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Treatment with a combination of mMAb17-1A, GM-CSF, alpha-Interferon and 5-Fluorouracil of patients with advanced colorectal carcinoma (CRC).

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Background: The mouse monoclonal antibody MAb 17-1A (IgG) recognizes the tumor associated antigen CO17-1A/ GA 733-3/Ep-CAM, which is abundantly expressed in CRC. The clinical effect of MAb17-1A alone in advanced CRC is modest. Combination with cytokines, as granulocyte/macrophage-colony-stimulating-factor (GM-CSF), improved significantly the clinical response, with some remarkable long-lasting complete remissions. Chemotherapeutics such as 5-fluorouracil (5-FU) can serve as immunomodulating agents. Based on previous clinical studies and on a preclinical animal model, we have conducted this phase II study in patients (pts) with advanced CRC.

Material and methods: Twenty-seven pts were included were treated with the following regimen: Recombinant human α -interferon (α -IFN)(3 X 10^6 IU) was given subcutaneously (s.c.) once daily day 1 through 5. 5-FU (500 mg/m^2) was administered as a daily intravenous (i.v.) bolus injection on days 4 and 5. Two days' rest was followed by GM-CSF (200 μ g/m^2 /day) s.c. once daily day 8 through 14. MAb17-1A (400 mg) was given as a single infusion on day 10. The treatment was repeated every 4th week until disease progression.

Results: Twenty-six of 27 pts were evaluable for response. 54% (14/26) (1 PR, 2 MR, 11 SD) had a clinical response. The median progression-free survival (PFS) and overall survival (OS) were 3.2 months (range 1-17 months) and 17.5 months (range 1-38.5 months), respectively, from start of MAb 17-1A therapy. Each pts received a median of 4 cycles (range 1-9 cycles). The total number of treatment cycles were 119. Treatment was well tolerated with no grade IV toxicity. There was a tendency to reduced frequency of side-effects related to the administrations of cytokines by increasing number of treatment cycles. Allergic reactions to MAb 17-1A increased by each treatment cycle but could significantly be reduced by lowering the MAb dose and increasing the infusion time. The intended full dose of MAb 17-1A had to be reduced at 71% of the infusions.

Conclusion: This phase II study was the 10th consecutive treatment protocols conducted and evaluated by the same group of clinicians evaluating the therapeutic effect of MAb17-1A in pts with advanced CRC. In the present study, the addition of 5-Fu, GM-CSF and α -IFN to MAb17-1A therapy seemed to augment the clinical effect of MAb17-1A in pts with advanced CRC. Compared to MAb17-1A therapy alone the clinical response rate increased from 15% to 54%. The majority of the responses was SD > 3 months. Furthermore, PFS was significantly improved from 1.5 months to 3.5 months. Based on the adjuvant studies using Panorex where this anti-EpCAM MAb seemed to have a clinical effect by itself, with an antitumor mechanism differ from conventional chemotherapeutics and on the results of the present study, it might be suggested to incorporate anti-EpCAM antibodies into current treatment protocols in pts with metastatic CRC.

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Serial monitoring of serum HER-2 extracellular domain (H-ECD) during herceptin-taxol chemotherapy (CT) for metastatic breast cancer (MBC) pts: preliminary results from the French experience (HER.ME.S protocol).

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Rationale: The H-ECD level is increased in the sera of pts with overexpressed MBC. Circulating levels of the H-ECD have been reported to be predict for response to CT. Study: The French protocol HER.ME.S is a phase IV, pharmaco-economic, on-line study in which we were interested to determine whether serum levels of H-ECD (Oncogene Science -Bayer Diagnostics-Elisa kit) would predict the course of disease in HER-2+ MBC pts. 180 HER-2+ MBC pts are planned to be treated in the HER.ME.S protocol with a combination of Taxol (175 mg/m2/3w or 80 mg/m2/w, 6 weeks/8) + Herceptin (4 mg/kg on week 1, followed by 2 mg/kg/w) or Herceptin alone, until progression or unacceptable toxicity. Since 09/01, 72 pts have been screened for HER-2 status; 35 of them were HER-2+. The H-ECD levels were determined during the following periods: pre-inclusion, inclusion (day 0), then days 28, 56, 84, 102 and 120 for HER-2+ pts. Evaluation of the disease was performed according to the RECIST criteria every 2 months until withdrawn of the study.

Results: Results are available today for all the 72 screened pts and the first 30 included pts. At the time of pre-inclusion, the median level of H-ECD for the 72 pts was 105 ng/ml (7-1500). Forty-eight pts (90% of them being HER-2+) were determined above 15 ng/ml (level generally considered as the cut-off value between normal pts and MBC pts). For the first 30 included pts, the median H-ECD level was 156 ng/ml (7-1500). For 16 pts with low metastatic spread (< 3 metastatic sites), the mean H-ECD level was 27 ng/ml (11 - 63). For 14 pts with high metastatic spread (3 or more metastatic sites), the mean H-ECD level was 297 ng/ml (7 - 1500). Nineteen pts were evaluated after one month of treatment: 53% responded, 37% were stable and 10% progressed. No progressive pts had a decrease in H-ECD level; among stable pts, the mean decrease of H-ECD level was 10% (-135% to 41%); no responder pts had an increase in H-ECD level and the mean decrease was 44% (6% - 89%).

Conclusion: ratio of H-ECD variation (initiation vs one month determination) seems to be an early predictive response factor.

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Human phase II/A study of a novel somatostatin analogue, TT-232 in malignant melanoma patients

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TT-232, a novel somatostatin analogue, with a five residue ring structure (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2) has shown a strong antitumor effect both in vitro and vivo. TT-232 has practically no growth hormone release inhibitory activity and it is a potent inducer of apoptosis. It seems to act via shortterm induction of tyrosine phosphatase and inhibition tyrosine kinase.

Patients (pts) with histologically proven advance stage malignant melanoma had been treated with 480 μg/kg TT-232. daily 4-hour infusion on the following days 1-7, 15-21. The next cycle has been started on day 29.

Between August 2001 and September 2002 twelve patients (male:6, female:6) were enrolled in the study. The median age was 52 yrs. (rage 27-72). The primary tumor localization was the following: trunk:7, head:1, ocular:1, upper extremity:1, lower extremity:2. All pts had previous surgery, 8 had radiotherapy, all received chemotherapy and 11 had immunotherapy.

Altogether 49 cycles had been given (range 2-8, median 4). One patient had partial remission (lymph nodes) and 3 pts had stable disease (hepatic, mediastinal, rertoperitoneal lymph nodes and pulmonary metastases).

Out of 343 days of treatment fever (gr.1-2) has been observed on 8 days. No other toxicity occurred.

Serum level of TT-232 has been measured with an enzyme immunoassay (ELISA) method. Blood samples were taken on day 1 at 0,2.4,8 hours. The free peptide concentration has raised during the first 2 hours. At 8 hours approx. 25% of the max. serum level was still present.

TT-232 seems to be a promising new agent in the treatment of malignant melanoma with no obvious toxicity.

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Positive correlation between expression of vascular endothelial growth factor (VEGF) and highest microvascular density in esophageal carcinoma

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Purpose: The prognosis and the characteristics are different according to the individual tumor in esophageal carcinoma. It has been suggested that the prognosis of macroscopically infiltrative type of esophageal carcinoma is worse than that of the localized type even if clinical stage was same. The aim of this study was to determine the cause of unfavorable prognosis in

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