when it was administered during the time period that tumor IFP was lowered by PGE1. In contrast, the same dose of 5-FU alone did not influence tumor growth, apoptotic index or carcinoma cell mass. The combination therapy with PGE1 and 5-FU lacked significant anti-tumor activity when administered at sufficiently separated time points. Neither blood vessel density nor the level of leukocyte infiltration were changed after treatment with 5-FU and PGE1. Thus, reduced tumor IFP enabled increased delivery and treatment efficacy of the low molecular weight cytostatic 5-FU in solid tumors as assessed by tumor growth retardation and tumor morphology. These effects did not depend on an immune response nor on changes in the tumor vasculature. In conclusion, we provide for the first time direct evidence that reducing IFP in experimental solid tumors increases the delivery and efficacy of a low molecular weight cytostatic. This concept is an important approach to increase efficacy of anti-cancer drug in solid tumors.

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Phase I/II Study of CT-2103 (Xyotax) in patients with recurrent ovarian cancer

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CT-2103, a polymer-bound form of paclitaxel, shows selective distribution to tumors and is soluble in aqueous solution. CT-2103 preferentially exposes normal organs to conjugated paclitaxel, which does not bind tubulin, thus minimizing systemic toxicity. In an open-label, multicenter phase I/II study, ovarian cancer patients (pts) who have failed primary therapy with paclitaxel/platinum are receiving a paclitaxel-equivalent dose of CT-2103 175 mg/m^2 as a 10-min IV infusion every 21 days. NCI CTC (version 2) and RECIST criteria are being used to assess toxicity and efficacy, respectively. Eighty-eight pts have received from 1 to 9+ cycles. Efficacy data for 43 and safety for all 88 are available; 29 pts are still receiving treatment. Patients range in age from 33 to 81 yr. Median number (range) of prior regimens received was 2 (1-7) in platinum-sensitive (PSens) pts, 4 (1-9) in platinum-resistant (PRes) pts, and 6 (2-9) in platinum-refractory (PRef) pts. In 22 PSens pts, 5 had PR and 8 had SD, with overall disease control achieved in 59% of these pts. In 21 PRes/Ref pts, 1 had PR and 8 had SD, with overall disease control achieved in 43% of these pts. PSens pts had median progression-free survival (PFS) of 3.3 mo; PRes/Ref pts had similar PFS of 3.1 mo. To date, six patients have died. No Grade 4 drug-related toxicity or treatment-related death has been reported, and growth factors for hematologic support have not been required. The majority of patients did not require premedications; 8 pts have required pretreatment in subsequent cycles for Grade 1-2 hypersensitivity. Three pts progressed from Grade 1 or 2 neuropathy at baseline to persistent Grade 3 neuropathy, and minimal hair thinning occurred in 5 pts, but no alopecia has occurred. CT-2103 is well tolerated in these patients with recurrent ovarian cancer and shows antitumor activity across all categories of chemotherapy sensitivity and resistance. Further studies of CT-2103 include (1) a phase II trial in patients with recurrent ovarian cancer conducted by the GOG and (2) a randomized, comparative phase III trial of CT-2103/carboplatin vs paclitaxel/carboplatin in patients with newly diagnosed ovarian cancer. (Supported by Cell Therapeutics, Inc.)

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Evaluation *in vivo* of new agents for drug-resistant ovarian and breast carcinomas

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Therapeutic resistance to Taxol is a major issue in a number of cancers, particularly breast and ovarian carcinoma. This resistance is multifactorial, including P-gp170-linked MDR and over-expression of HER-2/neu. We evaluated the efficacy of a paclitaxel-poly(L-Glu) copolymer (PGA-TXL) in a human ovarian carcinoma orthotopic xenograft model which reflects resistance to Taxol (HEY); we also evaluated PGA-TXL as well as a liposomal (SUV) formulation of dimethyl-sphingosine (L-DMSP; which induces apoptosis in a broad spectrum of tumor cell lines *in vitro*) in an orthotopic human breast adenocarcinoma model that over-expresses HER-2/neu (MDA-361). In the ovarian model, early treatment (Day 2 post-implantation) with multiple-dose MTD Taxol (10 mg/kg) i.p. achieved slight improvement in survival, but was not curative. However, treatment with a single dose (180 mg/kg, paclitaxel equivalents) of PGA-TXL i.p. markedly improved survival and induced some apparent cures. The higher tumor burden present on Day 7 rendered this model resistant to MTD Taxol administration at this time, but still responsive to PGA-TXL. For the breast model, treatment on Day 7 post-implantation, before tumors were palpable, with PGA-TXL resulted in subsequent tumor growth delay, regression, or even apparent cure. Treatment at this time with a multiple-dose MTD regimen of L-DMSP (4.5 mg DMSP/dose) i.p., caused a delay in or reduced subsequent tumor growth, but was not curative. When administered later after tumors grew to 5-6 mm diameter, PGA-TXL still caused tumor growth delay, but no cures were observed; administration of L-DMSP at this later time was not efficacious We conclude that formulation of paclitaxel with this poly(L-Glu) backbone substantially enhanced its potency, and rendered it active in drug-resistant human ovarian and breast models. Further, we conclude that DMSP as a liposomal formulation has some efficacy against this HER-2/neu over-expressing breast model: however, only when the tumor burden is low. (Supported in part by DOD grants BC980420, BC991113 and OC000036 to JK).

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Phase II Study of first-line chemotherapy using CT-2103 in patients with Non-Small-Cell Lung Cancer who are > or = 70 years of age or who have PS = 2

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Xyotax (CT-2103), a polymer-bound form of paclitaxel, shows selective distribution to tumors. Xyotax is soluble in aqueous solution and exposes normal organs to conjugated paclitaxel, which does not bind to tubulin, thus minimizing overall toxicity. Xyotax showed enhanced efficacy compared with paclitaxel/Cremophor in syngeneic and xenogeneic *in vivo* tumor models of lung tumors (data on file, CTI). An open-label, multicenter phase II study is currently under way in chemotherapy-naïve patients with non-small-cell lung cancer who are at least 70 years of age or who are 18 to 69 years of age and have ECOG PS = 2. Patients are receiving a paclitaxel-equivalent dose of Xyotax 175 mg/m^2 in a 10-min IV infusion every 21 days for 6 cycles. Dose reductions for defined toxicities are allowed. NCI CTC (version 2) are used for safety assessments. RECIST criteria are used for efficacy assessments, which are done after every second cycle. Thirty patients have been treated. Preliminary unmonitored data are available. The median age is 77 yr (range, 49-90 yr). Thirty-three percent of patients have PS=2. Thirteen patients (43%) achieved disease control (PR or SD). Nine patients completed the full course (6 cycles) of therapy. With a median follow-up of 6 months, median progression-free survival has not been reached. Twenty-four patients are alive. Treatment was well tolerated. One patient experience drug-related Grade 4 neuropathy and discontinued for disease-related symptom deterioration. One patient was hospitalized for drug-related fever. No other drug-related serious adverse events have been reported to date. The study will continue until an additional 30 patients have been treated.

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Development of MMPs specific peg-peptide-doxorubicin conjugates based on angiogenesis

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Introduction: Matrix metalloproteinases (MMPs) secreted from cancer cells play important roles during the tumor progression. They degrade extracellular matrix (ECM) mainly composed of collagen and facilitate tumor invasion and metastasis. Based on these phenomena, we designed type IV collagenases, MMP-2 and MMP-9, specific PEG-peptide-doxorubicin conjugates formed as micelle in aqueous system (Fig. 1).

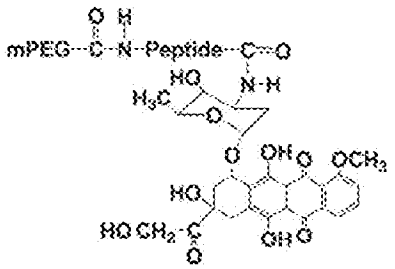

Figure 1. The structure of PEG-peptide-doxorubicin conjugates

Experimental methods: PEG-peptide-doxorubicin conjugates were prepared using peptides with the specificity for the matrix metalloproteinases MMP-2 and MMP-9. The secretion of MMPs from cancer cells was analyzed *in vivo* for the cell lines of LLC, MCF-7, and Head-Neck SCC by zymograpy. Using in situ zymography using gelatin-coated film, the expression of MMPs from tumor tissue inoculated in C57Bl/6 was observed. The distribution of MMPs in tumor tissue was observed immunohistochemically. The degradation behavior of our conjugates was observed by HPLC with the incubation time with enzyme and the concentration of active MMP-2. The cytotoxic activity of the conjugates compared to free doxorubicin was investigated by MMT assay.

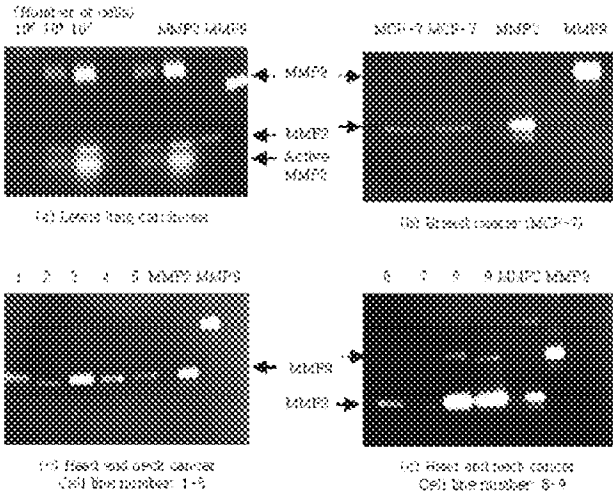

Figure 2. Screening of MMPs released from various cancer cell lines

Results and Discussion: Cancer cell lines were secreted two types of MMPs such as pro- and active-MMPs. Among the cell lines, LLC cells released pro- and active-MMP-2 and only pro MMP-9 (Fig. 2). It was observed that MMP-2 and MMP-9 distributed around the tumor from the immunohistogram of tumor tissue (Fig. 3) and the result from in situ zymography of tumor tissue tumor was showed similar tendency.

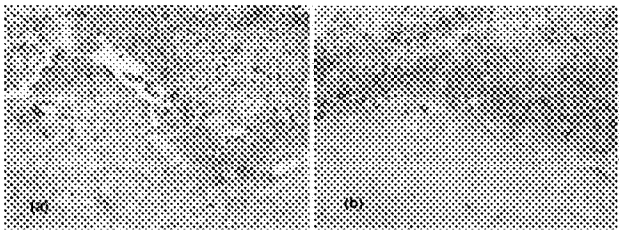

Figure 3. Ditribution of Lewis Lung. MMPs around Carcinoma tissue in C57BL/6 mice. (a) and (b) showed pro MMP-2, pro MMP-9 around tumor site, respectively.

We demonstrated by HPLC analysis that PEG-peptide-doxorubicin conjugates were degraded by the active MMP-2 to maximum 90 % depending on the concentraion of doxorubicin and the incubation time. Also the conjugates were less toxic in MMT assay because of their micelle form.

Conclusion: PEG-peptide-doxorubicin showed the selective degradation by the active MMP-2. Also the conjugates showed the reduced toxicity compare to free doxorubicin in MMT assay. As results, it is expected for PEG-peptide-doxorubicin conjugates to be degraded selectively by MMP-2 or MMP-9 in tumor region and then show the anti-cancer activity. Therefore, They are available to apply our conjugates as an anti-cancer drug to target cancer.

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Microsphere-encapsulated 4OH-Tamoxifen: a new sustained release delivery system with antitumour activity against DMBA-induced mammary carcinoma in sprague-dawley rats

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Introduction: Tamoxifen (TAM), a synthetic non-steroidal anti-estrogenic compound, is considered as the standard treatment of hormonodependent advanced breast cancer. One of its metabolites, 4-hydroxytamoxifen (4OH-TAM), may be responsible for a major part of the *in vivo* effects of TAM. However, the use of 4OH-TAM is limited by its very low solubility.

Aim: To investigate the efficacy of one single subcutaneous (sc) injection of 4OH-TAM encapsulated in microspheres (MS) that induce a sustained release. This experiment was done in comparison with daily repeated administrations of free 4OH-TAM (sc) and TAM (per os) in the model of 7,12-dimethybenz(a)anthracene (DMBA)-induced rat mammary tumours. Material & Methods: Biodegradable PLGA microspheres were obtained by solvent extraction method. Sprague-Dawley rats received TAM (10.0 mg/kg daily per os for 28 consecutive days), free 4OH-TAM (0.1, 1.0, 10.0 mg/kg daily sc for 28 consecutive days) and MS/4OH-TAM (single sc injection of 28.0 mg/kg) or were ovariectomised (OVX) 9 weeks following DMBA administration.

Results: No major toxicity was observed in all groups, except for animals treated with free 4OH-TAM, where a severe local necrosis due to the ethanol/water (65:35) vehicle was observed. The total number of tumours/animal and the sum of tumour volumes/animal were significantly ($p<0.01$) lower in TAM, MS/4OH-TAM groups and in OVX rats as compared to control. Moreover, the percentage of tumour reduction was significantly ($p<0.01$) higher in MS/4OH-TAM (67%) than in TAM (33%) treated-groups. MS formulation allowed obtaining a 4-weeks release of 4OH-TAM without any toxicity.

Conclusion: Our data demonstrated that this sc single administration MS delivery system gives an antitumour activity of 4OH-TAM equal/better than that of TAM. Therefore MS may be a promising new class of polymers suitable for 4OH-TAM targeted drug delivery.

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Interference with TGF-beta1 and -beta3 in tumor stroma lowers tumor interstitial fluid pressure independently of growth in experimental carcinoma

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Solid tumors are characterized by a high interstitial fluid pressure (IFP) which constitute a hydrodynamic barrier resulting in a low uptake of anticancer drugs. The aim of the present investigation was to study the role of members of the transforming growth factor (TGF)-beta family on the generation of a high tumor IFP. We used an *in vivo* model of human anaplastic thyroid carcinoma (ATC) and a specific inhibitor of TGF-beta1 and -beta3. Treatment of KAT-4 ATC tumors grown in athymic mice with 10 mg/kg of the TGF-beta inhibitor for 10 days resulted in a 48% reduction in tumor IFP compared to untreated control tumors. The mice that received the inhibitor had initially a higher tumor growth rate. However, at day 10 the apoptotic index, as well as the protein level of the cell cycle inhibitor p27Kip1, were higher in tumors from treated mice compared to the control mice. This was followed by a decreased tumor growth rate between days 15 to 29 in the treated mice. Since the KAT-4 cells *in vitro* did not respond to TGF-beta1 stimulation, measured as phosphorylation of Smad2 protein and growth inhibition, the effects observed in the tumors by the inhibitor are believed to be caused by the effects on the tumor stroma. Taken together, the present data indicate that members of the TGF-beta family are involved in the generation of the high tumor IFP observed in the ATC model, as well as in regulation of tumor growth, by changing the properties of the tumor stroma. These results identify TGF-beta1 and -beta3 as potential targets for novel anticancer treatment directed to the tumor stroma.