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.

427

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

C. Joscheck[1,5], W. Fiedler[4], P. Algenstaedt[2], I. Muller[1], C. Heintz[5,6], K. Lamszus[3], M. Brockmann[3], M. Krause[1], W. Ruther[1], N. Hansen-Algenstaedt[1]. *[1]University Hospital Hamburg-Eppendorf, Department of Orthopedic Surgery, Hamburg, Germany; [2]University Hospital Hamburg-Eppendorf, Department of Internal Medicine, Hamburg, Germany; [3]University Hospital Hamburg-Eppendorf, Department of Neurosurgery, Hamburg, Germany; [4]University Hospital Hamburg-Eppendorf, Department of Hematology & Oncology, Hamburg, Germany; [5]University Hospital Hamburg-Eppendorf, Center of Biomechanics, Hamburg, Germany; [6]AK Harburg, Hamburg, Germany*

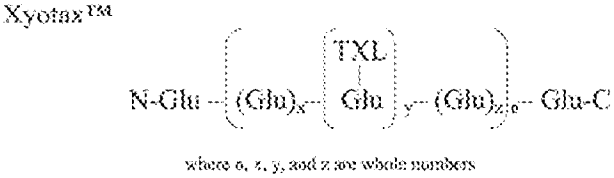
Background: Tumor vasculature is characterized by a heterogeneous vessel distribution, morphology and physiology. These vascular irregularities are responsible for barriers of drug delivery and hinder successful therapies. However, due to inherit problems for continuous non-invasive monitoring of microvascular 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.

428

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

S.A. Shaffer, C. Baker Lee, A. Kumar, J.W. Singer. *Cell Therapeutics, Inc., Seattle, USA*

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.

429

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

A. Salnikov[1], V. Iversen[3], C. Sundberg[1], L. Stuhr[3], M. Sjöquist[2], R. Reed[3], K. Rubin[1]. *[1]Uppsala University, Dept. of Medical Biochemistry and Microbiology, Uppsala, Sweden; [2]Uppsala University, Dept. of Physiology, Uppsala, Sweden; [3]University of Bergen, Dept. of Physiology, Bergen, Norway*

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