ciable binding to the other two lines. Despite the relatively lower cell-binding activities of the conjugates compared to unconjugated MAb, the conjugate concentrations required to result in 50% cell death (IC_{50}s) were significantly lower than the free drug (Table 1).

Table 1. IC_{50} (nM) values of paclitaxel (PTX), PTXC225, and PTXHer conjugates in MDA-MB-468, LNCAP, and DU145 cell lines.

Cell Line	PTX	PTXC225	PTXHer
MDA-MB-468	13.5	3.9	3.7
LNCAP	3.0	0.9	N/A
DU145	8.3	3.3	3.2

Furthermore, preliminary therapy experiments show stabilization of A431 human epidermoid carcinoma tumors in athymic nude mice treated with PTXC225 as compared relative to the C225 treated control. Based on the above binding and IC_{50} results, a controlled therapy experiment with DU145-implanted nude male mice and using PTXC225, is underway. These results may point to a MAb-mediated tumor-specific paclitaxel delivery which may be advantageous to the conventional systemic administration of this important drug.

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Dynamics of tumor cell induced angiogenesis and microcirculation from tumor onset until late stage tumor disease: barriers to drug delivery

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Background: Tumor vasculature is characterized by a heterogeneous vessel distribution, morphology and physiology. These vascular irregularities are responsible for barriers of drug delivery and hinder successful therapies. However, due to inherit problems for continuous non-invasive monitoring of microvascular properties, the dynamics of these barriers during tumor onset and tumor growth are poorly understood.
Methods: A dedifferentiated Angiosarkoma cell line (SV40, GFP transfected, HBMEC-1) was implanted in cranial window for continuous non-invasive intravital microscopy in 12 weeks old male SCID mice (n=30). For 85 days vascular parameters such as functional vascular density, velocity, leukocyte endothelial interaction (LEI), tissue perfusion rate (TPR), branching pattern, vessel morphology and vascular permeability were obtained using fluorescence microscopy, as described elsewhere (Hansen-Algenstaedt et al. Cancer Research 2000, Yuan et al. Cancer Research 1994). To demonstrate histomorphologic aspects, immunhistochemistry was performed. Anti-laminin staining was used for basal membran visualization. Electronmicroscopy was performed for high resolution analysis.
Results: Tumor cell implantation was accompanied by an immediate and significant increase in permeability of pre-existing vessels. Although permeability peaked on day 12 the initial increase was significantly pronounced during the first 48 h, reaching a plateau phase after day 8. Blood flow in newly formed vessels was detected 3 days after tumor cell implantation. Tumor vessels demonstrated an increasing permeability from day 13 until day 61. No further increase until the end of observation period was observed. LEI increased significantly in tumor vessels. Increase of TPR was observed only during tumor onset. Later stages were characterized by a steady state while tumor size increased constantly and a slight decrease of TPR on day 85 respectively. During initial tumor growth the vascular branching pattern and blood flow velocity were less heterogeneous than during later stages. Anti-laminin staining revealed that tumor cells did not participate in endothelial lining of tumor vessels. Electronmicroscopy revealed intraluminal abnormalities such as multiple intercellular openings and transluminal bridging.
Conclusions: The vulnerable tumor onset period is characterized by regular vascular morphology but increased vascular permeability of host vessels. These characteristics can be utilized for the delivery of large molecules during tumor onset. Later stages with established tumors and tumor vessels are characterized by heterogeneous vessel distribution and irregular vessel morphology leading to impaired drug delivery. Therefore therapies that equalize blood supply, such as anti-vascular therapies, can be helpful to normalize drug delivery for combined therapies.

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Proteolysis of xyotax by lysosomal cathepsin B; metabolic profiling in tumor cells using LC-MS

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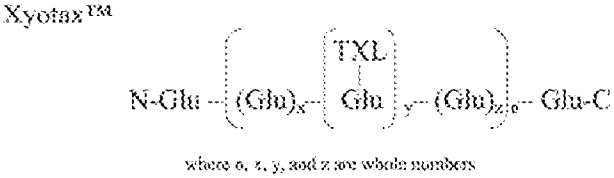
Xyotax(TM) (CT-2103) is a water-soluble polymer-drug conjugate that displays enhanced anti-tumor activity relative to paclitaxel (TXL) in a variety of preclinical models. Xyotax consists of a polydisperse poly-L-glutamic acid backbone, averaging 33 kD, in which paclitaxel molecules are esterified through the 2′ position on paclitaxel to the gamma-carboxylic acid residues of the PG polymer. Conjugation at 37% paclitaxel by weight results in approximately 1 paclitaxel molecule per 11 glutamic acid residues. It is currently under evaluation in Phase III clinical trials in multiple indications including colon, lung, and ovarian cancer. Tissue distribution studies in tumor bearing mice have led to the conclusion that Xyotax is biodegradable and undergoes degradation in part by proteolysis to form monoglutamyl-paclitaxel (2′-[L-gamma-Glu]-TXL), a chemically unstable species that is hydrolyzed to form paclitaxel and pyroglutamate. Although the specific mechanism(s) for this have not been fully elucidated, it has been proposed that principle uptake of amino acid polymers occurs by pinocytosis followed by transport to the lysosome for processing and degradation. Lysosomal cathepsin B, a cysteine protease which is highly expressed in a variety of tumor types and is associated with tumor cell invasion and metastasis, displays a high dipeptidase activity towards Xyotax resulting in abundant formation of diglutamyl-paclitaxel conjugates (i.e., 2′-[L- gamma -C-NH2-Glu-Glu]-TXL and 2′-[L-gamma-C-COOH-Glu-Glu]-TXL). We evaluated the ability of RAW264.7 (murine monocytic leukemia), HT-29 (human colon carcinoma), and NCI-H460 (human large cell lung) cell lines to metabolize Xyotax *in vitro*. Quantitative analysis was achieved using isotope labeling, reverse-phase HPLC, and electrospray ionization on a Micromass Quattro II mass spectrometer.



Time dependent generation of diglutamyl-paclitaxel, monoglutamyl-paclitaxel and free paclitaxel was observed for the cellular extracts over a 48 hour time period. Utilizing a cell permeable, selective inhibitor of cathepsin B, CA-074 Me, we find both limited and delayed proteolysis of Xyotax relative to control in the tumor cell lines studied. These data provide strong support for the biodegradability of Xyotax and suggest that release of free paclitaxel from CT-2103 may be increased in tumors with higher levels of cathepsin B expression.

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Increased sensitivity to chemotherapy during the window in time when tumor interstitial fluid pressure is lowered

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Chemotherapy against solid malignancies is often ineffective due to impaired transport of anti-cancer drugs into tumor tissue. This in part has been attributed to the pathologically increased tumor interstitial fluid pressure (IFP). We investigated the relevance of the pathologically high tumor IFP for efficacy of treatment with 5-fluorouracil (5-FU) in subcutaneous syngeneic PROb rat colonic carcinomas and chemically-induced rat mammary carcinomas. Prostaglandin E1 (PGE1) was used to acutely lower tumor IFP. IFP is transiently lowered following PGE1 administration, reaching a minimum after 10-15 minutes, and returning to the initial value after around 60 min. Lowering of IFP occurs without changes in blood flow or blood vessel permeability for albumin. 5-FU has a t1/2 of ~10-20 minutes in rats. By administering 5-FU at times when tumor IFP was lowered by PGE1, or alternatively, outwith those times, we could directly assess whether tumor IFP generates a functional barrier to chemotherapy. Lowering of tumor IFP with PGE1 increased capillary-to-interstitium transport of 5-FU as measured by microdialysis. A low dose of 5-FU had significant anti-tumor activity only

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).