MGMT gene in matched tumour tissue samples from patients with head and neck cancer.
Material and Methods: Methylation was analyzed in primary tumours and healthy tissue from 37 patients. DNA was isolated from the tumour samples by conventional phenol chloroform extraction. Methylation of the genes was analyzed by Methylation-Specific Multiplex Ligation Dependent Probe Amplification (MS-MLPA) using 32 different probes. Different regions of the genes were analyzed by using 21 probes. 11 probes which did not have recognition sites for the HhaI enzyme were used as reference probes. The PCR products were analysed by capillary electrophoresis using the ABI 310 genetic analyzer. Two samples from each patient were compared to each other. The signals were normalized by dividing each peak area to the area of the reference probes. A ratio higher than %20 was considered as methylation-positive.
Results: In 12 (%55) patients more than one gene was methylated while 10 (%45) patients displayed only one metyhlated gene. Methylation was not observed in the repair genes in 15 patients. The most frequently methylated gene was the MGMT gene (%43) followed by the MSH6 (%21) and MLH1 (%19) genes. The MGMT gene was also freuently methylated at more than one site.
Conclusion: Our results indicate that methylation of the mismatch repair genes is a frequent event in head and neck cancer and may play a role in the development of the diesase.

592 PI3K cooperates with TGFβ in the regulation of the TGFβ malignant autocrine loop in glioblastoma

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Human glioblastoma (GBM) is one of the most aggressive and recalcitrant human tumours and is virtually not curable. GBM presents a high TGFβ-Smad activity that confers poor prognosis. High expression of TGFβ2 in GBM is responsible for the increased activity of the TGFβ-Smad pathway. This increased secretion of TGFβ2 is caused by a malignant autocrine loop through which TGFβ induces its own expression. In this work we aimed to study the molecular mechanisms implicated in this malignant autocrine loop. Specifically we studied how TGFβ regulates the expression of TGFβ2 in GBM. Using GBM cell lines and GBM patient samples we have identified a new crosstalk between the PI3K and TGFβ signaling pathway at the level of TGFβ2 secretion. We demonstrate that hiperactivation of PI3K signaling increases TGFβ mediated expression of TGFβ2. These results have been confirmed in human GBM specimens. At the moment we are looking for the transcriptional complex that mediates this process. This work provides new molecular targets to restore normal TGFβ function as new therapeutic strategies against this disease.

593 Stressor effect of zoledronic acide in rabbit heart tissue

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Background: Treatment with a bisphosphonate was linked with a significantly increased risk for atrial fibrillation (AF) in a few studies. Once-yearly infusions of intravenous zoledronic acid (ZA) was also significantly increased serious AF in postmenopausal women with osteoporosis. In this study, in order to investigate of zoledronic acid on oxidative stress and antioxidant effect of rabbit in the heart tissue.
Material and Methods: In the study, 7 rabbits on the 100 mcg/kg given daily basis Zoledronic acid (ZA) group, control group (7 rabbits) was fed 28 days ad.lib at the same time. The MDA levels in the tissue of both groups were examined using Uchiyama and Mihara methods (1978). The method is based on the production of the pink compound producing maximum absorbance at 535 nm as a result of thiobarbutyric acid's reaction with MDA. The GSH level was examined using the Ellman method (Fairbanks and Klee, 1986). The level of NO was measured by reading the maximum absorbance at 545 nm after cadmium reduction of nitrate to nitrite (Cortas and Waked, 1990). All tissue were examined histopathologically. The data are presented in mean values and standard deviations. Normality test was done with Shapiro–Wilk method. Independent samples t-test was used for the statistical analysis. $P < 0.05$ was considered statistically significant.
Results: Our findings, ZA group MDA and NO levels were found statistically significantly higher when compared to control group ($P < 0.0001$), GSH levels were found to be lower in ZA group when compared to control as statistically significant ($P < 0.0001$).
Conclusions As a result, the rabbit heart tissue Zoledronic acid, induced oxidative stress, reduces antioxidant levels were observed. Regarding the safe use of these agents, further studies with antioxidant supplements are needed.

594 A novel form of cellular senescence induced by hyper-activation of the PI3K/Akt pathway

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In normal human cells, expression of oncogenes such as Ras and c-myc, results in p53-dependent senescence or apoptosis respectively, both of which are recognized as self-protection mechanisms against tumourigenesis. Senescence induced by oncogenic Ras follows a hyper-proliferative phase associated with accumulation of DNA damage. The DNA damage response triggers changes in global gene transcription leading to irreversible proliferation arrest. For cell transformation additional genetic alterations are required to bypass these proliferation arrest mechanisms. Enhanced activity of the PI3K/Akt pathway is detected in approximately 30% of human cancers with downstream effects on cell survival, proliferation, metabolism, cell migration and angiogenesis via effectors including GSK3b, MDM2, FOXO1/3a, TSC2 and p27. In this study we examine the senescence-like phenotype induced by hyper-activation of the PI3K/Akt pathway driven by expression of constitutively active (CA) Akt, mutant PIK3CA, or PTEN depletion in normal human fibroblasts. We have examined the accumulation of senescence markers including cell cycle inhibitors and senescence associated β-galactosidase activity, and markers of the DNA damage response. We have also investigated the additional genetic alterations required for bypass of CA-Akt induced proliferation arrest using SV40 T antigens and shRNA introduced into isogenic cell lines. Interestingly, we find that hyper-activation of the PI3K/Akt pathway results in a novel form of p53-dependent proliferation arrest that is not associated with an initial hyper-proliferative phase or DNA damage accumulation. Using chemical inhibitors, we have implicated the stress activated p38MAPK and the mTORC1 cell growth pathway as two key elements in the activation of p53 by CA-Akt. The formation of senescence-associated heterochromatic foci (SAHFs) is implicated in irreversible silencing of proliferation promoting genes. Notably, as compared to oncogenic Ras, heterochromatic reorganization was not detected upon expression of CA-Akt, which may affect the response to these cells in vivo. Given that pro-senescence therapies are being suggested as cancer prevention and treatment strategies, understanding the differences between types of oncogene-induced senescence will become increasingly important./

Monday 28 June 2010 09:45–17:30

Poster Session
Survivorship Research

595 The clinical and biological significance of the immunophenotypic assessment of CD81 in multiple myeloma clonal plasma cells

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Background: Although CD19 is typically down regulated in myelomatous plasma cells (MM-PC), we have recently shown that a minority of multiple myeloma (MM) patients (4%) express this marker at diagnosis, which correlates with adverse outcome. The CD19 expression is thought to be regulated by CD81, a tetraspanin involved in mechanisms of cell proliferation. However, phenotypic or genomic studies of CD81 expression in MM are scanty, and its potential prognostic value remains unknown.
Material and Methods: A total of newly diagnosed 36 smoldering MM (SMM) patients and 229 untreated symptomatic MM patients were included in this study, the latter group uniformly treated according to the Spanish GEM05>65y protocol. Expression of CD81 on MM-PC was assessed by multiparameter flow cytometry (MFC), staining BM samples using a four-color direct immunofluorescence technique that allowed the identification of MM-PC as well as CD81 surface expression. In a subset of patients (18 SMM and 23 MM) mRNA gene expression profiling (GEP) was performed on immunomagnetically enriched MM-PC.
Results: MFC studies detected positive staining for CD81 in MM-PC of 15/36 (42%) SMM and 90/229 (39%) MM patients. Interestingly, both SMM and MM CD81+ cases showed a higher frequency of CD19 expression on MM-PC compared to CD81− cases (13% vs. 0%, P=.08 and 7% vs. 1%, P=.01; respectively), in line with the regulatory role of CD81 over CD19. Concerning GEP analysis, we found a significantly (P=.003) lower relative expression of *CD81* mRNA in MM-PC of SMM (6.8) and MM (6.7) patients compared to normal PC (9.3), which could explain, at least in part, the absence of

CD81 on MM-PC surface in around half of myeloma cases. Accordingly, we found a significant correlation between GEP and MFC expression of CD81 ($r = .743$; $P < 0.001$), and CD81+ SMM and MM patients showed higher levels of relative expression of *CD81* mRNA compared to CD81− cases (7.5 vs. 6.6, $P = .03$ and 7.4 vs. 6.7, $P = .04$, respectively). No significant differences were found in baseline characteristics of CD81− vs. CD81+ SMM or MM patients, except for the % of MM-PC in S-phase (0.8 vs. 1.4, $P = .09$ and 0.9 vs. 1.4, $P = .003$ for SMM and MM, respectively). Finally, CD81+ SMM patients had a shorter time to progression to symptomatic disease than CD81− cases (median not reached – NR – vs. 25 months, $P = .04$); and also CD81+ MM showed significantly lower response rates (complete remission: 8% vs. 26%, $P = .01$), progression-free (median 23 vs. NR months, $P = .001$) and overall survival ($P = .03$) than CD81− cases.

Conclusions: Our findings uncover the existence of a phenotypic/genomic correlation of CD81 expression in MM-PC, which correlated with an adverse outcome in SMM and MM, supporting the clinical relevance of baseline routine BM evaluation by MFC in myeloma.

596 The effect of cabbage juice and it's active components on the protein level and expression of CYP1A1, CYP1A2 and CYP1B1 in MDA-MB-231 breast cancer cells

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Breast cancer is the most common malignancy in women. A major effort to reduce breast cancer mortality and morbidity is focused on development of better breast cancer chemoprevention. Although pharmaceutical agents have therapeutic and preventive roles in breast cancer, the use of compounds from natural products to prevent breast cancer is currently being explored. Among the promising food components being investigated to reduce breast cancer risk are phytochemicals found in cruciferous vegetables – indole-3-carbinol (I3C), diindolylmethane (DIM) and sulforaphane (SUL). In addition epidemiological studies have indicated that high intake of white cabbage may be associated with a lower risk of neoplastic diseases such as cancer of breast.

Our previous study showed that cabbage juice and it's potential active components affected the proteins level and expression of CYP450 isoenzymes involved in estrogen biosynthesis and metabolism in breast cancer estrogen dependent (MCF7) and human epithelial nontumourigenic (MCF10A) cell lines.

The aim of the present study was to determine the effect of cabbage juices, I3C, DIM and SUL on the expression profile of *CYP1A1*, *CYP1A2* and *CYP1B1* mRNA and proteins level in breast cancer estrogen independent (MDA-MB-231) cell line. Cells were treated with raw cabbage and sauerkraut juices (obtained from vegetables cultivated in industrial and ecological farms) or I3C, DIM and SUL at the concentrations relevant to those observed in human plasma. After 72 hours of incubation the screening of cDNA from total RNA was performed using real-time PCR assay with specific primers for *CYPs* and protein level was determined by Western blot analysis.

The most marked effect was observed on the mRNA level. The enhanced expression of *CYP1A1* and *CYP1B1* was noticed as a result of treatment of MDA-MB-231 cells with both doses of I3C and DIM. *CYP1B1* mRNA level was also increased by all tested cabbage juices. In case of *CYP1A1* expression the similar effect was observed after treatment with juices obtained from vegetables cultivated in ecological farms.

The results of this study provide additional data on the possible anticarcinogenic and antimutagenic activity of cabbage.

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597 Modulation of transcription factor Nrf2 as mechanism of chemoprotective effects of cabbage juices, indole-3-carbinol and phenethyl isothiocyanate in human hepatoma cells and rat liver

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Epidemiological studies suggest that the intake of *Brassica* vegetables is associated with a decreased risk of developing cancer. This effect is usually linked to the intake of glucosinolates and their metabolism to cancer preventive indoles, such as indole-3-carbinol (I3C) and isothiocyanates, such as phenetyl isothiocyanate (PEITC). These compounds lead to the induction of phase 2 enzymes of xenobiotic metabolism. In the last few years it was shown that the main role in the induction of phase 2 enzymes is played by the Nrf2 transcription factor. Nrf2 activates various genes encoding cytoprotective proteins which deactivate reactive electrophilic metabolites of xenobiotics, decompose reactive oxygen species and stabilize the cell redox potential. Our previous studies demonstrated that the administration of cabbage or sauerkraut juice increases the activity of glutathione transferase (GST) and changes the level of expression of its isoenzymes in rat liver.

In order to explain the mechanism of GST induction in this study the activation of Nrf2 was evaluated in rat liver and compared with the effect of cabbage and sauerkraut juices and I3C and PEITC in human hepatoma cells (HepG2). Male Wistar rats were treated by gavage with cabbage juices, I3C or PEITC for 4, 10 and 30 days. The HepG2 cells were incubated with cabbage juices, I3C or PEITC for 24 hours and Nrf2 levels were assessed by immunoblotting.

The treatment with either cabbage juices or pure compounds (I3C and PEITC) resulted in the translocation of Nrf2 protein from cytosol to the nucleus in both the *in vi*vo and *in vitro* model. Western blot analysis showed the most significant enhancement of nuclear Nrf2 levels in rat liver after 30 days of treatment with cabbage juice, I3C or PEITC. Cabbage juices and other tested compounds showed a similar effect in HepG2, but the highest level of Nrf2 in the nuclear fraction was observed in cells incubated with PEITC.

The results of our present and earlier studies indicate that the induction of GST through the activation of Nrf2 by cabbage juices may be responsible for their chemopreventive activity demonstrated by epidemiological studies and in animal models. However, the activation of Nrf2 in cancer cells may enhance resistance to chemotherapeutic drugs and should be taken into consideration in dietary recommendations for cancer patients.

598 DNA cancer vaccine mediated by polyethylenimine

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Background: Polycations have been widely used in non-viral gene delivery. This study attempts to investigate the adjuvant effect of polyethylenimine (PEI) on stimulation of antigen-specific immune response via DNA-based cancer vaccine.

Materials and Methods: Plasmid DNA encoding the ovalbumin (OVA) gene was complexed with polyethyleimine (PEI) and subcutaneously injected into the C57BL/6J mice. Cells were isolated from the secondary lymphoid organs and assayed by an OVA/K^b-specific murine cytolytic T-hybridoma. Specific lysis of the target cells was studied employing the *in vivo* cytotoxic T-lymphocyte (CTL) assay by labeling the target cells with CFSE (carboxyfluorescein succinimidyl ester), and the effect of PEI-mediated DNA vaccination on tumour growth was examined in the EL4 and EG7-OVA thymoma model. Both tumour volumes and the animal survival rates were routinely monitored. Animal care and experimental treatment were conducted in compliance with the institutional policies.

Results: Treatment of animals with the PEI-DNA complexes resulted in significant activation of an OVA-specific cytotoxic clone that recognizes the target cells through the class I major histocompatibility complex (MHC) molecules, illustrating the induction of class I-restricted antigen presentation *in vivo*. Antigen-specific cell lysis was demonstrated by the CFSE-based *in vivo* assays. Immunohistochemical staining showed that adjuvant-mediated DNA vaccination induced cell death and significant lymphocyte infiltration at the injection sites. Vaccination of the C57BL/6J mice with plasmid DNA/PEI complexes, either preceded or after the tumour challenges, lead to suppression of the tumour growth and prolonged the survival rate of the animals.

Conclusions: Our data illustrated the potential use of polycations in DNA-based cancer therapy for induction of antigen-specific immune response and CTL effect, resulting in the protective and therapeutic immunity in the experimental tumour models.

599 C-reactive protein; a potential marker of second cancer and cardiovascular disease in testicular cancer survivors?

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Purpose: C-reactive protein (CRP) is a marker of cardiovascular risk both in patients with cardiovascular disease (CVD) and in presumably healthy patients with normal LDL cholesterol while there is a conflicting evidence regarding CRP as a marker of future cancer. The aim was to assess whether CRP predicts CVD and consecutive cancer in testicular cancer survivors (TCSs).

Methods: During 1998–2001, 586 TCSs with a high sensitivity CRP $\leqslant$ 10 mg/L were identified median 11 (4−21) years after treatment (FU-1). A second follow-up survey (FU-2) was conducted median 8 (6−9) years after FU-1. At FU-2 we obtained information about post-FU-1 CVD (cardiovascular death, nonfatal myocardial infarction, stroke, revascularization or heart failure). Information about post-FU-1 non-germ cell cancer and cardiovascular death in all 622 patients were retrieved from the Cancer Registry of Norway.

Results: After FU-1 31 (5.3%) of 586 patients developed non-germ cell cancer (excluding localized prostate cancer) while 28 (4.9%) developed CVD.