lis, MN). Statistical analysis was performed by Cox's proportional hazard model. The pts population included 34 squamous, 26 adenocarcinomas, 12 large cell and 37 unspecified NSCLC. Nineteen pts had stage I-IIIA (94.7% underwent surgery), 29 had stage IIIB (55.2% underwent chemo-radiotherapy) and 61 had stage IV (65.6% pts received chemotherapy). At baseline, mean VEGF was 571 pg/ml (SD= 449). A difference was found for VEGF levels when measured at baseline or at 2 hours in a cohort of 68 samples (p=0.000009). VEGF positively correlated with white blood cells and platelets (r=0.24, p<0.0003; r=0.17, p=0.008) and negatively with Hb (r=-0.16, p=0.016). For stage, lymph nodes, metastasis (lung/brain) and co-morbidity (diabetes, arteriosclerosis, deep vein thrombosis) no association was detected. In 42 pts who were treated with Cisplatin and Gemcitabine, there was a trend for a better response in those with higher VEGF (61% vs 38%). Survival analysis was performed at a median follow-up time of 9 months and after 69 deaths. After adjusting for treatment, VEGF was associated with increased mortality risk (p=0.0004). Poor survival and high mortality risk were also associated with decreasing of Hb (p=0.0019) and low albumin levels (p=0.0004). VEGF was predictor of poor survival also in a multivariate model including treatment, Hb and albumin (Hazard Ratio 1.77, 95%CI from 1.01 to 3.10 for a variation of 1000 pg/ml, p<0.044).
Conclusions: A direct association between VEGF and mortality and an inverse correlation between Hb and VEGF were detected. The role of VEGF in response to chemotherapy could be due to its vascular permeability function. Measurements of serum VEGF should be performed at the same time point in order to reduce variability.

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Biomarkers (VEGF, BFGF) for assessing the biological activity of PTK787/zk222584 (PTK/ZK), a vascular endothelial growth factor (VEGF) receptor inhibitor, in tumours known to overexpress VEGF

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PTK/ZK is an orally active inhibitor of the VEGF receptor tyrosine kinases (flt-1/KDR), inhibiting VEGF-induced angiogenesis. It is known that such agents inhibit tumor growth, but not necessarily induce tumor regression. Thus, it is increasingly important to identify biomarkers that demonstrate the required drug-target interaction and the desired downstream biological effects. Two proangiogenic factors, plasma VEGF and bFGF (basic fibroblast growth factor) were assessed. Patients received a continuous daily dose of 50, 150, 300, 500, 750, 1000, 1200, 1500, or 2000 mg until progressive disease or toxicity. Samples were taken at predose, 10 hrs, day 8, day 15, day 15 + 10 hrs, day 22, and day 28 for each cycle. A total of 65 patients, with predominantly advanced colorectal cancer and glioblastoma, from 3 Phase I studies were evaluable for plasma VEGF and bFGF analysis. Using the 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) was 30% lower than on day 1. Dose proportional increase in exposure was observed up to 1000 mg. The terminal half-life was 3-6 hours. No dose-limiting toxicity was observed up to 2000 mg. The extent of rise for both soluble biomarkers, plasma VEGF and bFGF, was evaulated by: 1) the concentration at 10 hours post-dose ($VEGF_{10\,hrs}$, $bFGF_{10\,hrs}$) and 2) the maximum concentration within 28 days ($VEGF_{max0-28}$, $bFGF_{max0-28}$). An dose-dependent rise in both plasma VEGF and bFGF levels was observed within the first 28 days of treatment. The rise was more prominent in non-progressors than progressors. In non-progressors, plasma VEGF and bFGF levels increased, respectively, by 5 and 3 fold at doses >= 1000 mg. The rise in plasma VEGF and bFGF would be consistent with an increased expression of VEGF and bFGF by tumor cells in response to hypoxia induced by the reduction in tumor vascular permeability and vascularization induced by PTK7ZK treatment. The observed decline in plasma VEGF and bFGF is attributed to the death of tumor cells. These results are supportive of previous DCE-MRI results which showed a reduction in tumor vascular permeability and vascularization within 36 hours post first dose of PTK/ZK treatment. The soluble biomarkers, plasma VEGF and bFGF, may be useful as indicators for biological activity of anti-angiogenesis agents and consequently tumor response.

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Generation and characterization of monoclonal antibodies that antagonize the binding of VEGF-C to VEGFR-3 (Flt-4)

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VEGFR-3, a member of the vascular endothelial growth factor family of receptors, has been shown to be involved in the proliferation and survival of lymphatic endothelial cells. Experimental over-expression of its ligands, VEGF-C and VEGF-D, by tumor cells results in increased rates of tumor metastasis. Although VEGFR-3 is absent from normal vascular endothelium in adults, its expression has been reported in actively forming blood vessels in tumors. Thus, VEGFR-3 is a potential target for both anti-metastatic and anti-angiogenic therapy. We used phage display to generate fully human monoclonal antibodies to human VEGFR-3. One such antibody, HF4-3C5, demonstrates strong inhibition of soluble VEGFR-3 binding to immobilized VEGF-C. BiaCore analysis shows the K_D of VEGF-C binding to VEGFR-3 to be approximately 3.5 nM. Using the same technique, the apparent K_D of HF4-3C5 for VEGFR-3 is 56 pM, exceeding that of VEGF-C by about 100-fold. Deletion studies show that both VEGF-C and HF4-3C5 bind to the three N-terminal immunoglobulin-like domains of VEGFR-3. NIH-3T3 cells were transfected with expression vectors encoding either full-length human VEGFR-3 or a chimeric receptor in which the extracellular domain of human VEGFR-3 was fused to the transmembrane and kinase domains of cFms. Resulting stable cell lines responded strongly to VEGF-C but not VEGF-A in a mitogenic assay. This response was inhibited by greater than 90 % by HF4-3C5 but not isotype-matched control antibodies. Because HF4-3C5 does not bind to murine VEGFR-3, monoclonal antibodies to the mouse receptor are being generated for the purpose of conducting proof-of-principle studies. These experiments will evaluate anti-angiogenic and anti-metastatic efficacy of blocking the activation of VEGFR-3 in mouse tumor models.

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A phase I dose escalating study of the angiogenesis inhibitor thrombospondin-1 mimetic (abt-510) in patients with advanced cancer

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Thrombospondin-1 (TSP-1) is a naturally occurring protein inhibitor of angiogenesis. ABT-510, a thrombospondin-mimetic peptide that exhibits antiangiogenic activity in preclinical models, inhibits tumor growth in animal studies at concentrations * 200 ng/mL for 3 hours per day. We determined safety and pharmacology of subcutaneously (SC) administered ABT-510, either given as daily continuous infusion (CI) or as bolus injections once or twice daily (QD and BID) in a phase I study. Plasma samples for PK obtained on days 1 and 22 were analyzed by LC/MS/MS. In selected patients, PET-scans with $H_2{}^{15}O$ and ^{18}F-FDG were performed at days 1 and 22. Response was assessed after every 2 cycles of 28 days each. To date, 26 patients (pts) are treated with CI 100 mg/day (4 pts), bolus 50 mg BID (6 pts), 100 mg QD (6 pts), 200 mg QD (5 pts) and 260 mg QD (5 pts). Local CTC grade 2 skin infiltration at injection sites occurred in all pts of the CI cohort. CI dosing was stopped, while SC dosing continues. The most commonly observed adverse events include grade 1 and 2 fatigue, anorexia, insomnia, headache and nausea. One patient (NSCLC) with progressive disease had a hemorrhage in an unknown cerebellar metastasis after 32 days of ABT-510 treatment at 100 mg QD. Another patient (leiomyosarcoma) had a TIA after 21 days at 260 mg QD. Both SAEs were determined to be possibly related to ABT-510. No other clinically significant, treatment-related toxicities, nor cumulative toxicities have been observed to date. PK analysis on day 1 revealed rapid absorption with T1/2 of approx. 1 h, C_{max} of 955 $\pm$ 350, 1793 $\pm$ 549 and 3293 $\pm$ 1105 ng/mL and AUC of 2734 $\pm$ 728, 4168 $\pm$ 1335 and 8636 $\pm$ 1331 ng*h/mL for the 50 mg BID (N=5), 100 mg QD (N =3) and 200 mg QD (N=5) cohorts, respectively. PK data on day 22 showed similar results. Serial PET-scans have been performed in 4 patients to date. Stable disease according to RECIST-criteria was seen in 9 out of 23 evaluable patients for more than 2 cycles or 8 weeks. Five patients had stable disease > 16 weeks (different tumor types).