macroscopically infiltrative type of esophageal carcinoma from the viewpoint of tumor angiogenesis.

Methods and Materials: A total of 40 surgically resected esophageal carcinoma tissues without preoperative treatment were selected at random from macroscopically localized type (n=20) and infiltrative type of esophageal carcinoma (n=20). The highest intra-tumoral microvascular density, Ki67 labeling index, and expression of VEGF in each section were estimated. The highest microvascular density was estimated in a magnification of x200 field where showed the most developed neovascularization in the tumor.

Results: The highest microvascular density was significantly (p=0.0006) greater in the infiltrative type than in the localized type, and Ki67 labeling index (p=0.022) were significantly lower in the infiltrative type than in the localized type. The expression level of VEGF was significantly (p

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The Casein kinase 1 delta (CK1 delta) specific inhibitor IC261 impinges growth of pancreatic tumor cells and the expression of CK1delta in healthy young adult BALB/c mice

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Background: During the development of pancreatic tumors mutations in oncogenes and tumor suppressor genes and alterations in signal pathway occur. CK1 delta, especially CK1delta, mediated signals seem to play an important role in insuring genome integrity. Alterations in CKIδ mediated signals may play an important role in pancreatic tumorigenesis.

Methods and animals: CK1delta expression levels were analyzed in ASPC1, BXPC3, Capan1, Colo357, Panc1, Panc89, and PancTu1 by Western blotting. Cells treated with the CKIδ specific inhibitor IC261 were analyzed by FACS analysis at different time points (12, 24, 36, and 60 hours). In addition, immunofluorescence (IF) analyses were performed using a polyclonal rabbit anti pericentrin serum. Tissue specific distribution of CK1delta in perfusion fixed, paraffin embedded pancreatic tissue of 4 to 6 weeks old BALB/c control and IC261 treated (1mM) mice were detected by the CK1delta specific polyclonal antiserum NC10.

Results: Different time courses indicated good response between 0.4 and 1.6μM IC261 in PancTu1 cells. Therefore, different pancreatic tumor cells were treated with IC261 (1 μM). Our FACS analysis revealed a cell line specific sensibility towards IC261 which lead to cell death or to cell cycle arrest in a G1 like status. Furthermore, structural changes and amplifications of centrosomes could be detected by IF. Immunohistochemistry of CKIδ in the pancreas of young adult BALB/c mice revealed a finely granulated staining in the exocrine part in the cytoplasm of the acini cells, the intralobular, and interlobular ducts. The cytoplasm of cell types in the endocrine part was strongly positive. Inhibition of CKIδ activity by IC261 was accompanied with a reduced CK1' staining in the whole pancreas.

Discussion: Our results show that inhibition of CKIδ by IC261 differentially effects the growth the pancreatic tumor cells and reduces CKIδ levels in the pancreas of mice. Therefore, down regulation of CK1' could be used as a new approach in the treatment of pancreatic cancer.

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A novel adenoviral vector encoding angiogenin cDNA from cancerous liver tissue

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Background: Angiogenin is a 14 kDa protein with a potent angiogenic effect and a poor ribonuclease activity. In previous reports, these activities have been studied with the use of recombinant proteins. To further characterize these functions, we have constructed a replication-defective adenoviral vector with an angiogenin cDNA isolated from a hepatoma library (Neznanov N *et al.*, Mol.Biol Moscow 1990), which differs in its 5'UTR from the original cDNA reported in non-cancerous liver tissue (AV646980).

Material and Methods: 1) Construction of the adenoviral vector: the human angiogenin cDNA was isolated from the pBluescript SK-Angio plasmid with BamH1 / Xho1 restriction. This fragment was cloned into the pcDNA1 plasmid, further isolated by restriction with Not1/EcoRV and sublconed into the pAdTrack-CMV plasmid. All plasmids were evaluated by restriction analysis, PCR and/ or sequencing. The construction of E1a-, partially E1-b, and partially E3-deleted vectors based on human adenovirus type 5 Ad vectors was carried out as previously described (He, 1998). The resultant viruses were purified by ViraPrep columns© and quantitated by OD 260/280 and plaque assay. 2) Human fibroblasts and HeLa cells were infected at different m.o.i. (1 to 20) in serum free media for two hours; 3) Flow cytometry analysis was used to evaluate the transfection efficiency and survival rates; 4) RNA and protein extractions were carried out at 24 and 48 hours after infection, respectively; 5) RT PCR and further amplification of the 5'UTR and coding regions was achieved by two different sets of primers; 6) Western Blot analysis was carried out as described elsewhere using a polyclonal antibody against human angiogenin.

Results: An adenoviral vector containing the Green Fluorescence Protein and angiogenin genes under the transcriptional control of the CMV promoter has been constructed (Ad-Angio-GFP). The transfection efficiency of this virus was over 90% in fibroblasts and HeLa cells. No cytopathic effects were observed even at the highest dose tested (i.e. 20 m.o.i). The expression of the exogenous angiogenin gene was dose dependent in both cell lines. Interestingly, we could also amplify the 5'UTR of our angiogenin transgene in cDNA from non infected-HeLa cells, but not from intact fibroblasts' cDNA. The angiogenin protein could be tracked down by WB in both, cellular extracts and culture media. Thus, it seems that the angiogenin protein can be secreted by infected cells. The concomitant expression of GFP allowed us to monitor the expression of the transgenes in all conditions.

Conclusion: a novel adenoviral vector that expresses an angiogenin transcript found in cancerous liver tissue, has been constructed. The functional activity of the encoded protein is under study.

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Effect of retinoic acid analogue on tumor growth and angiogenesis

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Background: Retinoic acid (RA) can be regarded as a pharmacological agent that is commonly used for its ability to affect growth and differentiation of a variety of cell types, such as several tumor and endothelial cells. In the present work, we studied the effect of all-trans RA (ATRA) and its analogue EA4 on the growth of several human prostate normal epithelial and tumor cell lines in vitro, as well as the formation of new capillaries, in the in vivo chicken embryo chorioallantoic membrane (CAM). Methods ATRA was purchased from Sigma Chemical Co. and the modified steroid EA16 was prepared as described in literature. For the synthesis of the final esteric derivative EA4 the method of esterification with unsymmetrical anhydrite was applied. At first step the unsymmetrical anhydrite of ATRA with 2,4,6-trichlorobenzoylchloride was prepared and then by adding the EA16, under the appropriate conditions, the desired product EA4 was obtained. The biological evaluation of ATRA, EA16 and EA4 was performed on the human prostate cell lines PC3, LnCap and PNT1. The MTT assay was used to measure the number of cells after treatment with different concentrations of the various agents for several time periods.The effect of the agents on angiogenesis in the chicken embryo CAM, as well as on the morphology of the tissue, was estimated in tissue paraffin sections stained with haematoxylin and eosin.

Results: ATRA caused a slight decrease in the number of prostate cells only at the concentration of 10-5 M. Higher concentrations could not be tested because of solubility problems. The analogue EA4 significantly decreased the number of tumor but not normal prostate cells, in a dose-dependent manner. This decrease was significant even at concentrations lower than 10-7M of EA4 and was not due to the steroid component (EA16) of the molecule. ATRA and EA16 induced angiogenesis in the CAM and moreover, ATRA increased the layer of CAM keratinocytes and induced the deposition of fibrin matrix. EA4 had no effect on either angiogenesis or tissue structure in general.

Conclusions: The retinoid EA4 seems to be a promising agent for the inhibition of tumor prostate cell growth.

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Activity of trastuzumab plus vinorelbine in patients with erb-B2 overexpressing metastatic breast cancer

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Introduction: Trastuzumab (T) is an anti-erb-B2 humanized monoclonal antibody with activity in patients with erb-B2 overexpressing metastatic breast

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

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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.

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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.

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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.

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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