cancer. T is synergistic with several cytotoxic drugs such as vinorelbine (V), gemcitabine and taxanes.

Objective: We have assessed the activity of vinorelbine plus tratuzumab in patients with erb-B2 overexpressing metastatic breast cancer.

Patients and methods: From January 1999 to October 2002, 15 patients have been treated with Vinorelbine 30 mg/m2/week plus trastuzumab 4mg/kg (first week) followed by 2 mg/kg/week. All patients had +++ erb-B2by immunohistochemistry in the primary tumor.

Results: Overall, 297 treatment courses were given (median = 36 courses per patient, range 4-48+). Median age was 48 (range 31-67). Median time from diagnosis to first relapse was 20 months (range 11-63). Number of prior chemotherapies for metastatic breast cancer was 1 (0-3). The most relevant toxicity was grade 3 leukopenia requiring omission of Vinorelbine in 35 courses. Grade 1-2 neuropathy in most patients receiving more than 10 courses was treated with gabapentin and did no require treatment discontinuation. One patient achieved a complete response (6.6%), 9 patients had a partial response (60%), one patient (6.6%) had stable disease for more than 2 months and 4 (26.6%) had progressive disease. Median time to progression was 24 weeks (range 4-56). Median survival was 36 weeks (range 4-130+).

Conclusions: Weekly Trastuzumab plus Vinorelbine is an active and well tolerated treatment option for patients with erb-B2 overexpressing metastatic breast cancer

998 POSTER

A phase II study of a novel taxane BAY 59-8862 in patients with aggressive refractory non-Hodgkin's lymphoma

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BAY 59-8862 (BAY) is a novel second-generation taxane. Compared to paclitaxel and docetaxel, it is 20-30 fold more potent as a growth inhibitory agent against human breast and colon tumor cell lines expressing P-glycoprotein 170. BAY is also active against multidrug resistant human colon xenografts. This phase II study was conducted to assess the efficacy (response rate) and toxicity of BAY in patients (pts) with Aggressive Refractory Non-Hodgkin's Lymphoma. BAY (75 mg/m2) was administered intravenously over 60 minutes every 3 weeks. Pts eligible were pts with Aggressive Refractory Non-Hodgkin's Lymphoma and having received no more than 3 prior chemotherapy regimens; with performance status 0, 1 or 2, adequate hematology and biochemistry; and at least one bi-dimensionally measurable lesion. Between March 2002 and March 2003, 29 pts entered the study: 9 female, 20 male; median age was 60 years; performance status 0/1/2 was 9/18/2. All were eligible and evaluable for toxicity. To date 19 pts are evaluable for response (4 pts too early for assessment, 6 pts had no repeat imaging). Number of prior chemo regimens was 1 (4), 2 (13), 3 (11), 4 (1). The median number of treatment cycles was 2 with 5 pts receiving 4 cycles of therapy and 1 pt receiving 7 cycles of therapy. Common grade drug related effects (study dependent) included nausea (9), fatigue (8), vomiting (3), peripheral neuropathy (3), anorexia (3) and skin rash (1). Grade 3-4 hematologic toxicities included neutropenia (15), anemia (6) and thrombocytopenia (3). Seven minor responses were observed out of the 19 pts now evaluable for response. Four pts continue on treatment to date. The recruitment is currently on hold and the interim analysis ongoing.

999 POSTER

Immunomonitoring in stage II melanoma patients treated with adjuvant GM-CSF

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Background: The importance of GM-CSF in the adjuvant treatment of skin melanoma has been discussed. We evaluated immunologic effects of postoperative immunotherapy with GM-CSF in stage II melanoma patients (pts).

Methods: 15 pts with stage T N M of skin melanoma were treated with low-doses of GM-CSF (1 mkg/kg, s.c., three days per week) for 1 year after surgical excision of the primary tumor(study group). Results were compared with 15 pts who received no adjuvant treatment after surgery (control group). All patients had ECOG performance status of 0. The median age was 41.2 years in the study group and 47.1 years in the control group. The men-women ratio was 1:2 in both groups.

Results: Before treatment the level of NK cells in study group was 2 times higher than normal ($p<0.001$). The level of HLA class I molecules as well as CD4, CD22, CD38 molecules was reduced ($p<0.05$). The immunological values of all others were within normal values. During therapy with GM-CSF an increase of HLA class I molecules expression, activated lymphocytes (CD38), helper T cells (CD4) and B-lymphocytes (CD22) and a decrease of NK cells (29,3 vs. 12,1) were shown ($p<0.05$). The percentage of CD8+T cells was 32,7 and 21,4 before and after treatment, respectively (t=1,95). The CTL cells depression may be explained by their migration to lymph node tissue. We observed an escalation of monocyte and lymphocyte count in study group ($p<0.05$). Three-year overall survival was 84,6% in study group and 66,5% in control group. The time to progression was 422,7 months in the study group and 356,5 months in the control group.

Conclusions: The adjuvant immunotherapy with GM-CSF induces tumor-specific immune response with an increase of HLA class I molecules expression. Despite the fact that both groups developed regional and distant metastases, survival rate of the study group patients was higher.

1000 POSTER

Pioglitazone and rofecoxib combined with angiostatic scheduling of chemotherapy in far advanced malignancies

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Purpose: Combined tumor- and stroma-cell targeted therapies might control chemorefractory malignancies.

Experimental design: A phase II trial was initiated to analyze the activity of a continuously administered molecular-targeted therapy (daily 45 mg pioglitazone po and 25mg rofecoxib po) combined with sequentially added angiostatic scheduled chemotherapy, in metastatic neoplasias with intrinsic or acquired drug resistance: Indication group A (67 cases) received capecitabine 2x1g/m^2 po from day 14 to 28, every 3 weeks, indication group B (37 cases) trofosfamide 3x50mg po daily, day 14+.

Results: Up to now 104 patients (pts) with 21 different tumor types are evaluable. Major side effects (WHO grade 3 and 4) were due to hand-foot-syndrome in 7 cases. Clinical response (CR, PR, SD > 6 months) occurred in 28% of the patients in Group A and B, in 25 and 40% of the patients with acquired and intrinsic drug resistance, respectively. A more than 50% decrease of tumor-associated CRP levels during treatment with the biomodulators alone was significantly associated with clinical response, p= 0.001.

Conclusions: This is the first study to show that novel therapeutic approaches including anti-inflammatory, angiostatic and cytostatic therapy are effective, with manageable toxicity profile in a range of chemorefractory malignancies.

1001 POSTER

Novel strategy of mature dendritic cells generation, suitable for adoptive immunotherapy of the ovarian cancer patients

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Background: The significance of adoptive immunotherapy in clinical oncology now clearly is not determined. There is increasing clinical interest in dendritic cells (DC) that are capable to initiate antitumor immune responses. Modern strategies of the generation of mature DC, pulsed with tumor antigens, have been shown to be effective methods. However, search of more simple and convenient methods of DC generation is object for future studies.

Material and methods: As a sourse of DC we used exudate from abdominal cavity, collected during 15 hours after tumour removal. CD45$^+$ 14$^+$-cells, isolated by plastic adherence, were cultured with 1.000 U/ml human granulocyte-macrophage colony-stimulating factor and 100 ng/ml lipopolysaccharide within 7 days. After 5 days of incubation DC were loaded with autologous tumor lysate (0,5 μ g/ml of protein, 1 $\times$ 10^7 cells). Surface marker analysis of DC was performed by flow cytometry and mAb- anti-CD3, CD20, CD16, CD14, CD86, HLA-DR. Function of DC in vitro and cytokines presence in DC supernatant were determined by study of their ability to stimulate of autologous and allogeneic lymphocyte proliferation. Pilot study

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.