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).

Conclusion: In this ongoing study, ABT-510 can be administered safely at 200 mg/day SC. Plasma concentrations exceed efficacious concentrations in preclinical models.

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A novel anti-angiogenic/anti-metastatic peptide, ATN-161 (Ac-PHSCN-NH2), which targets multiple fully activated integrins including alpha-5 beta-1 and alpha-v beta-3, leads to increased anti-tumor activity and increased survival in multiple tumor models when combined with chemotherapy

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ATN-161, a novel anti-angiogenic/anti-metastatic peptide [Livant et al. (2000) Cancer Res 60: 309], is scheduled to enter into phase I clinical trials in October, 2002. We have hypothesized that one of the ways ATN-161 would be most effective in the clinical setting would be in combination with chemotherapy. Thus, we evaluated ATN-161 in combination with several chemotherapeutic agents in various models of tumor growth. ATN-161 (1-10 mg/kg, q3d)+ cyclophosphamide (160 mg/kg, day 1, 3, 5 after initiating treatment) synergistically reduced tumor growth rate of staged 3LL tumors resulting in increased survival of the combination therapy group as compared to vehicle controls or either drug alone. A similar result was obtained in the adjuvant setting in a DU145 tumor growth model. DU145 tumors were staged to ~1000 mm3 then surgically resected. Treatment with either ATN-161, taxol or the combination was initiated the day after surgery. The combination treatment group had the longest time to tumor recurrence. Some of the mice in this group had no recurrence and those tumors that did recur grew much more slowly than the tumors in the other treatment groups. The scheduling of ATN-161 treatment relative to chemotherapy was critical to the activity of the drugs in the combination regimen. Results in several other xenograft models (CWR22R, MDA-MB-231) using different combination regimens will also be presented.

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Pharmacokinetic/pharmacodynamic (PK/PD) relationships for the angiogenesis inhibitor ABT-510 in preclinical efficacy models

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ABT-510 is a subcutaneously (SC) administered nonapeptide thrombospondin analogue entering Phase II clinical development for treatment of advanced malignancies. The correlation of pre-clinical efficacy *in vivo* and pharmacokinetics was analyzed to help determine a PK target for human studies. Data were available from 11 murine models (melanoma B16F10, orthotopic (OT) bladder 253J B-V, OT bladder EJ-1, OT bladder HT-1376, OT breast MDA-MB-435, flank breast MDA-MB-435, flank breast MDA-435-LM, corneal micropocket assay, flank colon HC-T15, flank lung LX-1 and flank lung NCI-H460). ABT-510 was dosed SC by bolus (BID, QD, or QOD) or osmotic pump. Doses ranged from 0.1 to 200 mg/kg/day. Efficacy measures varied by model and included tumor volume, tumor weight, number of metastases, and VEGF or bFGF-stimulated new vessel density. PK were measured in satellite animals of the same strain or estimated from similar doses. For each model, a simple Emax equation was fitted to the efficacy and PK data. PK variables included Cmax, AUC, time/day where plasma concentration (Cp) > 10, 50, 100 or 200 ng/mL, as well as unit dose and dose/day. Selection of the best overall PK predictor of efficacy (time/day Cp>100 ng/mL) was made based on standard goodness-of-fit criteria. Results: Across all models where estimates were obtained, the mean Emax was 43% (ranging from 90% in melanoma to 6% in OT bladder 253J B-V). The Emax equation could not be fitted to data from four of the eleven models: flank colon, both flank lung and OT bladder HT-1376 models. The mean E50 was 1.1 h (ranging from 0.4 h in orthotopic bladder 253J B-V to 1.8 h in orthotopic breast). A target for clinical PK was defined as Cp>100 ng/mL for 3 h/day, which, on average, achieved 75% of Emax in the murine efficacy models. For comparison, in companion dogs with spontaneous solid tumors treated with ABT-510 SC 12.5 mg BID, Cp >100 ng/mL for >3 h/day was observed in all 10 animals for which PK data are available. Objective responses and disease stabilization have been observed in these pets. In humans, ABT-510 SC bolus doses of 100 mg QD achieve the target PK (Cp>100 ng/mL for >3 h/day) in both healthy subjects and patients with cancer.

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Minimization of the anti-angiogenic Histidine-Proline Rich Glycoprotein (HPRG) protein

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The potent anti-angiogenic activity of the abundant multi-domain plasma protein HPRG has recently been reported and localized to its Histidine-Proline Rich (H/P) domain (Juarez et al. Cancer Research; in press). HPRG is evolutionarily, functionally and structurally related to activated kininogen (HKa) which is also an anti-angiogenic molecule that stimulates endothelial cell apoptosis through binding to cell-surface tropomyosin (J-C. Zhang et al. Submitted). HPRG and the H/P domain, but not other domains of HPRG, also bind specifically and with high affinity to tropomyosin *in vitro*. The H/P domain of HPRG is composed of repetitions of the consensus sequence [H/P][H/P]PHG and using the *in vitro* tropomyosin binding assay, we have identified a 5-mer sequence that inhibits the binding of HKa to tropomyosin in the μM range. This pentapeptide also has moderate anti-angiogenic and anti-tumor activities in a Matrigel Plug model *in vivo*. The optimization of this 5-mer has been initiated and studies are currently underway to evaluate the activity of several of these optimized peptides *in vivo*. In addition, we have also prepared multimers of this sequence (4x, 3x, 2x). These peptides have increasing affinities for tropomyosin *in vitro* (4x>3x>2x) that correlate with increasing activities in a Matrigel Plug model *in vivo*. In conclusion, we report the identification of an active 5-mer consensus sequence and the development of several peptide lead series derived from the H/P domain of HPRG.

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A phase I study of TNP-470 continuous infusion alone or in combination with paclitaxel and carboplatin in adult patients with non small cell lung cancer (NSCLC) and other solid tumors

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TNP-470 (TNP) is a synthetic analog of fumagillin that demonstrates potent antiangiogenic properties. Previous studies suggest that the combination of TNP 60 mg/m^2 3 times per wk and paclitaxel (P) 225 mg/m^2 with or without carboplatin (C) AUC 5 is well-tolerated (ASCO 2000 and 2001). However, preclinical data suggest that TNP infused continuously (CI) may enhance antiangiogenic effects with manageable toxicity. This study was designed to determine the safety and MTD of TNP CI alone and with chemotherapy with or without a bolus of TNP. Single-agent TNP was administered in 28-day cycles and combination regimens in 21-day cycles.

Cohort	Patients (n)	P (mg/m^2)	TNP 1-hr (mg/m^2)	C (AUC)	TNP CI (mg/m^2/day)
A	6	–	–	–	10/28 days
C	7	–	–	–	10/5 days/wk
D	5	225	–	6	10/5 days/wk
F	6	200	–	6	2.5/5 days/wk
G	9	200	60	5	2.5/5 days/wk
H	6	200	60	6	2.5/5 days/wk

Thirty-nine subjects enrolled; 64% were chemonaive and 72% had NSCLC. Regimens in Cohorts A, C and D were not well-tolerated due to neurotoxicities and myelosuppression (80% grade 4 neutropenia in Cohort D). P and TNP were reduced in Cohort F resulting in improved tolerability. A TNP bolus was added in Cohorts G and H, with 55% and 66% of patients respectively completing * 4 cycles. Response rates in NSCLC patients in arms D-H was 47%. TNP CI 2.5 mg/m^2/day, with or without TNP 60 mg/m^2 1-hr infusion, appears to be well-tolerated when given in combination with P 200 mg/m^2 and C AUC 5-6, warranting further evaluation in NSCLC in a randomized setting.