number of animals used, expedite model implementation projects, refine current animal models and build on existing work. The net result would be a significant improvement in information sharing, much more efficient use of limited research resources and ultimately a decreased time to identify, develop and market new therapeutics for cancer.

292 Poster

ZNF23 induces apoptosis in human ovarian cancer cells

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The Krupple-associated box-containing zinc finger proteins (KRAB-ZFPs) constitute one of the largest families of transcription factors. Some members of this family play critical roles in regulation of development, cell proliferation and apoptosis. Many KRAB-ZFPs are implicated in malignancies. Previously, we cloned and characterized ZNF23, which was a member of this family. Its gene localized to the 16q22, a chromosomal region frequently altered in solid tumors. ZNF23 expression was reduced in human cancers and ectopic expression of ZNF23 inhibited cell growth by inducing cell cycle arrest. Here we showed that in ovarian cancer cells, ZNF23 expression also induced apoptosis. The protein level of ZNF23 was greatly down-regulated in 20 samples of ovarian tumors compared with that in 13 samples of normal ovarian tissues. In ovarian cancer cell line SKOV-3, restoration of ZNF23 expression led to apoptosis as demonstrated by activation of caspase-3, nuclear condensation and formation of a sub-G1 peak. This apoptotic process was accompanied by loss of mitochondrial membrane potential, cytochrome c release and caspase-9 activation. Furthermore, ZNF23-induced apoptosis was partially through down-regulation of Bcl-XL. Thus, our study suggested that ZNF23 also played an important role in the induction of apoptotic cell death, and pointed to the possibility that its down-regulation might render ovarian cancers to an increased survival capacity.

293 Poster

Endothelial cell KIT expression in pediatric brain tumors

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Background: Receptor tyrosine kinases expressed in vascular endothelial cells are potential targets for therapy with tyrosine kinase inhibitors. KIT (receptor of the stem cell factor, SCF) has recently been found in tumor vessel endothelial cells of glioblastomas. It is not known whether KIT is expressed in endothelial cells of pediatric brain tumors, and its role in tumor angiogenesis is unknown.

Methods: We evaluated KIT, phosphorylated (p-KIT), SCF, and VEGFR-2 (vascular endothelial growth receptor factor-2) expression in a series of 69 pediatric brain tumors (35 pilocytic astrocytomas, 12 medulloblastomas, 11 ependymomas, 7 dysembryoblastic neuroepithelial tumors (DNTs), and 4 gangliogliomas) using immunohistochemistry. The median age at the time of the diagnosis was 9 yrs (range, from 0 to 20). PDGFRA (platelet derived growth factor receptor alpha), KIT, VEGFR2, and EGFR (epidermal growth factor receptor) gene copy numbers were determined using chromogenic in situ hybridization.

Results: Marked endothelial cell KIT expression was found in 13 (37%) of 35 pilocytic astrocytomas, 6 (55%) ependymomas, and 1 (8%) medulloblastoma, whereas none of the DNTs or gangliogliomas showed marked expression. Twelve (60%) of the 20 tumors with marked expression expressed also markedly p-KIT as compared to only 7 (14%) of the 49 tumors that lacked marked KIT expression (P =.0001, chi-square test). Endothelial cell KIT expression was associated with age at diagnosis in the largest histological subset (pilocytic astrocytoma), where 11 (69%) of the patents aged 8 or less had marked endothelial cell expression as compared to only 2 (11%) of those older than 8 (P =.0004). In the entire series 16 (53%) of patients < 8 at diagnosis had marked KIT expression in tumor endothelial vessels as compared to 4 (10%) among those > 8 (P <.0001). No KIT, PDGFRA, VEGFR2, or EGFR gene amplifications were detected in the endothelial cells of any of the tumors.

Conclusions: Endothelial cells of pediatric brain tumors often express KIT that is activated (phosphorylated), whereas marked VEGFR-2 expression is rare. Patients diagnosed with pilocytic astrocytoma at a young age often have a tumor with a marked endothelial cell expression of KIT, whereas such ex-pression is usually not found in tumor vessels of pilocytic astrocytomas detected at an older age. These findings suggests that angiogenesis of pilocytic astrocytomas of young children may differ from that of older ones.

294 Poster

Heterocyclic thiosemicarbazones and their Zinc(II) complexes inhibited proliferation of four different neoplastic cell lines

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Background: It has been reported that thiosemicarbazones (TSCs) are potent antitumor agents. This class of compounds can suppress tumor growth by inhibiting the bioactivity of ribonucleotide reductase (RNR). RNR is the rate-limiting enzyme in DNA synthesis due to its low abundance in normal cells. In comparison with several other key enzymes, RNR shows the greatest increase in activity in tumor cells and, therefore, RNR is considered an important intracellular target for inhibiting cellular proliferation. The aim of this work was to asses the antiproliferative action of some newly synthesized heterocyclic thiosemicarbazones and their zinc (II) complexes, on four human neoplastic cell lines: human cervix carcinoma (HeLa), chronic myelogenous leukemia (K562), and breast carcinoma (MDA-MB-453, and MDA-MB-361).

Methods: A new series of thiosemicarbazones (TSCs) and Zinc(II) complexes were synthesized. The structures of all the compounds were determined by analytical and spectral (1H-NMR,13C-NMR, IR, MS and

XRF). methods. The compounds were incubated with target cells for 72 h. At the end of this incubation period, antiproliferative activity in vitro was determined by the MTT assay. In this work we have analysed the cell cycle phase distribution of compounds treated (2IC50) and untreated malignant cells, 24h, 48h, and 72h, after the treatment. After cell staining with propidium iodide cells were analyzed using a Becton Dickinson FAC-Scan flow cytometer.

Results: Results showed that the ligand as well as the complexes demonstrated excellent antiproliferative activity (IC50 values in range of nM, respectively) against all cell lines tested. Flow cytometric analysis showed that apoptosis was mostly induced in K562, HeLa, and MDA-MB-453 cells; MDA-MB-361 cells were with moderate sensitivity to the apoptotic action of compounds but, with marked increase in the percent of these cells in G1 phase.

Conclusion: This examination clearly indicated that thiosemicarbazones and their Zinc(II) complexes have a pronounced antitumor activity on the four neoplastic cell lines.

295 Poster

Synthesis and primary cytotoxicity evaluation of non-heterocyclic thiosemicarbazones and their metal complexes

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Background: A new series of thiosemicarbazones (TSCs) and metal complexes were synthesized as potential antitumor agents. The aim of this work was to test the cytotoxicity of this compounds on different human tumour cell lines.

Methods: The compounds have been identified by elementar analysis and spectroscopic techniques (1H-NMR, 13C-NMR, IR, MS and XRF). In vitro cytotoxicity of the thiosemicarbazones and complexes was tested by MTT assay against the target cells: human cervix carcinoma (HeLa), chronic myelogenous leukemia (K562), breast carcinoma (MDA-MB-453) and breast adenocarcinoma (MDA-MB-361). Compounds solusions were added to neoplastic cells grown in 96 flat bottomed wells, 20h after cell seeding. Cell survival was determined 72h after the continuous agent action.

Results: Compouds H2SalpipF, H2AppipF and its zinc(II) complexes were evaluated and all the compounds demonstrated the most marked effects towards used target cell lines.

H2AppipF exhibited potent cytotoxic activity against chronic myelogenous leukemia (K562), (IC50=0.76 microM) and cervix carcinoma (HeLa), (IC50=0.89 microM). The complexes of TSCs showed significant improvement in cytotoxic activity against all cell lines (compound Zn(HAppipF)2; IC50=0.24-0.8 μM), and breast carcinoma (MDA-MB-453), (compound Zn(HSalpipF)2; IC50=0.79 μM).

Conclusion: This new group of compounds may offer novel exploratory derivatives for future investigations in the treatment of cancer.

296 Poster

Nuclear c-Met and p-p38 at the invasive front of oral squamous cell carcinomas

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Background: The c-Met receptor tyrosine kinase is the receptor for hepatocyte growth factor. C-Met promotes cell motility, invasion and survival. Some studies report of nuclear c-Met expression, but the signalling outcome of the c-Met location is unknown. In the present study, we wanted to evaluate the nuclear expression of c-Met at the invasive front of oral squamous cell carcinomas (OSCCs). Furthermore, in an initial attempt to understand how other molecules e.g. activated p38 MAP kinase (p-p38), can influence the nuclear expression of c-Met in oral cancer, a 3D-co-culture organotypic oral cancer (OTOC) model was constructed.

Material and methods: By immunohistochemistry (IHC), the presence of nuclear c-Met and p-p38 was studied at the invasive front of 51 T1-T2 OSCCs. Furthermore, the nuclear expression of c-Met and p-p38 was studied in an OTOC model. In addition, the nuclear expression of c-Met was evaluated by IHC in OTOC models treated with a p38 inhibitor (SB203580).

Results: At the invasive front, 26 of the tumours showed nuclear c-Met expression while 25 tumours did not demonstrate nuclear c-Met. Thirty-five tumours demonstrated nuclear p-p38 staining at the invasive front while 16 tumours did not show nuclear p-p38. At the invasive front we found that when nuclear c-met was expressed in a high number of cells, the nuclear expression of p-p38 was much lower and vice versa (p=0.004). Inhibition of p38 in the OTOC model resulted in an increased number of cells with nuclear c-Met compared to the model without inhibition.

Conclusion: Nuclear c-Met and nuclear p-p38 was present in OSCCs. Either the expression of nuclear c-Met or nuclear p-p38 dominated at the invasive front. Inhibition of p38 increased the number of cancer cells expressing nuclear c-Met in the OTOC model. Our results suggest an association between c-Met and p-p38.

297 Poster

p75NTR and pattern of invasion predict poor prognosis in oral squamous cell carcinomas

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Background: The high rate of treatment failure for patients with oral squamous cell carcinomas (OSCCs) indicates the need for better prognostic markers to identify tumours not responding to today's treatment. Thus, studies of the biological characteristics in OSCCs are of importance, also with regard to development of new treatment approaches.

Material and methods: In 53 T1-T2 OSCCs we evaluated the prognostic value of p75 neurotrophin receptor ($p75^{NTR}$) known to induce both cell survival and cell death. The results were related to conventional prognostic systems (TNM staging and WHO grading) and invasive front grading/IFG. Immunohistochemically, we assessed the expression of $p75^{NTR}$ both in central/superficial tumour parts and at the invasive front in OSCCs. Hematoxylin and eosin stained sections were graded according to WHO and IFG. Endpoint was disease-free survival.

Results: All tumours expressed $p75^{NTR}$. $p75^{NTR}$ both in central/superficial tumour areas and at the invasive front was associated with poor prognosis (p=0.03, and p=0.02 respectively). Tumours with marked cell dissociation (IFG parameter) was associated with poor prognosis (p=0.03). In tumours showing both $p75^{NTR}$ at the invasive front and marked tumour cell dissociation, the average risk of recurrence was increased about 17 times compared to tumours with low $p75^{NTR}$ expression and collective invasion (p=0.01). Conventional prognostic systems were not of prognostic significance.

Conclusion: $p75^{NTR}$ was expressed in all OSCCs. The $p75^{NTR}$ expression and the pattern of invasion were significantly associated with a poor prognosis, and both were better prognostic factors than conventional prognostic systems. The combination of $p75^{NTR}$ expression and the pattern of invasion strongly increased the identification of patients with low disease-free survival.

298 Poster

Cold stress induces apoptosis in a multidrug resistant leukaemic cell line by a caspases-dependent mechanism

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The acquisition of a multidrug-resistant (MDR) phenotype that renders tumoural cells insensitive to anti-neoplasics is one of the main causes of failures on chemotherapy of human malignancies. Thus, the discovery of new stimuli able to induce cell death on drug resistant cells are fundamental on the design of new approaches to eliminate drug-resistant tumours. We have found that leukaemic cells with MDR phenotype are sensitive to hypothermia-induced cell death only when those cells are exposed to temperatures below 4 ºC for a long period of time (up to 12 hrs). We have analyzed cell surface exposure of phosphatidylserine by using Annexin-V-FITC and Propidium Iodide (PI) staining, and found that cold exposure significantly induced early apoptosis (as defined by Annexin V-positive/PI-negative) on MDR cells in comparison to their sensitive counterparts. Furthermore, we have determined the role of individual caspases such as the effector caspase-3 and the initiators caspase-8 (extrinsic pathway) and caspase-9 (intrinsic pathway) by using selective inhibitors of each one of these ptoreins and our results showed that the cold-induced cell death mechanism is caspases-dependent.

Together, these findings indicate that acquisition of MDR phenotype by leukaemic cells is accompanied by pleiotropic changes that result on reduced tumour capacity to survive under stress conditions such as hypothermia. Hence, these studies on MDR-tumours may assist in the design of specific therapeutic strategies that could complement current chemotherapy treatments.