**Conclusion:** 5-FU+INDO combination significantly increased the proliferation inhibition effect of 5-FU monotherapy on high COX-2 protein expressing HCA-7 colorectal cancer cell lines and xenografts.
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**358** POSTER

**Regulatory pathways of plasma membrane integrity in necrotic leukemia cells**

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Tumor cells died by apoptosis may evoke immune tolerance in the host, while necrosis leads to a proinflammatory response against the tumor. Thus instead of enforcing induction of apoptosis, provoked necrosis may help to establish a more effective cancer therapy. While apoptotic pathway has become increasingly well defined, little is known about the types and regulation of necrotic cell death pathways. Despite the idea that necrosis is an uncontrolled form of cell death, accumulating studies have suggested that necrotic cell death can be a regulated event. Recent studies describe several modes of necrotic cell death like secondary necrosis, PARP mediated necrosis or autophagic necrosis. Most recently a potent new pharmacon, necrostatin-1 was discovered that was suggested to halt specifically the death receptor mediated necrosis-like cell death form, termed necroptosis, in caspase compromised cells; although the target of necrostatin was not determined. Earlier we have established a model system to investigate the caspase independent cell death mechanisms in U937 leukemia cells applying non selective caspase inhibitor (z-VD.fmk, 5 μM) and flow cytometry to detect plasma membrane damage (R. Mihalik et al, CDD, 2004, 11:1357). In this model system at 20 hrs treatment condition we found that: (1) h.r.TRAIL (48 ng/ml), staurosporine (STS 1 μM) and $H_2O_2$ (250 μM) induced secondary necrosis (after caspase activation) was inhibited by PARP inhibitors (PJ-34, 1 μM; DPQ, 10 μM). (2) In the presence of caspase inhibitor, TRAIL-induced necrosis was completely abrogated by necrostatin-1 while STS- and $H_2O_2$-induced necrosis only partially. (3) Necrostatin-1 and 3-methyladenin (10 mM; an inhibitor of autophagy) additively protected cells from necrosis induced by STS or $H_2O_2$. (4) Geldanamycin (1 μM), by down regulating the expression of RIP1, rendered caspase-compromised cells resistant to TRAIL- and STS-induced necrosis completely but only partially of $H_2O_2$-induced necrosis. (5) Geldanamycin and PJ-34 together conferred complete resistance to $H_2O_2$-induced necrosis in the presence of caspase inhibitor. (6) Geldanamycin has no significant effect on secondary necrosis induced by either drugs.
In conclusion, our results indicate that necrosis can be induced in U937 leukemia cells at least three distinct molecular signal pathways. These forms may have different relevance to rising the immune response against leukemia cells.
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**359** POSTER

**Expression profile of BRAF, RKIP, P53 and the AKT family genes in endometrial cancer and atypical endometrial hyperplasia**

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**Background:** Aberrations in mediators or downstream effectors of the RAS/RAF/MAPK and PI3K/AKT signaling cascades have been suggested to increase the risk of developing endometrial cancer. However there is limited information regarding these genes expression profile and their association with the malignant transformation of the endometrium.
**Material and Methods:** In the present study we evaluated the mRNA expression pattern of BRAF, RKIP, P53 and the AKT family genes (AKT1, AKT2, AKT3) by Real-Time PCR in tissue samples of 4 patients with complex atypical endometrial hyperplasia (AEH), 26 patients with endometrial cancer and adjacent normal tissues of all patients.
**Results:** Transcript levels of all genes were found to be similar in endometrial cancer and adjacent normal tissue samples. Cancer specimens exhibited similar mRNA levels with AEH cases. Interestingly, BRAF mRNA was not expressed in 39% of the endometrial cancer tissues and in 25% of the AEH cases ($P = 0.033$, $x^2$ test), while its inhibitor mRNA (RKIP) was present in all cases. P53 transcript levels were detectable only in 19% of endometrial cancer tissues, and not in AEH cases ($P < 10^{-5}$). AKT1 was the predominant family member whose mRNA was expressed in all cases, whereas AKT3 exhibited mRNA expression only in 11% of cancer cases and not in endometrial hyperplasia. No association was observed between all genes mRNA levels and tumor histological type, FIGO staging or grade. A disruption of co-expression patterns was displayed in cancer compared to adjacent normal specimens. BRAF mRNA was positively correlated with AKT1 and marginally negatively correlated with P53 in the normal but not malignant endometrium ($P = 0.017$, $P = 0.056$ respectively, Spearman Correlation). Only in the cancer specimen group however, AKT3 transcript levels correlated negatively with BRAF and P53 mRNA ($P = 0.018$ and $P = 0.005$ respectively). AKT1 mRNA was co-expressed with RKIP in both cancer and normal specimens.
**Conclusions:** Deregulation of the mRNA co-expression profile of mediators or downstream effectors of the RAS/RAF/MAPK and PI3K/AKT signaling cascades may be associated with the development of endometrial carcinoma.

**360** POSTER

**Can antisense oligonucleotides specific to mutated K-ras gene inhibit the tumor growth, invasiveness, and MMP-2 and MMP-9 expression in hamster pancreatic cancer model in vitro and in vivo?**

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**Background:** Matrix metalloproteinases (MMP), especially MMP-2 and MMP-9, are thought to play major roles in pancreatic cancer growth and metastasis. Ras activates a multitude of downstream activities with roles in cellular processing, including invasion and metastasis. Therefore, antisense oligonucleotides (ASO) targeting this K-ras gene may be a therapeutic approach.
**Aim:** To elucidate the effectiveness of this gene therapy in hamster experimental cancer model.
**Materials and Methods:** HaP-T1, a cell line derived from BHP-induced pancreatic cancer was used. Transfection with ASO were performed. MTT and MTT agarose assays were done. Chemoinvasion assay was performed. MMP-2 and MMP-9 production by the cell lines was determined by gelatin zymography. For in vivo experiments, subcutaneously resected tumors were implanted orthotopically in Syrian golden hamsters, which were divided in 3 groups: (A) Positive control (PC), (B) Sense treated hamsters (STH), and (C) Antisense treated hamsters (ATH). Oligonucleotides were administered for 2 weeks. Follow up was done. Five animals of each group were sacrificed at Days 10, 17, 24, 31, and 38, to study the local response and metastatic sites. Five animals of each group were left to study the survival time. Specimens were studied histopathologically. Orthotopic pancreatic tumor MMP production was measured by gelatin zymography.
**Results:** ASO inhibited the tumoral growth and invasiveness. They downregulated active forms of MMP-2 and MMP-9 in a dose dependent manner in vitro. Positive controls, STH, and ATH survived in average 72.7, 74.3, and 82.7 days, respectively. Spontaneous lymph node metastases were found from 31 days in ATH group, while PC and STH groups showed metastases and direct invasion to adjacent organs from 17 days. After death, metastatic sites were similar in the 3 groups. ASO downregulated the activation of MMP-9, more than MMP-2 in vivo.
**Conclusions:** These experiments suggest that ASO targeted K-ras gene may be a good choice in the management of pancreatic cancer because of the suppression of tumor growth and invasiveness in vitro and in vivo. ASO also downregulated the activation of MMP-9 and MMP-2 in vivo.

**361** POSTER

**N-glycolyl sialic acids as a cancer vaccine target: developing of a mouse B16 melanoma model by transient or stable expression**

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**Background:** Sialic acids are normal components of the glycocalyx in most normal cells that participate in biological processes such as migration, adhesion and specific receptor recognition. N-glycolyl sialic acids (NeuGc) are a subset of these molecules synthesized by the enzyme CMP-NeuAc hydroxilase in murine cells. Although normal human cells do not express NeuGc, it has been described that the antigen can be detected in the cell membrane in melanoma and breast cancer. These facts support the idea to use NeuGc as a target for cancer vaccines in human beings. On the contrary, mouse B16 melanoma cells, as well as most murine tumors,

do not express NeuGc, making difficult the development of appropriate preclinical tumor models. Our aim was to obtain B16 melanoma cells with transient expression of NeuGc by in vitro antigen incubation or by stable overexpression of CMP-NeuAc hydroxilase.
**Materials and Methods:** Transient expression was obtained by in vitro incubation with mucin, a NeuGc-rich compound. Stable expression were done by molecular techniques in order to isolate and amplify the murine CMP-NeuAc hydroxilase sequence from normal liver. The cloning and transfection were done using the invitrogen cloning TOPO system.
**Results:** Incubation of B16 cells with mucin induced the presence of this antigen in B16 cell membrane during 48 hours. Preincubation with mucin caused an enhancement in tumor cell adhesion on plastic surfaces. In vivo, mucin-incubated B16 cells showed a rapid subcutaneous primary tumor formation and an increase in the metastatic ability after endovenous injection in syngeneic C57Bl6 mice. Transfected B16 cells showed the presence of CMP-NeuAc hydroxilase mRNA and the presence of the NeuGc antigen in tumor cell membrane. We observed an increase of in vitro proliferation and cell adhesion in transfected cells as compared with control non-transfected B16 cells. Interestingly, stable NeuGc expression was associated with a weak tumorigenicity in syngeneic mice after subcutaneous implantation of transfected B16 cells and a decrease of lung metastasis.
**Conclusions:** Taken together, the results indicate that the presence of NeuGc modulates positively in vitro proliferation and adhesion of mouse melanoma cells, but stable expression of the antigen may induce a negative selection during tumor progression in immunocompetent mice.

**362** POSTER

**Pepsinogen C gene polymorphism and breast cancer: Influence on the overall survival**

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**Background:** Pepsinogen C gene (PGC) has been associated with hormonal control, and therefore the role of its protein has been investigated in breast cancer. We have studied the influence of an insertion/deletion polymorphism in the Pepsinogen C (PGC) gene, in the clinical outcome of breast cancer patients.
**Material and Methods:** The study was performed with 172 blood samples of breast cancer patients. The 6 polymorphic alleles were amplified using PCR: allele 1 (510 bp), allele 2 (480 bp), allele 3/4 (450/460 bp), allele 5 (400 bp) and allele 6 (310 bp).
**Results:** Our results indicate that patients carrying the allele 6 present a higher 5-year survival mean (83.4% of 6 allele carriers were alive at 5 years versus only 68.6% of non-carriers, $p = 0.001$), suggesting a role for this polymorphism in the outcome of breast cancer patients.
**Conclusions:** We hypothesize that PGC polymorphism can be a predictive biomarker in breast cancer, contributing to an individual profile of great interest in clinical oncology.

**363** POSTER

**Genomic instability in non-small cell lung cancer assessed by arbitrarily primed polymerase chain reaction**

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Lung cancer is the most common cause of neoplasia-related death worldwide. One of the crucial early events in carcinogenesis could be the induction of the genomic instability phenotype. The high incidence of genomic instability in lung cancers has been well established, and in some cases it has been associated to prognosis. We investigated genomic instability in patients with non-small cell lung cancer (NSCLC). Instability was correlated with patients' age at diagnosis, gender, NSCLC subtype, histological grade and stage, tumor necrosis and lymph node invasion.
DNA from tumor and corresponding normal tissues of 30 patients with NSCLC was isolated and amplified with five arbitrary primers using arbitrarily primed polymerase chain reaction (AP-PCR).
Four out of five tested primers produced informative sequence alterations differentiating normal tissue from NSCLC. Comparing AP-PCR profiles of normal and tumor tissue we identified significant genomic instability in most cases. Two types of electrophoretic changes were detected, qualitative changes (structural DNA alterations) and quantitative changes (chromosomal gains and losses). Genomic instability was represented as the frequency of DNA alterations. Genomic instability resulting from the total number of DNA changes was significantly higher in patients older than 50 ($P < 0.05$). Frequency of DNA alterations calculated from qualitative changes was significantly different between patients with adenocarcinoma and patients with squamous cell carcinoma ($P < 0.05$). ANOVA revealed a significant correlation between the total number of DNA changes and histological grades ($P < 0.006$) as well as between quantitative changes alone and histological grades ($P < 0.016$). Post hoc comparisons showed significant difference between the frequencies of DNA alterations in grade groups 1 and 2 ($P < 0.05$) and in groups 1 and 3 ($P < 0.005$), as well as in grade groups 2 and 3 ($P < 0.05$). Most importantly, genomic instability decreased with increasing tumor grade.
Our results suggest that high frequency of genomic instability in early stages of cancer development may be involved in progression of NSCLC. Lower degree of genomic instability in advanced stages of NSCLC (histological grades 2 and 3) could be considered as a marker of poor prognosis. Our study shows that AP-PCR is an effective method for the identification and analyses of genomic instability in NSCLC and may provide insight into the molecular mechanism of lung carcinogenesis.

**364** POSTER

**Importance of the pro-apoptotic Bcl-2-like protein Bak for radiation- and hypoxia-induced apoptosis**

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The disruption of mitochondrial homeostasis is the key event in DNA damage- and stress-induced apoptosis. It involves breakdown of the mitochondrial membrane potential and release of pro-apoptotic factors from the mitochondrial intermembrane space with subsequent activation of the caspase cascade and execution of apoptosis. The mitochondrial homeostasis is controlled by pro- and anti-apoptotic proteins of the Bcl-2 family that either antagonize (Bcl-2, Bcl-$x_L$) or activate (Bax, Bak) downstream signalling events.
To gain further insight into the mechanisms of radiation- and hypoxia-mediated cytotoxicity at the level of the mitochondria we tested in how far crucial pro-apoptotic Bcl-2 proteins, namely Bak and Bax, are involved in apoptosis-induction using Jurkat T-lymphoma cell clones being either negative for Bax but expressing Bak (Jurkat Bak positive), or being negative for both, Bax and Bak (Jurkat Bak negative) Induction of apoptosis by hypoxia and irradiation was determined in Jurkat Bak positive and Jurkat Bak negative cells by flow cytometry (breakdown of the mitochondrial membrane potential, nuclear fragmentation), fluorescence microscopy (nuclear condensation), and Western blotting (activation of caspase-9, caspase-3, caspase-8 and cleavage of the caspase-substrate PARP).
Our results provide evidence for Bak-dependent pro-apoptotic effects of hypoxia and irradiation at the level of the mitochondria. While lack of Bax was not sufficient to inhibit radiation- and hypoxia-induced apoptosis in Jurkat cells expressing Bak, absence of Bak strongly reduced mitochondrial alterations compared to Bak-positive cells and completely abrogated treatment-induced caspase activation.
From these data we conclude that the pro-apoptotic Bcl-2 homologue Bak is essential for radiation- and hypoxia-induced apoptosis in Bax-deficient Jurkat T-lymphoma cells.

**365** POSTER

**Origin of 5-ALA-induced PpIX at brain tissues surrounding tumor (in vitro photometrical study)**

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**Background:** In surgical treatment of malignant glioma, we experienced the fluorescence of 5-aminolevulinic acid (5-ALA)-induced protoporphyrin IX (PpIX) at a non-tumor department, brain without blood-brain barrier (BBB), edematous brain tissues surrounding tumor and so on.
**Materials and Methods:** In vitro, we cultured several kinds of brain tumor cell lines (C6 rat glioma, U87delta human glioma, U251 human glioma and IOMM-Lee human malignant meningioma) and exposed to different condition of 5-ALA including culture medium. After this, fluorescent degree of the medium and cells were each measured by means of photometrical assay, and analized quantitifically with fixed-quantity of intracellular and extracellular PpIX.
**Results:** Comparing the fluorescence degree of a cell, C6 and U87delta had a peak in the vicinity of 0.5mM 5-ALA, but U251 and IOMM-LEE had not the peak. In addition, comparing the fluorescence degree of a nutrient medium, we recognized a peak in the vicinity of 0.5mM 5-ALA entirely.