to determine efficacy of DC-immunotherapy has been undertaken in 7 advanced ovarian cancer patients. On average (5-11,2) $\times$ 10^6 DC were administered by intravenous infusion.

Results: DC were identify by morphology method and had CD3$^-$CD20$^-$CD16$^-$CD14$^-$HLA-DR^{++}CD86^{++}-phenotype. DC were able to stimulate of autologous and allogeneic lymphocytes proliferation at the ratio 1:100 or 1:300 (DC: lymphocyte). DC supernatant caused the autologous and allogeneic lymphocytes proliferation at the phytohemagglutinin level. In 1 month after DC infusion lymphocytes of the patients acquired ability to react to autologous tumour lysate by proliferation response. At the same time increased number of CD3$^+$, CD4$^+$, CD16$^+$, CD11b$^+$, CD38$^+$, but not CD8$^+$-lymphocytes in peripheral blood was found.

Conclusions. Sufficient numbers of mature DC from exudate cells were obtained after short-term in vitro generation. DC were verified by phenotype and their function in vitro. Based on obtained results, this strategy represent a promising approach for the adoptive immunotherapy of advanced ovarian cancer patients.

1002 POSTER

Evaluation of RFLP, DNA sequencing, PCR-SSCP and line probe assay for HPV genotyping

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Cervical cancer is one of the most common malignancy both in incidence and in mortality in women worldwide. In Croatia, in 1993, cervical cancer took the third place following breast and stomach cancer. Therefore, early detection of HPV infection, as a leading cause of cervical cancer, is of major importance. In Croatian population, approximately 40% of samples positive by polymerase chain reaction (PCR) with universal HPV primers (MY09/MY11 and L1C1/L1C2) remained undetermined with type-specific primers for HPV 6/11, 16, 18, 31 and 33. The aim of this study was to identify HPV genotypes (HPV X) that remained undetermined, in order to determine the prevalence of other supposed high risk HPV types other than 16, 18, 31 and 33. For that purpose, we compared restriction fragment length polymorphism (RFLP) analysis, DNA sequencing (Alf-express system, Amersham Pharmacia Biotech), PCR-single-strand conformational polymorphism (PCR-SSCP) analysis and line probe assay (LiPA, Innogenetics). MY09/MY11 amplicons were analysed by RFLP using DdeI, DraI, PstI, Sau3AI, BamHI, HaeIII and/or RsaI restriction enzymes, DNA sequencing and PCR-SSCP. Amplicons obtained with general primer set, SPF10, which allows identification of 25 HPV genotypes, were hybridized according to the manufacturer's instuctions (LiPA). Out of 35 HPV X samples, single HPV infection was determined in 20 (57%) and 22 (63%) cases, multiple infections in 4 (11%) and 9 (26%) cases, by RFLP and LiPA, respectively. The most frequently observed types were HPV 53 and 58, both in 5 cases (14%). RFLP and LiPA did not allow the identification of HPV types in 11 (32%) and 4 (11%) cases, respectively. The remaining HPV-positive unresolved specimens were identificated by DNA sequencing. PCR-SSCP analysis was used to confirm multiple infections detrmined by RFLP and LiPA. The advantage of RFLP and DNA sequencing of PCR products over LiPA is the ability of genotype larger number of HPV types. However, multiple HPV infections can not be discriminated clearly enough. PCR-SSCP analysis proved to be a good method of choice for confirmation of multiple HPV infections. Yet, our preliminary results should be confirmed on a larger number of samples.

1003 POSTER

Chemopreventive administration of EB1089, a vitamin D analogue, on spontaneous mouse mammary and hepatocellular carcinoma.

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Background: EB1089, an analogue without the acute side effects of Vitamin D, exerts strong antiproliferative activities on malignant cells, including breast and hepatoma cells in vitro and in vivo. It also induces cell cycle arrest and apoptosis in premalignant conditions, suggesting its application in chemopreventive trials. We examined the possible chemopreventive effect of EB1089 on spontaneous mouse mammary (SMMC) and hepatocellular carcinoma (HCC) incidence on C H/Sy mice. SMMC constitutes one of the most widely used model systems, in which a confluence of hormonal and viral agents are implicated. C H/Sy mice develop a high incidence of SMMC and HCC between 8-12 months.

Materials and Methods: A total of 95 virgin female mice, 16 weeks old, were used. EB1089 injections of 0.5 1/4 g/Kg of body weight were given intraperitoneally every other day for 2, 4 and 6 months to 51 mice (18, 19 and 14 mice respectively). The remaining 44 mice were divided into 3 control groups, accordingly, and injected with the vehicle solution only. The mice were sacrificed when they appeared moribund. The rest were sacrificed at the age of at least 80 weeks. Urine samples were collected during the experimental period and blood samples just before sacrifice for calcium levels evaluation. A full autopsy was performed and mammary and liver tissues were processed for histological examination.

Results: The results obtained show that 62.75% of treated mice developed mammary carcinoma compared to 38,64% of the control group. On the same time a 3.9% of treated mice developed hepatocarcinomas, exclusively in the 2 month group, compared to 36.4% of hepatocarcinomas in the control group. Urine calcium levels increased significantly immediately after commencing the treatment with EB1089 in all groups, remained very high during the whole treatment period and gradually decreased at the end of the treatment, until they reached calcium levels of the control groups. Blood calcium levels of treated groups were higher, statistically significant different from those derived from the control groups.

Conclusion: Our results suggest that the administration of EB1089 has no chemopreventive action on the incidence of SMMC, it rather promotes tumor progression. It causes a very statistically significant ($p<0.0001$) inhibitory effect on HCC incidence of C H/Sy mice. These effects could be useful only as a potential application on the chemopreventive control of HCCs.

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1004 POSTER

Effects of docetaxel on apoptosis-related proteins in patients with adenocarcinoma of the esophagus.

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Bcl-2 and its homologs Bcl-x, Bax, and Bak are key regulators of apoptosis. The apoptotic pathway is central activity of cytotoxic chemotherapy. In vitro studies of taxanes have shown modulation of this pathway by phosphorylation of Bcl-2. This clinical study was designed to examine the effects of docetaxel on apoptosis-related proteins in patients with adenocarcinoma of the esophagus. Presented are the results of tissue studies obtained before and one day following docetaxel chemotherapy. Twenty-four (24) stage II-III esophageal cancer patients were enrolled into two consectutive novel tri-modality therapy programs. Endoscopic biopsies were taken prior to and one day following the initial dose of 80 mg/m2 of docetaxel. Seventeen (17) patients had specimens obtained pre-treatment and one day post-treatment with docetaxel. Biopsies were divided into 3 parts: Part 1 was fixed and analyzed for confirmation of tumor, p53 status, and apoptosis (TUNEL staining); Part 2 was flash frozen at -70°C for subsequent lysis and western blotting for Bcl-2, Bcl-x, and MPM-2; and Part 3 was disaggregated, filtered, and fixed in ethanol for analysis of cell cycle phase and concurrent MPM-2 expression. The amount of protein in each lysate was assayed, and an equal amount of protein was loaded in each well. Loading controls of 500 - 50,000 MCF-7 cells were run on the same gel to define a relative expression level for each sample. Samples were also probed for the pro-apoptotic Bax protein. Patient characteristics: 19 males, 5 females; 4 pts had stage IIA, 2 stage IIB, 18 stage III, median age 66 years (range 34-81). All pts had adenocarcinoma. All tumors exhibited Bcl-2 by Western blot with no consistent band shift observed due to therapy. All tumors expressed Bax at approximately the same level, and no changes occurred as a result of therapy. Also, there was no evidence of bands with retarded mobility in any of the samples. The absence of any evidence for Bcl-2 phosphorylation initially suggests that docetaxel did not get to the tumor, or did not have the anticipated effect in vivo. However, clinical antitumor activity was noted in these patients and the data from cell culture experiments has shown that this Bcl-2 phosphorylation only occurs in G2/M phase of the cell cycle. In vivo, the majority of cells are not replicating, hence far fewer cells are likely to be arrested in G2/M. Accordingly, it is unlikely that enough cells would have accumulated in G2/M following docetaxel to cause a significant phosphorylation of Bcl-2. Clearly, more sensitive assays of mitotic arrest such as posphoBcl-2 specific antibiodies would establish whether docetaxel function through the expected mechanism in vivo. Funded in part by NIH CA23108 and Aventis Pharmaceuticals.