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Antiangiogenetic therapy with pioglitazone, rofecoxib and metronomic trofosfamide in advanced malignant vascular tumors

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Purpose: Systemic therapy options for advanced angiosarcomas are limited and the prognosis is infaust. The idea of angiostatic therapy following the paradigm of metronomic dosed chemotherapeutics combined with pro-apoptotic biomodulators has not yet been considered in these patients. Therefore, in a phase-II-study, the efficacy of metronomically scheduled low dose trofosfamide in combination with the PPARγagonist pioglitazone and the selective COX-2 inhibitor rofecoxib was evaluated in advanced vascular malignancies.

Experimental Design: Six patients with advanced and pretreated, but progressive malignant vascular tumors (5 angiosarcomas and 1 haemangioendothelioma) received a combination of pioglitazone (45 mg/d p.o.) plus rofecoxib (25 mg/d p.o.) and after 14 days additionally trofosfamide (3 x 50 mg/d p.o.). The therapy was administered continuously until progression was observed. If necessary, doses were modified according to side effects.

Results: Two patients responded with complete and one with partial remission, three achieved stable disease. Median progression free time was 7.7 month (2-15 month). Side effects were generally mild (WHO grade 1-2). Hospitalisation was not necessary.

Conclusions: This new triple combination of low-dose metronomic trofosfamide, pioglitazone and rofecoxib might represent a feasible new alternative in the palliative treatment of advanced malignant vascular tumors.

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Preliminary results of a phase I study of rhumab VEGF (bevacizumab) with concurrent radiotherapy (XRT) and capecitabine (CAP) in locally advanced pancreatic cancer

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Background: Bevacizumab is a humanized monoclonal antibody that prevents the binding of VEGF to its receptors. Preclinical studies show that antiangiogenic therapy can increase tumor perfusion (reduce hypoxia), radiosensitivity, and chemosensitivity. Results from phase I/II studies of bevacizumab in combination with radio- and chemotherapy show promising activity against many different tumor types, including pancreatic cancer. This phase I trial was designed to study the safety of bevacizumab plus chemo-radiation in locally advanced pancreatic cancer. Planned correlative studies included functional CT to evaluate blood flow.

Methods: The study was designed to accrue thirty patients with locally advanced, unresectable pancreatic cancer based on CT criteria. Bevacizumab (5 mg/kg IV) was administered to all patients 2 weeks prior to the start of XRT (50.4 Gy treating the primary and gross adenopathy), then every 2 weeks thereafter (2.5 mg/kg IV). CAP was administered concurrently with radiotherapy (650 mg/m^2, then 825 mg/m^2 PO BID, days 1452). Patients with stable or responding disease were offered maintenance bevacizumab (5 mg/kg IV q 2 wks) until progression. Functional CT was performed on days 0, 14, and at the time of restaging (5 weeks after XRT).

Results: To date, nine patients have been treated. There has been no significant hematologic or gastrointestinal toxicity, thrombosis, proteinuria, or hypertension. One patient had a tumor-related duodenal ulcer that bled two weeks after discontinuing therapy. It subsequently healed after the tumor responded to therapy. One of three evaluable patients completing therapy had a partial response and two had stable disease. One patient with stable disease had a Ca 19-9 level of 1000 that dropped to 177 (nadir not reached). Results from perfusion imaging indicated increased blood flow after the initial dose of bevacizumab.

Conclusion: Treatment with this novel combination of bevacizumab and chemoradiation is well tolerated and has activity in pancreatic cancer patients. We plan to escalate the dose of bevacizumab at 2.5 mg/kg increments to10 mg/kg. The data produced will be used as the foundation a randomized RTOG study. Updated data will be presented at the meeting.

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Antitumor activity of trastuzumab (Herceptin®) in human gastric cancer models

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Background: Trastuzumab (Herceptin®) is widely used as a standard therapy for patients with HER2 overexpressing metastatic breast cancer. Since HER2 overexpression has also been reported in gastric cancer, we explored the antitumor activity of trastuzumab in human gastric cancer xenograft models.

Results: Among 9 human gastric cancer xenograft models examined, NCI-N87 and 4-1ST models showed HER2 overexpression. Both lines were HER2 positive in immunohistochemistry (HercepTest®) and showed gene amplification in fluorescence in situ hybridization (PathVysion™) (8.4 and 5.3 signal ratio, respectively). When trastuzumab was administered i.p. twice a week, at an initial dose of 20 mg/kg and maintenance doses of 10 mg/kg, to mice bearing these HER2 positive xenografts, significant antitumor activity was observed. Tumor growth inhibition in NCI-N87 and 4-1ST was 73% and 61%, respectively. In contrast, trastuzumab administered to mice bearing HER2 negative-gastric cancer xenograft, GXF97, did not show any significant antitumor activity. Activity of trastuzumab in combination with standard agents used for gastric cancer chemotherapy was also examined. Trastuzumab administered in combination with either paclitaxel, docetaxel, 5'-DFUR or capecitabine in the NCI-N87 model, or in combination with CDDP or CPT-11 in the 4-1ST model showed potent antitumor activity which was significantly greater than that of either single agent in each case.

Conclusions: Trastuzumab showed significant antitumor activity in HER2 positive-human gastric cancer xenograft models, both as a single agent and in combination with standard agents used for chemotherapy of gastric cancer. These preclinical findings support clinical evaluation of the antitumor activity of trastuzumab in patients with HER2 positive-gastric cancer.

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Induction of apoptosis in herpes simplex virus thymidine kinase/aciclovir transfected experimental breast cancer models.

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Background: The induction of apoptosis of tumor cells by suicide-gene system HSV-TK/ACV was studied in MCF-7 and T47D human breast cancer cells. The mechanism of TK/ACV apoptosis induction considered to be mediated by caspase cascade activation following formation of DISC-complex, TRAIL receptor activation and mitochondrial activation.

Material and methods: Cultures MCF-7 and T47D were transfected with pIRES2-EGFP-TK and control pIRES2-EGFP and pEGFP-N1 vectors. The expression of thymidine kinase in subcloned cultures was shown by RT-PCR 24h after transfection. Then cells were treated with acyclovir and different inductors of apoptosis: TNF-alpha and TRAIL (TNF-related apoptosis induced ligand). DNA fragmentation out of and under the death receptors blockage by anti-APO-1, anti-TNF-alpha antibodies was measured by flow cytometry analysis of propidium iodide-stained cell nuclei. Annexin-V staining was used as a more relevant method for apoptosis detection. Caspase inhibitor zVAD-fmk was used in order to modify the effect of apoptosis inductors, caspase-8, caspase -9 expression was detected by immunoblot assay.

Results: The induction of apoptosis by ACV was evident in pEGFP-TK cells compare to control cultures. Aciclovir induced apoptosis in subcloned pEGFP-TK MCF-7 and T47D cultures in dose-dependent mode; TNF-alpha and TRAIL increased HSV-TK/ACV-induced apoptosis. The blockage of CD95, TNF receptors did not decrease TK/ACV-induced apoptosis effectively. Caspase inhibitor zVAD-fmk can greatly decrease apoptosis induction in pEGFP-TK cells as was demonstrated in both PI, Annexin-V flow cytometry analysis and immunoblot of caspase-8, -9 expression.

Conclusions: The mechanism of HSV-TK/ACV apoptosis induction involves different interchanging ways of receptors activation and caspase signaling. Contrariwise to non-specific caspase inhibition the blockage of one or more of death receptors did not cause the blockage of apoptosis in pEGFP-TK MCF-7 and T47D human breast cancer cells. The sensitization of HSV-TK/ACV cells for CD95, TNFR1,and TRAIL-receptor-induced apop-

tosis is due to up-regulation of the receptors and downstream sensitization of the respective pathways.

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The impact on human renal cell carcinoma cell line by transfection of Cox-2

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Background: Cyclooxygenase (Cox)-2 is an inducible isoenzyme of Cox that catalyzes the rate-limiting step in arachidonate metabolism. Many studies have indicated that Cox-2 plays an important role in carcinogenesis in several neoplastic diseases. We had reported there is Cox-2 expression in some human renal cell carcinoma (RCC). In present study, the impact on human RCC cell line by transfection of Cox-2 were investigated.

Materials and Methods: The expression vectors containing full-length sense and antisense cDNA of Cox-2 were constructed using the mammalian expression vector, pTargeT (Promega). Transfection of OS-RC-2, the human RCC cell line which overexpress Cox-2, with pTargeT/*Cox-2 sense*, pTargeT/*Cox-2 antisense* or vector control was done using LIPOFECTAMINE PLUS. The expression of Cox-2 in transfectants were detected by Western Blot and the production of PGE2 and VEGF by transfectants were examined by ELISA. Sensitivity of transfectants to apoptosis inducer, butyric acid, was observed by Fluorescence-activated Cell Sorting (FACS). The expression of CD44 in transfectant were detected by FACS, and the expression of MMP2 and MMP9 in transfectant were detected by FACS and zymography. The tumorigenicity of the transfectants were observed in nude mouse.

Results: The expression of Cox-2, PGE2 and VEGF were increased in sense transfectant and remarkable decreased in antisense transfectant. The sensitivity of antisense transfectants to apoptosis inducer was significantly higher than sense transfectant and the parental OS-RC-2. Although no difference of the expression of MMP2 and MMP9 observed between sense and antisense transfectant, there was significant difference of the expression of CD44 between sense and antisense transfectant. The result of tumorigenicity showed that overexpression of Cox-2 in sense transfectant can enhance tumorigenicity contrast to blocking Cox-2 expression in antisense transfectant.

Conclusions: Cox-2 expression may be related with some RCC carcinogenesis. Blocking Cox-2 expression in RCC cell line through anti-sense strategy suppressed growth of the cells in vitro and in vivo, as well as increased sensitivity of the cells to apoptosis inducer. These findings are suggestive of a new therapeutic strategy for some RCC through targeting Cox-2 expression.

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Interaction between Interferon-beta and cellular DNA-repair

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Background: Pretreatment of tumor cells with Interferon-beta (IFN) has reported radiosensitizing activity, associated with a decline of the shoulder and an increase of the alpha-component of the survival curve. We tested the possibility of an interaction between IFN-treatment and the regulation of the repair of radiation-induced damage.

Materials and Methods: The following cell lines were used: MCF-7, WiDr, ZMK-1, A549 expressing either wild type or mutated TP53, and DNA-PK-proficient M059K and DNA-PK-deficient M059J cells. Cells were incubated with IFN 24 h before irradiation, and cycling or confluent cultures were used. HDR irradiation was either given as single dose between 1 and 6 Gy or as split dose. LDR irradiation was given at total doses of 5.45 Gy and 12.5 Gy. DNA rejoining was measured by constant gel electrophoresis. The repair capacity of M059-K cells was inhibited by wortmannin treatment. Cytotoxicity was evaluated by a standard colony-forming assay; and survival curves were fitted by the linear-quadratic equation. Sensitizer enhancement ratios were calculated, and isobologram analysis was applied to test the IFN-radiation interactions. Apoptosis was determined morphologically.

Results: Sublethal damage repair was strongly inhibited after IFN treatment, with recovery ratios decreasing form 1.14 to 0.96 in cycling cells and from 1.59 to 1.00 in confluent cells. LDR irradiation of WiDr cells resulted in an inverse dose rate effect, which, after IFN-treatment, increased dramatically to a sparing ratio of 0.222 for cycling cells. There was no increase in initial DSBs and no alteration of DNA rejoining after IFN treatment. M059J cells showed a supraadditive, M059K cells an additive IFN-radiation interaction. In repair-inhibited M059K cells, we found an increase in IFN-induced radiation cytotoxicity. The TP53-status did not influence IFN-induced radiosensitization of A549 cells. Incubation with 3000 I.U./ml IFN enhanced the radiation-induced apoptosis in MCF-7 and ZMK-1 cells, but not in A549 cells.

Conclusions: In general, the sensitizing ability of IFN was higher in cycling cells compared to confluent cells and did not depend on the TP53-status. Increased radiation-induced apoptosis may play a role at high IFN concentrations. All results are pointing towards an interaction between IFN, or IFN-induced proteins and the regulation of the repair of radiation-induced damage as the predominant mechanism of IFN-related radiosensitization.

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Molecular mechanisms of G1 arrest of Antineoplaston AS2-1 against colon cancer.

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Introduction: Antineoplastons are naturally occurring peptides and amino acid derivatives found in human blood and urine, were first described by Burzynski in 1976. The small peptides reportedly control neoplastic growth and have minimum adverse effects. However, the molecular mechanisms by which Antineoplastons exert antitumor effects are not known. Characteristics of Antineoplaston were consider now that antagonism against l-glutamine and cell growth arrest to intercalation between DNA base pair, interaction of oncogenes and activation of suppressor genes to normalization of methylation status. In the present study, we have investigated the antiproliferative effect of Antineoplaston AS2-1 against colon cancer, and its influence to cell cycle.

Methods: We tested effects of Antineoplaston AS2-1 on *in vitro* and *in vivo* cell growth activity using human colon cancer cells (KM12SM, SW620, SW1417, Colo205). And we analyzed cell cycle of the Antineoplaston treated cells by FACS and investigated expression of cell cycle related factors by Western Blot.

Results: Antineoplaston AS2-1 inhibited the proliferation of all human colon cancer cells in a dose and a time dependent manner *in vitro*. Antineoplaston AS2-1 also inhibited the growth of implanted human colon cancer (KM12SM, SW620) in nude mouse in a dose and a time dependent manner *in vivo*. The cell cycle analysis demonstrated cell arrest at the G1 phase by treatment with Antineoplaston. The protein levels of cyclin-dependent kinase (cdk)-2, cyclin E, cdk-4, and cyclin D in the cells decreased and the levels of p16 and p21 increased in a time and dose dependent manner by Antineoplaston treatment. Antineoplaston AS2-1 also down-regulated the levels of the phosphorylated Rb protein.

Conclusion: Antineoplaston AS2-1 shows antiproliferative effect through the G1 cell arrest in colon carcinoma.

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Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune responses and clinical outcome

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Purpose: Many human cancers are associated with mutations in dominant and recessive oncogenes including *K-ras* and *p53* and frequently express mutant K-ras and p53 that are uniquely present in a patient's cancer cells but not in the normal tissue. Immunization with individual patient tumor-specific mutant peptides was evaluated for clinical use to induce tumor-specific cytotoxic T lymphocytes (CTL) against the tumor while avoiding immune reaction against normal organs. The aim was to assess 1) cellular immunity specific to an individual patient's tumor, 2) to assess whether such immunity can be induced or boosted by immunization with a synthetic peptide specific to the mutation in K-ras or p53, 3) to assess the toxicity of oncopeptide immunization, and 4) to monitor clinical outcome.