

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 its 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 its 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 nontumorigenic (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 phenethyl 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 vivo* 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 polyethylenimine (PEI) and subcutaneously injected into the C57BL/6J mice. Cells were isolated from the secondary lymphoid organs and assayed by an OVA/K<sup>b</sup>-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  $\leq 10$  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.

Cox regression analyses showed that patients with CRP  $\geq 1.5$  mg/L had 2.21 (95% CI 1.04–4.70) times higher risk of developing non germ cell cancer and 2.79 (95% CI 1.22–6.34) times higher risk for CVD compared to patients with a CRP  $< 1.5$  mg/L at FU-1. Radiotherapy was associated with 2.56 (95% CI 1.19–5.51) times higher risk for developing non-germ cell cancer in comparison to patients treated with surgery with or without chemotherapy.

**Conclusion:** In long-term TCSs CRP may serve as a potential marker of cardiovascular events and a second cancer.

#### **[600] The association of biomarkers of insulin resistance and obesity to prostate tumour markers**

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Prostate cancer incidence is expected to rise among developing nations secondary to increased prevalence of obesity and the elderly. Many case-control studies have been done associating obesity to increased prostate cancer risk; very few studies have been conducted correlating tumour markers of prostate cancer to biomarkers of obesity and insulin resistance using a homogenous cancer-free cohort. This study aims to fill this gap. A total of 219 consenting adult Arab men aged 30–70 years old were included in this cross-sectional study. Demographics were noted and anthropometrics measured. Fasting blood samples were extracted and measured glycemic and lipid profile using routine lab methods. Serum adipocytokines and inflammatory markers were measured using multiplex assays. Prostate tumour markers including tPSA, fPSA and PTHrP as well as endoglin were measured using enzyme linked immunosorbent assays. Among all subjects, age, triglycerides and waist-hip ratio were significantly and positively associated with circulating tPSA levels ( $p$ -values  $< 0.01$ ). Systolic blood pressure, adiponectin, aPAI-1, TNF- $\alpha$  and IGF all had inverse significant associations to tPSA. Stepwise linear regression revealed that adiponectin, IGF, WHR and PTHrP predicted 30 % of variance in tPSA levels ( $p$ -value  $< 0.0001$ ). In conclusion, the significant association of several biomarkers of insulin resistance and obesity, specifically adiponectin and waist-hip ratio strengthens the link between insulin resistance and visceral adiposity to prostate cancer development among Arab men.

#### **[601] Association of regret with utilization of counseling about reproductive loss: a survey of reproductive-aged patients in California with leukemia**

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**Background:** Rates of treatment-related-infertility in reproductive aged women with leukemia – and the possible effects of such infertility on post-treatment quality of life – are poorly understood. So, too, is the availability and utilization of fertility preservation counseling and treatment on a public health scale in the United States.

**Material and Methods:** We are conducting a retrospective cohort study using a written survey instrument. Women aged 18 to 40 at diagnosis of leukemia between 1993 and 2007 were identified in the California Cancer Registry – a collection of all cancer diagnoses in California, a state with a diverse population of 40 million.

The surveys are being sent out in 5 year segments: (1) Diagnosed between 2003–2007, (2) 1998–2002, (3) 1993–1997. The instrument contains questions about treatment history, menstrual history, family size, parenting desires, fertility counseling, decision-making about fertility preservation, and incidence of infertility. Patients are also asked to complete the following: (1) A Decision Regret Scale (DRS), (2) The Reproductive Concerns Scale, (3) WHOQOL-BREF, and (4) Satisfaction with cancer and fertility treatment scales. These scales have all been previously validated. Patients are being contacted by mail with the written survey and given a follow-up phone call. All surveys (N = 750) will have been sent by April, 2010.

**Results:** We report preliminary data on 37 returned surveys (sent to patients diagnosed from 2003–2007). Their average age at diagnosis was 30.5 years. 34 women underwent treatments with the potential to compromise fertility (i.e., systemic chemotherapy, or a radiation field that includes the abdomen/pelvis). 16 received Bone Marrow Transplant (BMT); see the tables.

Table 1: Rates of utilization of fertility preservation services

Counseled about risk of BMT to fertility by oncology team	94%
Counseled about the risk of non-BMT treatment to fertility by oncology team	28%
Desire (more) children	57%
Considered fertility preservation before cancer treatment	21%
Actually visited a fertility doctor before cancer treatment	5%
Had fertility preservation treatment before cancer treatment	5%

Table 2: Decision Regret Scale (DRS), as it relates to decision to have undergone (or not) fertility preservation treatment (5 Least Regret, 25 Most)<sup>a</sup>

Desire children	Counseled by oncologist	
	Yes	No
Yes	13.5 [5 to 22]	18.5 [15 to 25]
No	10.3 [10 to 11]	11*

<sup>a</sup>19 participants without children before diagnosis; overall DRS = 13.9 [5 to 25]

\*Only one value in range.

**Conclusions:** Our preliminary data suggests decision regret scale scores may be lower if counseled regarding the potential for reproductive loss prior to treatment.

#### **[602] Extracapsular extension of axillary lymph node metastases in HER-2 receptor positive and negative breast cancers: prognostic importance**

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**Background:** Studies on the association of HER-2/neu with the axillary lymph node metastasis are controversial. Amplification of the protein product of the HER-2/neu oncogene in primary breast cancer specimens is associated with an adverse prognosis.

**Methods:** From January 2000 to December 2008, 504 breast cancer patients operated in General hospital "Sveti Vracevi" in Bijeljina. We selected 253 (50.2%) patients with breast cancer who had metastases to axillary lymph nodes.

**Results:** Extracapsular extension (ECM) was found in 103 (40.7%). The patients were identified and divided into two groups: patients in the HER-2 positive group (38 patients) and HER-2 negative group (65 patients). ECM was seen in 10 of 16 (62.5%) patients in the HER-2 positive group compared with 5 of 17 (29.4%) in the HER-2 negative group ( $P = 0.059$ ). Total number of lymph nodes showing ECM were also significantly more in the HER-2 positive group (48 of 81, 59.25%) vs. (13 of 60, 21.66%) in the HER-2 negative group ( $P < 0.001$ ). With a median follow-up of 96 months factors with independent prognostic value for disease-free survival by multivariate analysis included HER-2/neu overexpression with extracapsular extension ( $P < 0.005$ ), pN category ( $P < 0.01$ ), presence of lymphovascular invasion (LVI;  $P < 0.005$ ), and ECM ( $P < 0.001$ ). An independent negative prognostic effect on overall survival was observed for HER-2/neu overexpression with extracapsular extension ( $P < 0.05$ ), pN category ( $P < 0.05$ ), and presence of LVI ( $P < 0.005$ ) and ECM ( $P < 0.001$ ).

**Conclusions:** In patients whose tumours expressed HER-2/neu who had positive lymph nodes and extracapsular extension prognosis was significantly worse compared with those who were HER-2/neu negative and lymph node positive with extracapsular extension. These findings have led to the conclusion that HER-2/neu overexpression is associated with a more aggressive subtype of cancer.

#### **[603] Long-term accumulation of platinum (Pt) and its impact on self-reported neuro- and ototoxicity in cisplatin-treated testicular cancer survivors (TCSs)**

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**Background:** Platinum (Pt) induced neuro- and ototoxicity (NTX) are important, long-term complications of chemotherapy for TC. Although residual Pt can be measured in serum for many years after the completion of cisplatin-based chemotherapy, its impact on NTX has not been assessed.

**Material and Methods:** 165 testicular cancer survivors (TCSs) treated with Pt provided blood samples at survey I (1998–2002) and reported NTX during both survey I and II (2007–2008) 5–28 years after therapy. Symptom severity was self-reported by a four categorical scale.

Serum Pt concentrations were quantified using a Perkin Elmer DRC II Inductively Coupled Plasma-Mass Spectrometer. We assessed associations between applied cumulative Pt dose, residual Pt levels and NTX at survey I and II using ordinal logistic regression analyses.

**Results:** Both initial Pt dose and residual Pt concentration were associated with NTX (Table).