week on, these parameters were back to control levels. The modulation of oxidative state of C57/B6 lung was not accompanied by histological modifications. Surprisingly, we did not observe the same results in the susceptible A/J mice.
In the present study, we have shown that urethane modulates redox components of the lung and this effect seems to be strain dependent. It is known that urethane-treated A/J mice will develop lung adenocarcinoma within 16−20 weeks after the first injection and that only a very small percentage of C57/B6 will develop lung cancer under the same conditions. Therefore, we put forward the idea that the ability of the resistant mice to up regulate a proper stress response at an initial stage act as a protective mechanism against carcinogenesis. On the other hand, the apparent lack of response observed in susceptible mice might mitigate the establishment of a chronic nocive environment that would contribute to the development of lung adenocarcinoma.

588 Aloe vera and honey solution decreases cell proliferation and increases apoptosis susceptibility in tumour tissue while avoids liver damage

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Background: Cancer is diagnosed in approximately 11 million people and is responsible for approximately 8 million deaths worldwide every year. Researches in cancer control have shown the importance of co-adjuvant therapies. *Aloe vera* may reduce tumour mass and metastasis rates, while honey may inhibit tumour growth.
Materials and Methods: This study verified the influence of *Aloe vera* and honey on tumour growth evolution accessing cell proliferation rate (Ki67-LI) and apoptosis susceptibility (Bax/Bcl-2 ratio) in tumour and liver tissue from adult rats at 7, 14 and 20 days of Walker 256 carcinoma (sc) implant. Tumour-bearing Wistar rats were distributed into two groups: *Aloe vera* and honey-treated group (WA) received a gavage with a 670 ml/kg dose of *Aloe vera* and honey solution daily, while non-treated group (CW) received only 0.9% NaCl solution in the same dose.
Results: The effect of *Aloe vera* and honey against tumour growth was observed through WA versus CW, showing decrease in tumour relative weights (CW-7d = 0.79±0.32; WA-7d = 0.68±0.43; CW-14d = 4.14±2.08; WA-14d = 3.17±1.38; CW-20d = 7.57±2.98; WA-20d = 5.16±2.46 (%)), lower cell proliferative rates (Ki-67 LI: CW-7d = 71.0±10.9; WA-7d = 51.4±18.1; CW-14d = 69.6±13.5; WA-14d = 37.2±16.4; CW-20d = 59.1±22.7; WA-20d = 32.0±3.3), and increase in apoptosis susceptibility (Bax/Bcl-2 ratio: CW-7d = 0.39±0.05; WA-7d = 2.35±0.08; CW-14d = 0.55±0.24; WA-14d = 2.48±2.16; CW-20d = 0.15±0.06; WA-20d = 1.20±0.80). In contrast, we observed that the *Aloe vera* and honey treatment leaded to increase in hepatocytes proliferation in early stages of tumour development (CW-7d = 12.6±3.3; WA-7d = 19.9±1.8; CW-14d = 7.2±1.4; WA-14d = 10.2±1.7; CW-20d = 10.9±1.8; WA-20d = 7.8±1.5) and decrease in their apoptosis susceptibility at 14th day of tumour implant (Bax/Bcl-2 ratio: CW-7d = 0.93±0.53; WA-7d = 0.86±0.62; CW-14d = 4.06±2.39; WA-14d = 0.88±0.63; CW-20d =3.53±3.24; WA-20d = 3.34±0.88), suggesting a possible protective effect in liver tissue, which is commonly harmed by tumour effects.
Conclusion: These data suggest that *Aloe vera* and honey affected tumour and host in a different way, inducing some benefits to host tissue while promoted damages in tumour evolution. Indeed, there are a large number of complex mechanisms involved in tumour growth, apoptosis and host health maintenance that can be modulated by *Aloe vera* and honey.

589 Extracts from endemic plant Helichrysum zivojini suppress survival of malignant cells

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Background: A wide variety of compounds and extracts from medicinal plants are in the center of attention of modern anticancer research as potential bioactive agents which might be used in future for the suppression of initiation, promotion and/or progression of malignant diseases. In this study our main goal was to investigate the anticancer properties of endemic plant species *Helichrysum zivojini* collected in Macedonia.
Material and Methods: The aerial parts of the plant were air-dried, powdered, and successively extracted with solvents of increasing polarity to obtain hexane, dichloromethane, ethyl-acetate, *n*-butanol and methanol extract. The cytotoxic activity of five obtained extracts was tested against selected cancer cell lines: human cervix adenocarcinoma HeLa, human breast adenocarcinoma MDA-MB-361, human malignant melanoma Fem-x, human myelogenous leukemia K562, unstimulated and stimulated for proliferation by phytohemagglutinin normal human immunocompetent peripheral blood mononuclear cells (PBMC) using MTT test. The mode of K562 cell death was analyzed morphologically.
Results: All investigated extracts exerted a selective dose-dependent cytotoxic action against all used target cancer cell lines and to PBMC stimulated for proliferation, but cytotoxic action was not as pronounced to normal, rested PBMC. The very prominent cytotoxic effect was observed against K562 cell line (IC_{50} values ranging from 11.78±0.94 to 74.88±7.57 μg/ml). Moreover, cytotoxicity of different extracts of *Helichrysum zivojini* was significantly stronger toward HeLa, Fem-x and K562 cancer cell lines than toward healthy immunocompetent PBMC stimulated for proliferation. It should be stressed that these extracts in whole exhibited weaker cytotoxic effect against unstimulated PBMC in comparison to stimulated PBMC. Morphological evaluation by microscopic examination of acridine orange and ethidium bromide stained K562 cells pre-treated for 48 h with plant extracts applied at a double $IC50_{72h}$ concentrations, demonstrated that all five extracts induced apoptotic cell death.
Conclusion: Results from this research show that extracts prepared from endemic plant species *Helichrysum zivojini* possess very pronounced anticancer potential, which could be attributed to the observed very selective antiproliferative and apoptotic effect, specially exerted to malignant cells.

590 Anticancer activity screening of Thai medicinal plants in human leukemic cell line MOLT-4

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Many phytochemicals have been proved to be a good candidate for anticancer drug. Eleven Thai plants were thus selected based on local usage for anticancer activity investigation. The 50% ethanol-water crude extract were prepared from *Rhus javanica* (stem), *Pinus kesiya* (branch), *Cratoxylum formosum* (stem), *Acorus tatarinowii* (leave & rhizome), *Tetracera loureirii* (vine), *Abrus pulchellus* (stem), *Catymbium speciosum* (rhizome), *Amomum villosum* (leave & rhizome), *Glochidion daltonii* (stem), *Rhus succedanea* (stem), and *Cladogynos orientalis* (arial part). The anticancer activity was determined from cytotoxicity and apoptosis induction in leukemic MOLT-4 cell and Vero cells. Cytotoxicity was tested by using Neutral red assay. An alkylation reaction with nitrobenzylpyridine (NBP), a nucleophilic DNA model was also examined. Apoptosis induction was evaluated from DNA fragmentation by using gel electrophoresis. Results showed that the plant that showed strong cytotoxic (IC_{50} < 100 μg/ml) and high selectivity (SI > 3.0) at 24 h and 48 h was *T. loureirii* (IC_{50} of 53.9±5.4 and 68.4±7.4 μg/ml, respectively). While *A. pulchellus*, and *P. kesiya* showed strong cytotoxic and high selectivity only at 48 h (IC_{50} of 71.7±4.2 μg/ml and 74.0±7.5 μg/ml, respectively). The plants that showed strong cytotoxic but less selectivity at 24 h and 48 h were *G. daltonii* (95.5±6.4 μg/ml and 61.0±3.9 μg/ml) and *C. speciosum* (99.4±3.6 μg/ml and 86.9±9.1 μg/ml). *C. formosum* possessed strong cytotoxicity (76.2±4.1 μg/ml) only at 48 h. Other crude extracts were found to be moderate cytotoxic (100 μg/ml ⩽ IC_{50} ⩽ 500 μg/ml) or inactive (IC_{50} > 500 μg/ml). The crude extracts illustrated different alkylating activity and only *A. tatarinowii* (leaves) showed no alkylating activity. The first 4 plants, *C. formosum, G. daltonii, R. succedanea, and T. loureirii*, showed high alkylating activity with 36, 22, 16, and 16% compared to melphalan, a positive control. Alkylating activity also indicated the presence of some electrophilic substance in the crude extract which alkylate with the nucleophilic site of NBP. Interestingly, almost of crude extract exhibited DNA ladder at 24 h except *T. loureirii*, *G. daltonii*, and *C. orientalis.* To be concluded, *A. pulchellus*, and *P. kesiya* showed high potential anticancer activity. While, the other plants that exhibited apoptosis induction were also of interest for further study. The active compound contributed to the activity and detailed mechanism of action will be further carried on.

591 Methylation of the mismatch repair genes in head and neck cancer

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Background: The Mismatch Repair System (MMR) plays a crucial role in the maintenance of genomic stability and increases the fidelity of DNA replication by eliminating mismatches which occur during the replication process. The MMR system incorporates several genes and has been conserved from procaryotes to eucaryotes. Aberrant methylation of the CpG islands at the promoter region of the genes is an epigenetic change that leads to transcriptional silencing of tumour suppressor genes. However, transcriptional silencing of the MMR genes in head and neck cancer has not been investigated throughly. In this study we investigated methylation of six MMR genes and the

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