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.

235

Role of Id proteins in tumor angiogenesis

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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.

236

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

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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.

237

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

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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.

238

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

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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-

unteers. Pharmacokinetics was linear at the investigated doses with maximum plasma concentrations (Cmax) seen at the end of the 30 min. infusion. Within 4-6 h thereafter concentrations declined rapidly and the mean terminal phase half-lives were in the range of 10-12 h. Renal excretion of the parent compound is less than one percent of the dose. The systemic safety profile of WX-UK1 was highly acceptable at all dose levels. No changes in vital signs, ECG parameters, general safety laboratory parameters and adverse event profiles were observed which could be attributed to the administration of the study drug. Evaluation of regular inspections of the infusion site during and after infusion gave no indication of substance related local intolerance reactions. For the coagulation parameters PT, aPTT and TT minor increases were observed at doses of 0.05 mg/kg and higher (mean increases of 6-13% after WX-UK1 compared to 0-5% with placebo) at the end of infusion. Except for aPTT and PT (2.6% and 4.6% above the upper limit of normal) at Cmax all parameters remained within normal limits. All values returned to baseline within 15 min. and were regarded not clinically relevant. Bleeding times remained unchanged and there was no indication of a drug-induced hemolysis. The promising results of this phase I healthy volunteer study warrants further development of WX-UK1 as an anti-metastatic compound for the treatment of solid, malignant tumors. A phase I/II trial in gastric, pancreatic, ovarian and head & neck cancer patients will be launched in Q3 2002.

239

Non-clinical assessments of safety profiles of S-3304, a matrix metalloproteinase inhibitor

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To assess safety profiles of S-3304 in non-clinical settings, we conducted single and multiple dose toxicity studies in rats and dogs, reproductive toxicity studies in rats and rabbits (seg I, seg II), and genotoxicity studies in three systems (reverse mutation, chromosomal aberration and micronucleus tests). Safety pharmacology was also investigated. All these studies were conducted in accordance with Good Laboratory Practice and were approved by Animal Care and Use Committee. Oral single-dose toxicity studies with doses of 2000 mg/kg showed that S-3304 was well-tolerated and exerted no apparent abnormalities in rats and dogs. Oral one-month, three-month and six-month repeated-dose toxicity studies in rats showed no toxicologically significant findings with the doses up to 1000 mg/kg/day. The no-observed-adverse-effect level (NOAEL) in each rat study was estimated to be 1000 mg/kg/day. In oral one-, three- and twelve-month repeated-dose toxicity studies in dogs, reversible increases in plasma ALAT and ALP concentrations were observed at doses of 600 mg/kg/day, 200 mg/kg/day and 300 mg/kg/day, respectively. The NOAELs in these studies were 200 mg/kg/day, 70 mg/kg/day and 70 mg/kg/day in the one-, three-, and twelve-month studies, respectively. Several tests showed that S-3304 has no genotoxic potential *in vivo* as well as *in vitro*. In fertility and early embryonic development study in rats, the NOAELs were estimated to be 1000 mg/kg/day for fertility, development of embryos and general toxicity in males, and 100 mg/kg/day for general toxicity in females due to a decrease in the body weight gains. In teratogenicity study in rats, the NOAELs were estimated to be 1000 mg/kg/day for reproduction and development of embryos or fetuses, and 300 mg/kg/day for general toxicity in dams due to a decrease in food consumption. In the rabbit teratogenicity study, the NOAELs were estimated to be 1000 mg/kg/day for general toxicity in dams and developmental toxicity in embryos or fetuses, and 300 mg/kg/day for reproduction due to abortions in 2 dams. In the safety pharmacology, S-3304 antagonized drug-induced contractions of isolated gastrointestinal tissues at 10 and 100 μM, and inhibited gastric emptying in rats at 300 mg/kg p.o. However, S-3304 did not show any significant effects in general activity, central/autonomic nervous, respiratory/cardiovascular, digestive and renal systems. The data supported the initiation of clinical studies of S-3304.

240

Inhibition of VEGF binding to HUVEC receptors and of heparanase by the nonanticoagulant and antiangiogenic heparin derivatives ST1514 and ST2184

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Heparin, in addition to its anticoagulant effect, displays many other biological properties including modulation of growth factor activity and inhibition of the heparanase. However, heparin-based therapy in cancer is limited due to its anticoagulant activity. We have synthesized novel heparin derivatives with the aim to abolish the anticoagulant effects of heparin and to inhibit the heparin-binding growth factor activity. Several *in vitro* and *in vivo* tests were carried out for the identification and characterization of the most active compounds. The anticoagulant properties of the heparin were completely abolished in ST1514 and the corresponding low-molecular weight derivative ST2184. However, the compounds retained the ability to bind FGF-2 as the original heparin, but had significantly reduced capacity to induce FGF-2 dimerization. Receptor binding studies showed that both ST1514 and ST2184 were able to prevent the binding of VEGF165 to cell surface receptors in human umbilical vein endothelial cells (HUVEC) with an IC_{50} equal to 14 μM and 22.4 μM, respectively. Scatchard analysis of binding studies showed that ST2184 decreased three times the number of VEGF apparent receptors (KDR) but did not alter the receptor ligand affinity. The heparanase-inhibiting effect of compounds ST1514 and ST2184 was tested using recombinant human heparanase (Hpa1) and sulfate labeled, naturally produced extracellular matrix substrate. Both compounds were highly effective, yielding 95-100% and 70-80% inhibition of heparanase activity (i.e., release of labeled heparan sulfate degradation fragments) at 1 and 0.2 ug/ml, respectively. In the mouse Matrigel plug implanted subcutaneously, ST1514 and ST2184 treatment (25mg/kg s.c. twice daily for 7 days) caused a significant ($p<0.01$) reduction of the hemoglobin content in FGF-2-containing pellets. In conclusion, ST1514 and ST2184 represent heparin derivatives, devoid of anticoagulant effects, with potential antiangiogenic and antimetastatic properties.

241

The effect of nitric oxide on cyclooxygenase-2 expression is mediated through the activation of guanylate cyclase in head and neck cancer cell lines

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The over-expression of cyclooxygenase-2 (COX-2) in head and neck squamous cell carcinoma (HNSCC) was previously reported. Nitric oxide (NO) was also known to be simultaneously produced by inducible nitric oxide synthase (NOS) when COX-2 was over-expressed in many cancer cells. Since up-regulation of COX-2 by NO was reported in inflammatory responses, we hypothesized that NO may increase the expression of COX-2 in cancer cells. We investigated the cross-talk between nitric oxide and prostaglandins (PGs) pathway in HNSCC cell lines (SNU-1041, SNU-1066, and SNU-1076). When adding a NO donor, 50-500 μM SNAP, PGE2 level was increased 2-20 times through the increase of COX-2 expression. This increase of COX-2 expression by a NO donor or PMA was blocked in various degrees by NO-scavengers (PTIO, C-PTIO), NOS inhibitors, L-NAME and 1400W. Also, the increased expression of COX-2 in basal level was inhibited by NOS inhibitors. When treating with dibutyryl-cGMP, its effect on COX-2 expression was similar to one by SNAP in SNU-1041. COX-2 expression induced by SNAP was inhibited by ODQ, a guanylate cyclase (GC) inhibitor. These results imply that endogenous or exogenous NO activates GC and the increase of cGMP induces new sigaling to up-regulate the expression of COX-2 in HNSCC cell lines. We observed that there was this interaction between NO and COX-2 in three additional HNSCC cell lines (PCI-1, PCI-13, and PCI-50) and other types of cancer cells with the detectable expression of COX-2. We suggest that blocking NO production will be able to be a potent strategy for inhibition of COX-2 expression itself in some cancers. (This study was supported in part by 2001-2002 BK21 project for Medicine, Dentistry and Pharmacy)