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Endosialin/TEM-1 glycoprotein: a novel therapeutic target for mesenchymal malignancy

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The endosialin/Tumor Endothelial Marker-1 (TEM-1) transmembrane glycoprotein was found to be selectively expressed by endothelial cells and fibroblasts. We detected endosialin/TEM-1 protein in malignant cells of mesenchymal origin and modeled TEM-1-positive soft tissue malignancy in vitro and in vivo. We surveyed human soft tissue tumors by immunohistochemistry and human sarcoma cell lines by PCR and flow cytometry. We characterized endosialin/TEM-1 protein expression in vitro in sarcoma cell tube formation assays and in vivo in a xenograft tumor model.

Endosialin/TEM-1 protein expression was studied in 30 human sarcoma biopsies, including 10 malignant fibrous histiocytomas, 5 Ewing sarcomas, 5 angiosarcomas, 4 rhabdomyosarcomas, 2 pleiomorphic sarcomas, 1 synovial sarcoma, 1 Kaposi's sarcoma, 1 leiomyosarcoma and 1 liposarcoma. Endosialin/TEM-1 was detected in 23/30 biopsies in vascular/perivascular cells, stromal cells and/or malignant cells. In cell lines, we detected endosialin/TEM-1 protein in 6/14 human sarcoma cell lines, in A673 Ewing sarcoma, HOS osteosarcoma, Hs414T and Hs93T fibrosarcoma, Hs729T rhabdomyosarcoma and SW872 liposarcoma cells. In contrast, we detected endosialin/TEM-1 mRNA in only 1/27 human cancer cell lines of epithelial and neural origin, in SKNAS neuroblastoma cells, which also expressed the protein. We did not detect endosialin/TEM-1 protein in 8 human cancer cell lines of hematopoietic origin.

We sought to model TEM-1-positive malignancy in vitro and in vivo using the A673 Ewing sarcoma cell line. A673 cells formed a network when plated on Matrigel that was similar to those formed by endothelial cells. In co-cultures with TEM-1-negative human microvascular endothelial cells (HMVEC), A673 cells associated with HMVEC to form networks. In vivo, A673 cells were implanted into BALB/c nude mice subcutaneously. Immunohistochemical analysis of A673 tumor sections showed clear staining for endosialin/TEM-1 protein, demonstrating that expression was maintained in vivo.

Our work documents TEM-1-positive mesenchymal neoplasia and offers models to unravel its cellular and molecular features. TEM-1 may be a useful therapeutic target in this disease, one that may provide a multi-pronged therapeutic approach by targeting at once several of the malignancy-driving cellular components of mesenchymal disease: vascular/perivascular cells, stromal cells and malignant cells.

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Characterization of a novel and selective inhibitor of checkpoint kinase 1: breaching the tumor's last checkpoint defense against chemotherapeutic agents

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Many tumors are defective in the tumor suppressor p53 and therefore lack a functional G_1 checkpoint. In these tumors, however, the S/G2 checkpoints remain intact and, in response to DNA damage, arrest cell cycle progression to enable DNA repair. Checkpoint kinase 1 (Chk1) is a key element in the DNA damage response pathway and plays a crucial role in the S/G_2 phase checkpoints. Inhibiting Chk1 represents a therapeutic strategy for overriding the tumor cell's last checkpoint defense against the lethal damage induced by DNA directed chemotherapeutic agents. Inhibiting Chk1 may define a molecular based strategy to selectively target tumors with intrinsic checkpoint defects while minimizing toxicity in normal cells. PF-00477736 is a potent, selective ATP-competitive Chk1 inhibitor from the diazapinoindolone series that inhibits Chk1 with a $K_i = 0.49$ nM. Checkpoint abrogating activity was demonstrated by an increase in phospho-histone H3 and by increased sub-G1 population. PF-00477736 abrogates cell cycle arrest induced by DNA damage and enhances cytotoxicity of chemotherapeutic agents, including gemcitabine, irinotecan and carboplatin. The addition of PF-00477736 to gemcitabine-treated cells decreased Chk1 and CDK1 phosphorylation, increased the presence of the hyperphosphorylated form of Cdc25C and increased cyclin B, phos-gamma-H2AX and apoptosis; all of which provide evidence of checkpoint abrogation, cell cycle progression through metaphase of mitosis, increased DNA damage and cytotoxicity. PF-00477736 chemopotentiation shows selectivity for p53-defective cancer cell lines over p53-competent normal cells. In Colo205 xenografts PF-00477736 enhanced the antitumor activity of gemcitabine in a dose-dependent manner demonstrating a 100% time-to-progression enhancement ratio and 1.2 logs of cell kill compared to 0.27 with gemcitabine alone. In HT29 xenografts PF-00477736 potentiated irinotecan with tumor growth delay and numbers of log cell kill consistently doubled. Tumors from combination studies showed dose-dependent increase in apoptosis and inhibition of Chk1 phosphorylation. PF-00477736 combinations were well tolerated. The clinical attractiveness of combinations with PF-00477736 includes the potential for enhancing the efficacy of widely used chemotherapeutics, improved quality of life due to preferential killing of cancer cells and a broad range of therapeutic opportunities amenable to rational combinations enabling new possibilities for treatment.

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Inhibition of N-cadherin enhances anti-tumor activity and overcomes chemoresistance in regional melanoma therapy

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Malignant transformation in melanoma is characterized by a phenotype "switch" from E-cadherin to N-cadherin which is associated with increased motility, higher grade and invasiveness of the tumor cells as well as changes in intracellular signaling pathways leading to increased proliferation, survival and angiogenesis and decreased apoptosis. We hypothesized that targeted inhibition of N-cadherin adhesion complexes using systemically administered ADH-1, a novel cyclic pentapeptide currently in Phase II clinical trials, could sensitize melanoma tumors to the cytotoxic effects of regionally administered chemotherapy.

We utilized a rat xenograft model of extremity melanoma to evaluate the effects of ADH-1 used in combination with regional isolated limb infusion (ILI) with melphalan (LPAM). Two well characterized human melanoma-derived cell lines were selected for *in vivo* study: (1) DM366 which has high N-cadherin and no detectable E-cadherin expression (measured by Western blot and immunohistochemistry) and is sensitive to LPAM and (2) DM738 which expresses moderate E-cadherin, low levels of N-cadherin, and is resistant to LPAM.

LPAM in combination with ADH-1 significantly reduced tumor growth compared to LPAM alone in both LPAM sensitive tumors (DM366) and in LPAM resistant tumors (DM738). Measured at day 30, the change in tumor volume with LPAM alone (90 mg/kg) was +154% for xenograft DM366 and +432% for xenograft DM738. When LPAM was used in combination with ADH-1 (100 mg/kg, dosed bid $\times 3$ days starting one hour pre-LPAM infusion) percent change in tumor volume measured at day 30 was −91% for xenograft DM366 ($p < 0.03$) and +42% for xenograft DM738 ($p < 0.005$). ADH-1 used as a single agent (dosed as above one hour before infusion with saline) did not reduce tumor growth (see Table 1). The frequency of complete (CR) and partial (PR) response rates at day 30 were 100%/0% (CR/PR) for DM366 tumor bearing animals and 33%/66% for DM738 tumor bearing animals when treated with ADH-1 in combination with LPAM. In 100% of ADH-1/LPAM treated DM366 animals, complete tumor regression was durable at >60 days after infusion.

Table 1

	Xenograft				
	DM366		DM738		
N-Cadherin expression	high		low		
E-Cadherin expression	none		moderate		
LPAM sensitivity	Sensitive		Resistant		
Treatment	LPAM (ILI)	LPAM (ILI) + ADH-1 (IP)	LPAM (ILI)	ADH-1 (IP)	LPAM (ILI) + ADH-1 (IP)
% ↑ tumor vol. at day 30	154.1	−90.6	432	484.6	41.9
Student t-test to LPAM	–	$p < 0.03$	–	$p = 0.46$	$p < 0.005$
PR Rate at day 30 (%)	25	40	0	0	33
CR Rate at day 30 (%)	0	60	0	0	0
Regression (%)	75	100	16.7	0	100

Targeted therapy using an N-cadherin antagonist in combination with melphalan can dramatically augment the anti-tumor effects of chemotherapy and overcome chemoresistance. Targeting N-cadherin is a novel approach to not only optimizing regional melanoma therapy but has potential applicability to systemic therapeutic strategies as well.