clearance resulted in a dose proportional area under the concentration-time curve (AUC) 3× higher in the CI infusion schedule versus the short infusion studies. Sequential CT and FDG PET scans were acquired to assess the effects of rHu-Endo on tumor blood flow and tumor glucose metabolism, respectively. At the 60 and 120 mg/m^2/d dose levels, a substantial decrease in FDG metabolism was observed; while blood flow estimated by first pass metabolism did not significantly change over a 28 day period. Prior to starting rHu-Endo, one patient had two FDG PET scans over a 28 day period. During this drug-free period, blood flow to analyze metastatic lesions increased by 41% from baseline. Following two cycles of rHu-Endo, blood flow decreased by 47% from baseline. This trial is currently accruing patients at the higher dose levels.

Dose Level (mg/m^2/day)	Cycle 1 Mean CI (ml/min/m^2)	Single Day Mean AUC (mg/ml·min)	Cycle 1 Mean AUC (mg/ml·min)	Cycle 1 Mean Est. Css (mcg/ml)
30(n=6)	135.9 ± 77.1	0.26 ± 0.10	7.11 ± 2.63	0.16 ± 0.07
60(n=5)	131.1 ± 12.8	0.46 ± 0.04	12.45 ± 1.19	0.32 ± 0.03
120(n=5)	114.0 ± 25.9	1.10 ± 0.24	29.57 ± 6.43	0.76 ± 0.17

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Structure-growth regulatory potency relationship investigation of TIMP-1 (tissue inhibitor of metalloproteinases) C-terminal domain fragments

H. Suli-Vargha[1], N. Mihala[1], J. Bodi[1], F. Timar[2], J. Olah[2], A. Jeney[2].
[1]Hungarian Academy of Sciences, Research Group of Peptide Chemistry, Budapest, Hungary; [2]Semmelweis University, Institute for Pathology and Experimental Cancer Res, Budapest, Hungary

Although TIMP-1 is widely known as a common matrix metalloproteinase (MMP) inhibitor, originally it was identified as a growth factor and is able to stimulate the growth of certain cell lines. It is noteworthy that TIMP-1 looses its growth stimulatory activity upon complex formation through its C-terminal domain with proMMP-9, but reduction and alkylation does not affect it. Assuming the importance of the C-terminal domain sequences in the growth stimulatory activity, peptide fragments related to this domain were synthesized and subjected for studies on SAR. In resting MCF-7 cell cultures TIMP peptides at the early treatment period induced higher DNA content without augmenting cell population, and at later non-apoptotic type of cell death was observed. The abolishment of DNA content elevation in the presence of EGF may indicate the participation of a cell surface receptor in the action of the peptides. TIMP peptides increased MMP-2, but reduced MMP-9 production in the HT-1080 cell cultures. The above data indicate that in growth factor deprived circumstances C-terminal fragments of TIMP-1 cause cell death and modulate the equilibrium between MMP-2 and MMP-9. No conclusion can be drawn from the SAR investigations for the presence of a well defined active center in the TIMP-1 C-terminal domain, it may rather be supposed that pharmacophores at different positions of the molecule are involved in the growth modulating activity of TIMP-1.

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Radiation and the endothelium: the importance and the modulatory effects of VEGF, bFGF, alphavbeta3 and the extracellular matrix components on ionizing radiation-induced endothelial cell damage

A. Abdollahi[1], K. Lipson[2], K. Weber[1], T. Trinh[1], A. Howlett[2], M. Wannenmacher[1], J. Debus[1], P. Huber[1]. *[1]University of Heidelberg-DKFZ, Radiation Oncology, Heidelberg, Germany; [2]Sugen, Inc, South San Francisco, USA*

In recent decades, radiation research primarily concentrated on the cancer cell compartment. Much less is known about the effect of ionizing radiation on the endothelial cell compartment and the complex interaction between the tumor and its microenvironment, which includes the ECM, cytokines, integrins and endothelial cells. Here we report that ionizing radiation is a potent antiangiogenic agent that inhibits endothelial cell survival, proliferation, tube formation and invasion. VEGF and bFGF were able to reduce the sensitivity of endothelial cells to radiation-induced damage, and this radioresistance could be reversed by the receptor tyrosine kinase inhibitors SU5416 and SU6668. Endothelial cells were found to be more sensitive to ionizing radiation than PC3 prostate cancer cells. IR upregulated VEGF and bFGF in PC3 cells and, interestingly, VEGFR2 and the integrin alphavbeta3 in endothelial cells. In a co-culture system, irradiation of the prostate cancer cells enhanced endothelial cell invasiveness through a Matrigel matrix. Because of the observed upregulation of alphaVβ3, we explored the modulatory role of ECM components on endothelial cell proliferation, plating efficiency and clonogenic survival. We observed that fibronectin and collagen I increased endothelial cell proliferation and survival without significantly affecting radiosensitivity. In contrast, laminin enhanced intrinsic radiosensitivity. Together these findings form the basis of a complex model of multifactorial communication between the tumor and its microenvironment that is modulated by ionizing radiation. This model may help us to better understand how tumors protect their microvasculature from radiation-induced damage. Simultaneously, our results rationalize concurrent administration of angiogenesis inhibitors and radiotherapy in cancer treatment.

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The alpha-v beta-3 antagonist S-247 inhibits the growth of primary renal tumor and spontaneous lung metastases in the RENCA model

C.R. Morris[1], X. Wang[1], W.F. Westlin[2], D.M. Meyer[2], R. Pili[1]. *[1]Johns Hopkins University, Oncology, Baltimore, USA; [2]Pharmacia, St. Louis, USA*

Tumor angiogenesis is a multistep process requiring migration, attachment and survival of endothelial cells. The integrin receptors play an essential role in tumor angiogenesis. Integrin receptor antagonists have been shown to inhibit tumor progression and metastases in preclinical models, and are currently in clinical development. In this study we assessed the effects of the alpha-v beta-3 (avb3) integrin antagonist S-247 in a murine model of renal tumor and spontaneous lung metastases. Murine syngeneic renal cell carcinoma cells (RENCA) were injected orthotopically into the renal capsule of Balb/c female mice. On day 4, animals were randomly assigned to control group and the experimental group. S-247 100 mg/kg in saline solution was administered by gavage twice a day. On day 22 all mice were sacrificed, and primary kidney tumors and lung metastases were analyzed. S-247 induced 49-68% inhibition of the primary tumor as compared to control ($p<0.01$). S-247 treated mice developed also significantly fewer spontaneous lung metastases than controls (up to 98% inhibition of microscopic lung colonies; controls 65; S-247 1.6; $p<0.01$). Preliminary immunohistochemical staining for CD31 and smooth muscle actin showed a reduction of microvessel and pericyte density in the in primary tumors of S-247 treated mice. Studies to evaluate the therapeutic effect of S-247 on established lung metastases in an "intervention" model are in progress. Imaging PET studies will be presented at the meeting. In conclusion, the avb3 integrin antagonist S-247 demonstrates significant anti-tumor and anti-metastatic activity in a murine model for renal cell cancer. Agents such as integrin receptor antagonists may represent an effective treatment in patients with renal cell carcinoma.

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A phase I study of the heparanase inhibitor PI-88 given subcutaneously (sq) in patients (pts) with advanced solid malignancies

S. Holden[1], M. Basche[1], C. O'Bryant[1], M. Morrow[1], S. Grolnic[1], M. Persky[1], C. Deem[1], K. Roberts[2], K. Ribbons[2], S. Eckhardt[1].
[1]University of Colorado Cancer Center, Developmental Therapeutics, Aurora, USA; [2]Progen Industries Limited, Darra, Australia

Heparan sulfates of the extracellular matrix (ECM) bind and sequester proangiogenic growth factors (GFs), such as bFGF and VEGF. Heparanase, which is overexpressed in many cancers, facilitates tissue remodeling and GF release from the ECM, and thereby promotes angiogenesis. PI-88 is a highly sulfated oligosaccharide that interferes with GF binding to heparan sulfates and inhibits heparanase. PI-88 inhibits both angiogenesis in the chick CAM assay, and tumor growth and metastasis in murine syngeneic and human xenograft tumor models. This ongoing phase I study is designed to evaluate the safety, pharmacokinetic (PK) behavior, and biological effects of PI-88 (80-250 mg) when given SQ on days 1-4 and 15-19 of a 28-day cycle to pts with advanced cancer. Dexamethasone 20 mg PO is given on days -1, 1, 14, and 15 for prophylaxis against immune-mediated thrombocytopenia. The rationale for this regimen included the convenience of SQ administration, as well as the possible identification of a distinct toxicity profile from that associated with prolonged intravenous administration. Thus far, 18 pts (median age 60 [range 19-77]; median PS 1) have received 40 courses (crs, range 1-11). Toxicities have included bruising at injection sites (gr 1, 36 crs), pain at known tumor sites (gr 1-2, 12 crs; gr 3, 2 crs), fatigue (gr 1-2, 13 crs), and peripheral neuropathy (gr 1-2, 4 crs). Two SAEs have occurred (pneumonia, second malignancy); neither is considered related to study drug. All

pts but one have had elevations in glucose (gr 1-2, 31 crs; gr 3-4, 5 crs) that are felt to be related to dexamethasone. No hematologic or dose-limiting toxicity has been encountered. Although there have been no partial or complete responses, 3/15 evaluable pts have maintained stable disease for 2, 4, and 10 crs. One pt with melanoma refractory to biochemotherapy has had a decrease in the size and number of pulmonary metastases. Preliminary PK analysis shows no evidence of drug accumulation with chronic dosing. These results indicate that PI-88 administered SQ on this schedule is well-tolerated, achieves plasma concentrations capable of biologic activity, and demonstrates antitumor activity. Updated biologic correlate analysis will be presented. Dose escalation will continue until identification of an MTD, after which phase II studies in melanoma with biological as well as clinical endpoints are planned.

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Role of Id proteins in tumor angiogenesis

V. Mittal[1], R. Schoer[2], M. Ruzinova[3], R. Benezra[4]. [1]*Cold Spring Harbor Laboratory, Cancer Genomics, Cold Spring Harbor, USA;* [2]*Cold Spring Harbor Laboratory, Cancer Genomics, Cold Spring Harbor, USA;* [3]*Memorial Sloan Kettering Cancer Center, Cell Biology, New York, USA;* [4]*Memorial Sloan Kettering Cancer Center, Cell Biology, New York, USA*

Pathological angiogenesis is a hallmark of cancer. The helix-loop-helix Id proteins (Inhibitor of differentiation/DNA binding) are essential for angiogenesis of tumors. Blood vessels in Id knockout (Id1-/-; Id3+/-) mice lack the ability to branch and sprout to support growth or metastasis of tumors, and any tumor growth present show defective vascularization and extensive necrosis. To examine the molecular mechanisms by which Id exerts its effects on angiogenesis we used PTEN+/- mice that are genetically predisposed to lymphoma formation. Gene expression patterns of lymphomas derived from PTEN+/- Id wild type mice and from PTEN+/-, Id1-/-Id3+/- mutant mice were compared by using high-density DNA arrays. Comprehensive data analysis (including error model building, cis-regulatory element analysis) unraveled markers that are differentially regulated in the absence of Id expression. We identified both novel genes and genes known to be previously involved in the process of angiogenesis. Array data was validated by independent methods such as RT-PCR and Northern blotting. To demonstrate that the differentially expressed transcripts were derived from the endothelium of the blood vessels and not from contaminating tumor cells in situ hybridization or immunohistochemistry on Id wild type and Id mutant tumor tissues was performed. Functional assays to determine *in vivo* roles of these candidate genes in angiogenesis are underway.

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Preclinical evaluation of the tyrosine kinase inhibitor SU11248 for the treatment of breast cancer

T.J. Abrams[1], L.J. Murray[1], N.K. Pryer[1], R. Garcia[1], O. Potapova[1], A.D. Laird[1], J.,W.C. Manning[1], E. Pesenti[2], J.M. Cherrington[1]. [1]*SUGEN, Inc., Preclinical Research and Translational Medicine, South San Francisco, USA;* [2]*Pharmacia Corp., Nerviano, Italy*

SU11248 is a potent inhibitor of the receptor tyrosine kinases Flk-1/KDR, PDGFR and Kit, all of which are expressed in human breast cancer and/or its supporting tissues. Angiogenesis is believed to play an important role in breast cancer, as microvessel density and elevated plasma VEGF levels have been reported as negative prognostic indicators. Additionally, we have demonstrated PDGFRb expression and phosphorylation in a majority of human breast cancer specimens by western analysis. Immunohistochemical analyses demonstrated the presence of PDGFRa, KDR, and their ligands in human breast cancer tumor samples as well. To support the potential use of SU11248 in breast cancer treatment, we are evaluating it as a monotherapy and in combination with other agents in preclinical models. In a transgenic model of mutant Ras-driven breast cancer (MMTV-Ha-Ras), SU11248 was administered orally to mice with established mammary tumors. Daily treatment of 40 mg/kg for 20 days resulted in tumor regression. In the MX-1 human breast cancer xenograft model in athymic mice bearing established subcutaneous tumors at the start of therapy, 40 mg/kg/day of SU11248 treatment resulted in significant tumor inhibition (52% inhibition, p = 0.02) as compared to controls. SU11248 and docetaxel (Taxotere®), an anti-mitotic microtubule inhibitor, have both been shown to prolong survival in murine breast cancer models. Therefore, combination therapy of these two agents is of interest in examining efficacy for the treatment of breast cancer. In the MX-1 model, a 3-arm dose-response study was performed in mice treated with SU11248 (40 mg/kg/day), docetaxel (10 mg/kg once per week for 3 weeks), or their combination, yielding 53% (p = 0.02), 73% (p = 0.0007), and 89% ($p < 0.0001$) inhibition of tumor volume, respectively, after 20 days of dosing. The combination therapy resulted in enhanced *in vivo* anti-tumor activity as compared to each treatment alone. The combination of SU11248 and docetaxel was well-tolerated. Additional combination studies with SU11248 are currently in progress. SU11248 is currently in Phase I clinical trials in patients with advanced cancers.

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Defining the biologically active dose for PTK787/ZK222584 (ptk/zk), a vascular endothelial growth factor (VEGF) receptor inhibitor, based on the assessment of two biomarkers [dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), plasma VEGF] in two phase I studies

B. Morgan[1], J. Drevs[2], W.P. Steward[1], L. Lee[3], C. DiLea[3], D. Marme[2], K. Mross[2], A. Thomas[1], D. Laurent[4], M. Dugan[3]. [1]*Royal Hospital, Leicester University, Leicester, United Kingdom;* [2]*Albert-Ludwigs-University Hospital, Tumour Biology Center, Frieburg, Germany;* [3]*Novartis Pharma, Translational Development, East Hanover, USA;* [4]*Schering AG, Berlin, Germany*

PTK/ZK is an orally active inhibitor of the VEGF receptor tyrosine kinases (flt-1/KDR), inhibiting VEGF-induced angiogenesis. Selecting the target dose may be difficult as the biologically active dose is likely to be lower than the maximum tolerated dose. Two biomarkers, DCE-MRI and plasma VEGF, were evaluated to identify the biologically active dose. PTK/ZK treated patients received a continuous daily dose of 50, 150, 300, 500, 750, 1000, 1200, 1500, or 2000 mg until progressive disease or intolerable toxicity. Pharmacokinetic (PK) samples were taken at predose, and days 1, 15, and 28. DCE-MRI was performed at baseline, day 2 (d2) and end of cycle 1 (ec1). The contrast enhancement for tumor was assessed by calculating the bi-directional transfer constant (ki) and expressed as a percentage (%) of baseline. Plasma VEGF was sampled at predose, 10 hrs, days 8, 15, 15 + 10 hrs, 22, and 28. Two Phase I studies (n=76), provided 22 evaluable patients with colorectal cancer and liver metastasis for DCE-MRI analysis, and 63 evaluable patients with advanced cancers for plasma VEGF and PK analysis. Using SWOG criteria, non-progressive disease was defined as >= 2 months stable disease. PTK/ZK was rapidly absorbed with T_{max} of 1 to 2.5 hours. At steady-state (day 15), the exposure (AUC) reduced by 30%. Dose proportionalilty was observed up to 1000 mg. The $t_{1/2}$ was 3-6 hours. No dose-limiting toxicity was observed up to 2000 mg. A significant relationship exists between the reduction in % of baseline ki and dose (d2:p=0.01; ec1: p=0.0003), AUC (d2:p<0.0001; ec1:p=0.003), C_{min} (d2:p=0.0003; ec1:p<0.0001), and liver disease size at end of cycle 2 (d2:p=0.004; ec1:p=0.0001). Non-progressors had significantly greater reduction in mean ki (d2: p=0.004; ec1: p=0.006). A 50-60% reduction in ki was associated with non-progressive disease, and exposure-response modelling suggests a target exposure of 114 hr·μM. Accounting for PK variability, a dose with the lower limit of exposure at 114 hr·μM should be the optimal dose, and thus 1200 mg/day is recommended as the biologically active dose. Supporting the selected dose is the dose-dependent rise in VEGF for non-progressors; these patients who received >= 1000 mg achieved up to 5 fold rise in VEGF. The rise in VEGF would be consistent with an increased expression of VEGF by tumor cells in response to hypoxia induced by the reduction in tumor vascular permeability and vascularization with PTK/ZK treatment.

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First dose in man phase I study of the anti-metastatic uPA inhibitor WX-UK1

R. Bartz[1], S. Ullrich[1], W.A. Schmalix[1], J. Knoeller[2], O.G. Wilhelm[1]. [1]*Wilex AG, Clinical Development, Muenchen, Germany;* [2]*FOCUS, GmbH, Bioanalytics, Neuss, Germany*

The plasminogen activation system with its key components urokinase-type plasminogen activator (uPA), the cell surface receptor uPAR and the inhibitor PAI-1, plays an important role in cancer cell invasion and metastasis. Inhibition of this system results in reduction of primary tumor growth and metastasis and is therefore an attractive target for therapeutic intervention. WX-UK1 is a synthetic inhibitor of uPA and the first representative of this substance class in clinical development. In this first dose in man, double-blind, randomized, three-way cross-over, placebo-controlled, phase I study pharmacokinetics, pharmacodynamics and safety and tolerability was investigated. Six escalating i.v. doses of WX-UK1 were administered in the range of 0.01-0.3 mg/kg and each dose was given to six healthy, male vol-