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PEGylated DX-1000: pharmacokinetics, anti-tumor and anti-metastatic effects of a specific plasmin inhibitor

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Plasmin is a serine protease predominantly present in the body in its inactive zymogen form, plasminogen. In tumors, activation of plasminogen mainly occurs locally by urokinase (uPA) overproduced by cancer or stromal cells. Using phage display, we have identified a TFPI-derivative Kunitz domain protein, which is a specific inhibitor of plasmin (Ki = 88pM) referred to as DX-1000. DX-1000 has been produced in Pichia pastoris. DX-1000 was tested in several functional cell-based activity assays and demonstrated potent inhibitory activity. DX-1000 specifically blocked plasmin-mediated proMMP-9 activation in tumor cells. When evaluated in vitro in a chemoinvasion assay, DX-1000 efficiently inhibited the invasiveness of uPA-expressing HT-1080 cells (cell invasion reduced by 46% and 66% with 1 and 100 nM DX-1000, respectively). We demonstrated that DX-1000 was able to efficiently inhibit tube formation of HUVECs (IC50 = 1.39±0.28 nM) and mouse endothelial cells (IC50 = 16.6±0.1 nM) highlighting the usefulness of this plasmin inhibitor as an effective inhibitor of angiogenesis. DX-1000 holds great promise as a candidate for treating cancer. However, due to its low molecular weight (7 kDa), the protein exhibits a rapid plasma clearance rate in vivo (β Phase half-life ~27 minutes in mice and 1 hour in rabbits) thereby limiting its therapeutic effectiveness. PEGylation overcomes these shortcomings. By increasing the molecular mass of DX-1000 from 7 to 27 kDa (5k PEG added at 4 sites), we dramatically improved pharmacokinetics of DX-1000. PEGylation of DX-1000 substantially prolongs in vivo circulation and stability (β Phase half-life ~13 hours in mice and 59 hours in rabbits) while having no effect on the activity or potency of DX-1000 as a plasmin inhibitor in several in vitro cell-based assays. Interestingly, PEGylated DX-1000 significantly reduced primary tumor progression by 45% and reduced liver and lung metastasis by 40% in a MDA-MB-231 orthotopic model in mice. Studies are ongoing to elucidate the cellular mechanism involved in the observed anti-tumor and anti-metastatic activities. In conclusion, we have generated a longer half-life version (through PEGylation) of our potent plasmin inhibitor without altering in vitro activity and demonstrating in vivo anti-tumor and anti-metastatic activities.

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The effects of endothelial cells on tumor cell gene expressions

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Background: The effect of tumor cell derived factors on angiogenesis has been well established. In contrast, little is known about the role of factors secreted by endothelial cells on tumor cell phenotype. The role of endothelial cells in tumor biology has been considered primarily lining the vessels required for the influx of oxygen and nutrients to tumor cells. To know the effect of endothelial cells on tumor cell gene expressions, we performed a co-culture system between endothelial cells and tumour cells.
Material and Methods: Human dermal microvascular endothelial cells (HDMEC; Clonetics) stably transduced with Bcl-2 (HDMEC-Bcl-2), empty vector controls (HDMEC-LXSN), and Human squamous cell carcinoma cell lines, UM-SCC-17B and OSCC3, were used. When indicated, endothelial cells (HDMEC) were cultured in the lower chamber, and tumor cells (UM-SCC-17B or OSCC3) in the upper chamber of a non-contact co-culture system for 24−48 h. These cells were separated by the 1 μm pore membrane of transfer wells. We cultured cells in 1 μg/ml polyclonal anti-human VEGF, or 21 μg/ml polyclonal anti-human VEGFR1 (Flt-1) antibody to neutralize the activity of these signaling molecules, and measured VEGF protein expression by ELISA. Total RNA was extracted from each cell line and Bcl-2, CXCL8, CXCL1 expression levels were examined by RT-PCR or real time PCR.
Results: Bcl-2, CXCL8 and CXCL1 mRNA expressions in tumour cells were upregulated when these cells were co-cultured with HDMEC-Bcl-2 as compared to co-culture with empty vector HDMEC-LXSN. Both squamous cell carcinomas studied here (UM-SCC-17B and OSCC3) expressed VEGFR1, and Bcl-2 mediated the upregulation of VEGF expression in endothelial cells. Blockade of the VEGF signalling pathway with a neutralizing antibody inhibited the ability of HDMEC-Bcl-2 to induce Bcl-2, CXCL8 and CXCL1 expressions in UM-SCC-17B cells. Likewise, blockade of VEGFR1 prevented upregulation of Bcl -2, CXCL8 and CXCL1 expressions in the tumour cells co-cultured with HDMEC-Bcl-2.
Conclusions: These data suggested that the endothelial cells may secrete factor(s) that directly influence gene expression levels in the tumour cells. These data also demonstrate that VEGF signalling through VEGFR1 is a key event in the process through which endothelial cells modulate gene expression in tumour cells.

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A phase I study examining weekly weight based or fixed dosing and pharmacokinetics (PK) of a novel spectrum kinase inhibitor, XL999, in patients (pts) with advanced solid malignancies (ASM)

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Background: XL999 is a small molecule inhibitor of multiple kinases involved in tumor cell growth, angiogenesis, and metastasis, including VEGFR2 (KDR), PDGFRα/β, FGFR1/3, FLT-3, and SRC. A phase I study of XL999 administered as a 4 hr infusion every 2 weeks in pts with ASM showed that the maximum tolerated dose (MTD) was 3.2 mg/kg and the plasma $t_{1/2}$ was approximately 24 hrs. Three pts dosed at 0.2–1.6 mg/kg had partial responses and 9 pts had stable disease (SD) >3 months. Based on these data, a weekly (wkly) weight based and fixed dosing schedule with PK monitoring was further explored.
Methods: XL999 at 2.4 mg/kg or 200 mg was administered to pts as a 4 hr infusion on day (d) 1 and d8 with toxicity assessment. PK sampling was performed on d1 & d15. Pts received further doses of XL999 wkly in the absence of unacceptable toxicity, disease progression, or accumulation based on PK results.
Results: As of June 1, 2006, 12 pts were enrolled and received XL999 weekly as initial treatment at 2.4 mg/kg or 200 mg fixed dose. Of 8 pts treated at 2.4 mg/kg, none had experienced any G2 or worse drug related adverse events. One asymptomatic pt at 2.4 mg/kg with non-specific ECG changes after d1 was discontinued from study. One pt receiving 200 mg experienced symptomatic hypotension and non-specific ECG changes on d1 and was discontinued. Four of 8 evaluable pts had SD for >2 months. In patients with complete d1 & d15 PK at 2.4 mg/kg (n = 5) or 200 mg (n = 4), there was moderate interpatient and intrapatient variability, with no evidence of drug accumulation on repeat dosing.

Averages for weekly dosing PK parameters[a]

Dose level	Cycle	t 1/2 (hr)	Cmax (ng/mL)	AUC_{0-inf} pred (hr ng/mL)	CL pred (L/hr)
2.4 mg/kg	1	20.8 (16%)	513 (34%)	5939 (45%)	40.9 (36%)
	2	23.2 (28%)	670 (41%)	7359 (35%)	32.8 (41%)
200 mg	1	36.1 (54%)	528 (44%)	7737 (52%)	32.1 (51%)
	2	29.2 (11%)	491 (42%)	6476 (44%)	34.6 (32%)

[a]%CV in parentheses.

Conclusions: XL999 administered wkly as a 4 hr infusion at a dose of 2.4 mg/kg or 200 mg appears to be well tolerated with no evidence of drug accumulation. Drug clearance appears independent of weight and supports the use of fixed dosing.

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Preclinical studies in a murine tumour model on the efficacy of combining radiation with angiogenesis inhibitors and vascular disrupting agents

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Background: Targeting tumour vasculature is becoming an increasing popular therapy, but the ability of angiogenesis inhibitors (AIAs) and/or vascular disrupting agents (VDAs) to inhibit tumour growth is limited. As such, these vascular targeting agents (VTAs) are now being combined with other therapies, (i.e., radiation). The aim of this study was to investigate the potential of combining both AIAs and VDAs with radiation.
Materials and Methods: A C3H mammary carcinoma grown in the right rear foot of female CDF1 mice was used in all experiments. Treatments were performed when tumours had reached 200 mm^3 in size. The AIA and VDA were TNP-470 (Takeda Chemical Industries) and combretastatin A-4 disodium phosphate (CA4DP; OXiGENE, Inc.),