inases grade 2 toxicity (2 subjects) and symptoms including headache, blurred vision, abdominal pain and vomiting (3 subjects). One of these was randomized to placebo. In the 3200mg bid group, all subjects randomized to S-3304 had hepatic transaminases increased to grade 1 or 2. The correlation between plasma drug concentration and raised transaminases is yet to be determined. Only the minority of subjects reported mild myalgia/arthralgia symptoms, and this did not interfere with their normal daily activities. AUC0-12,ss and Cmax,ss of S-3304 increased with dose level but less than proportionately (see Table).

Table. Steady-State Pharmacokinetic (PK) parameters of S-3304 (mean ± SD)

Dose level	800 mg*	1600 mg*	2400 mg*	3200 mg**
$AUC_{0-12,ss}$ (μg*hr/mL)	411±117	466±80	634±170	943±317
C_{max} (μg/mL)	80±20	93±13	120±21	140±27
$T_{1/2}$ (hr)	14.2±1.2	14.8±3.4	15.9±1.8	ND

*Day 28; **Day 14 (Dose discontinued before Day 28); ND: not determined

Pharmacokinetic parameters of metabolites will also be analysed.

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Dose and schedule optimization of 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

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ATN-161 (Ac-PHSCN-NH2) is currently completing pre-clinical development with the initiation of a phase I trial anticipated in October, 2002. Previously published data has demonstrated the ability of this peptide to inhibit tumorigenesis, angiogenesis and metastasis of subcutaneously inoculated tumors in a syngeneic (Mat LyLu) model of prostate cancer [Livant et al. (2000) Cancer Res 60: 309]. In addition, ATN-161 has been shown to inhibit angiogenesis in liver metastasis from intrasplenically injected CT26 mouse carcinoma cells [Stoelzing et al., Clin Cancer Res (2001) 7: 3656s]. We have extended these results to a syngeneic Lewis Lung Carcinoma (3LL) model and have observed that ATN-161 inhibits tumor growth as effectively as metronomically administered cyclophosphamide (170 mg/kg q6d) in the early 3LL model (ATN-161 given on or before day 6 after tumor cell inoculation). We have used this 3LL model to optimize dose as well as schedule. The inhibition of 3LL tumor growth by ATN-161 observed a U-shaped dose response with 1-10 mg/kg being the optimal dose. No anti-tumor effects were observed at 0.2 mg/kg and very little effect was observed at 100 mg/kg, the highest non-toxic dose tested, with escalating anti-tumor effects observed at 50, 25 and 12.5 mg/kg, respectively. This U-shaped dose response was confirmed using the CT26 metastasis model. At the optimal dose of ATN-161 (1 mg/kg), significant inhibition of liver metastasis was observed whereas no activity was observed at the high dose of ATN-161 (100 mg/kg) tested. Schedule optimization was also evaluated using the 3LL model. The optimal schedule was determined to be ATN-161 (1 mg/kg) q3d. These results will provide rationale for the starting dose and schedule for phase I trials.

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In vitro antiangiogenic activity of thalidomide analogues

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Thalidomide, also known as (alpha-(N-phthalimido)-glutarimide), is currently in Phase II clinical testing as a single agent or in combination with chemotherapy against a number of solid tumors such as gliomas, prostate and renal cell carcinomas. The resurgence of interest in thalidomide can be attributed to its antiangiogenic activity, which was shown to be mediated by a metabolilte – 5'OH thalidomide. Using the backbone of the metabolite, we synthesized 118 unique thalidomide analogues and examined their antiangiogenic activity *in vitro*. Preliminary experimental data selected seven of these analogues for further evaluation. In the rat aortic ring assay, six of the seven analogues significantly inhibited microvessel outgrowth at 12.5-200 uM. Thalidomide failed to block angiogenesis at similar concentrations. Subsequently, the effects of these analogues on human umbilical vein endothelial cell (HUVEC) proliferation and tube formation were studied. Six of the seven analogues demonstrated antiproliferative action in HUVECs. Cell proliferation was not affected by thalidomide. Interestingly, all seven analogues as well as thalidomide suppressed tube formation. Analogues in the tetrafluorophthalimido class showed the highest potency and efficacy in all three assays. Taken together, our results support the further development of thalidomide analogues as antiangiogenic agents. The *in vivo* toxicology and therapeutic potential of the described analogues in the treatment of prostate cancer are presently under investigation.

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VEGF-Trap: a novel, potent VEGF blocker with anti-tumor effects

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Vascular endothelial growth factor (VEGF) plays a critical role during the normal process of angiogenesis required for embryonic development, and plays a key role in the pathological angiogenesis that occurs in a number of diseases, including cancer. One of the most effective ways to block the VEGF-signaling pathway is to prevent VEGF from binding to its normal receptors by administering decoy soluble receptors. By determining the requirements to maintain high affinity while extending *in vivo* half-life, we were able to engineer a very potent VEGF blocker with desirable pharmacokinetic properties. The resulting VEGF Trap, a soluble decoy receptor created by fusing the ligand-binding immunoglobulin domains of VEGF receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2) to the constant region (Fc) of human IgG1, is the highest affinity VEGF blocker described to date, with an affinity for VEGF of 1-5 pM. The VEGF-Trap effectively suppresses tumor growth and vascularization *in vivo*, resulting in stunted, and almost completely avascular tumors. VEGF Trap mediated blockade may be superior to that achieved by other agents, such as monoclonal antibodies targeted against the VEGF receptor. The VEGF Trap is currently undergoing a Phase I clinical trial.

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Tumor genotype, RRM1 expression, and outcome of patients with lung cancer

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We have described frequent allele loss on chromosome segment 11p15.5 and its association with metastatic spread and shortened survival in patients with non-small cell lung cancer. Patients with stage I disease, i.e. absence of spread to lymph nodes, and allele loss had survival comparable to patients with stage II disease, i.e. cancer present in lymph nodes. We have recently reported the complete genomic sequence and transcript map for the minimal region of allele loss, and it encompasses the complete gene for the regulatory subunit of ribonucleotide reductase (RRM1). However, functional inactivating mutations were not found by screening a subset of NSCLC. Our recent cell biological studies have provided evidence for a functional role of RRM1 in suppression of cell migration, invasion, and *in vivo* metastasis formation, that is independent of an alteration in the deoxynucleotide (dNTP) pool. Other investigators have reported reduced anchorage independent growth in ras-transformed mouse fibroblasts transfected with RRM1 and a role for RRM1 in microtubule nucleation of centromeres in Xenopus. Here, we measured the level of expression of RRM1, compared to the expression of RRM2 (catalytic subunit of ribonucleotide reductase) and p53R2 (catalytic subunit involved in dNTP supply for DNA damage repair), and investigated the association with allele loss, RRM1 promoter polymorphisms, and survival. Tissue specimens from 51 patients undergoing resection for NSCLC were collected and immediately frozen in liquid nitrogen. Total RNA was extracted, reverse transcribed, and used for real-time quantitative PCR (ABI Prism 7700). Primers and probes for the genes RRM1, RRM2, and p53R2 were designed to cross introns, and the amplicons were 95 bp, 90 bp, and 105 bp respectively. Gene expression was normalized using 18S rRNA as reference. We found that RRM1 expression was associated with RRM1/D11S4932 allele loss, with a median RRM1 value of 3.8 in specimens with allele loss compared to 43.7 in those without allele loss. RRM1 expression was also associated with the A/C promoter polymorphism, with a median RRM1 value of 12.9 in patients with the CC allelotype, 72.8 in those with the AA allelotype, and 22.8 in heterozygotes (AC allelotype). RRM1 and RRM2 expression were highly correlated

($p<0.001$), but RRM1 and p53R2 expression were not significantly correlated. There was a trend towards longer survival in patients with increased RRM1 expression.

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Inhibition of VEGFR tyrosine kinase by ZK 222584/ ptk 787 (PTK/ZK) combined with fractionated radiotherapy (RT) in human squamous cell carcinoma (hSCC) in nude mice

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Purpose: To investigate the effect of the antiangiogenetic substance PTK/ZK, a specific inhibitor of VEGFR tyrosine kinases, on the growth rate of different hSCC and on the growth delay after fractionated RT of hSCC.
Materials and methods: Five hSCC lines (FaDu, UT-SCC-14, UT-SCC-33, UT-SCC-15, MKG7) were transplanted s.c. in NMRI nu/nu mice. Presence of murine VEGFR mRNA was confirmed by RT-PCR. At a mean tumor diameter of 6 mm animals were treated daily with PTK/ZK (joint development of Schering and Novartis; 50 mg/kg bodyweight per os) or with carrier (control). In a second set of experiments FaDu and UT-SCC-14 tumors were irradiated with 15 fractions of 2 Gy under ambient conditions (200 kV X-rays, 0.5 mm Cu, 1.2 Gy/min). PTK/ZK was given either before (4-8 days), during (15 days), or after (45 days) the course of fractionated RT.
Results: PTK/ZK was well tolerated. A significant decrease of growth rate in tumors treated with PTK/ZK was observed in 3 of the 5 hSCC. For the combination experiments with RT a non-responding (FaDu) and a responding (UT-SCC-14) tumor model were chosen. Short-term application of PTK/ZK before and during fractionated irradiation did not significantly change the growth delay of FaDu and UT-SCC-14 tumors. In both tumor models the longer application of PTK/ZK after fractionated RT showed a significant increased growth delay compared with irradiated controls. In UT-SCC-14 a significant increase in local tumor control was observed.
Conclusions: Short term neoadjuvant or simultaneous application of PTK/ZK did not decrease the efficacy of fractionated RT in non-responding FaDu and responding UT-SCC-14 tumors. Adjuvant application improved the effect of RT in both tumor models, i.e. also in FaDu tumors in which PTK/ZK alone had no effect. This might suggest enhanced sensitivity of irradiated tumor vessels to VEGFR-inhibition. Supported in part by Schering AG, Berlin, Germany.

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Use of a novel, hepatocyte growth factor-induced transcript, Mig-7, as a marker for circulating and migrating cancer cells

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Although various molecular markers have been used for the detection of circulating cancer cells in the blood or of migrating cancer cells in tissue surrounding tumors, many have been found to be limited to certain types of cancers or not to be specific for cancer cells. Hepatocyte growth factor, also known as scatter factor (HGF/SF), has been shown to cause migration of many different types of cancer cells upon activation of the c-Met protooncogene receptor. HGF/SF has also been shown to cause epithelial to mesenchyme transition so that migrating cancer cells are difficult to detect in the stroma surrounding the tumor. Both HGF/SF and c-Met have been localized to the invasive edge of tumors. Because HGF/SF and c-Met are found in normal cells as well as in the bloodstream, they themselves do not make good markers for migrating and circulating cancer cells. Our laboratory has isolated a novel, HGF/SF-induced transcript, now called Mig-7 that is specific to migrating cancer cells. We hypothesized that circulating cancer cells could be detected in the blood using Mig-7 as a marker. Under Internal Review Board approval, we isolated total RNA from the blood of treated and untreated metastatic cancer patients (breast, endometrial, and lung) and compared transcripts to those from normal individuals. By RT-PCR, we detected Mig-7 mRNA in 66.7% of blood samples from untreated patients (n=3) and a complete absence of Mig-7 transcripts in treated (n=2) or normal individuals (n=3). Our second hypothesis was that Mig-7 is a marker for epithelial to mesenchyme transitioned migrating cancer cells in normal tissue surrounding tumors. We have tested tumor samples from metastatic cancer patients and were able to detect various levels of Mig-7 mRNA in 100% of the tumor samples (n=4). Negative control was negative for Mig-7 expression and positive controls showed that RNA was intact and that there was no DNA contamination. Results from a cancer-profiling array of various cancer types show that Mig-7 expression is detected in pathologist evaluated "normal" tissue surrounding tumors. In conclusion, Mig-7 may be used as a broad spectrum, cancer cell-specific marker to detect circulating and migrating cancer cells.

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Endothelial precursor cells from human bone marrow: target for anti-angiogenesis therapy

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Tumor vasculature has been a potential target for anti-cancer therapy. While blood vessels can be derived from nearby existing vasculature, more recent evidence is suggesting that endothelial precursor cells (EPCs) derived from bone marrow or mobilized into peripheral blood may play a role in neoangiogenesis. Endothelial precursor cells from bone marrow expressing CD34 and AC133 markers of endothelial cell lineage were stimulated in culture with vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and heparin on fibronectin-coated flasks. Within two weeks, cells that had been maintained in suspension became adherent and elongated. As EPCs continued to differentiate and proliferate, expression of AC133 and CD34 was downregulated while expression of VEGFR2/FLK-1 was upregulated. Cells also expressed CD105, a common stem cell marker and protein expressed in vascular endothelial cells. Expression of additional endothelial markers such as VE-cadherin, CD31, and von Willebrand factor was also investigated. In addition to stimulation with VEGF and bFGF, the roles of epidermal growth factor (EGF), platelet derived endothelial cell growth factor (PD-ECGF), and transforming growth factor beta (TGF-b) were subsequently explored to determine their effects on cellular differentiation. These endothelial progenitor cells can form tubule networks on Matrigel, and possess migratory and invasive properties *in vitro*. Lectin-binding and acetylated LDL uptake have also been investigated. Because EPCs may be involved in the development of tumor vasculature, the response of these precursor cells to cancer cells in various settings was demonstrated *in vitro* in a novel tumor spheroid assay. Endothelial precursor cells from bone marrow or mobilized into circulation with cytokines can be stimulated by pro-angiogenic factors into differentiating into a more mature cell type that possesses properties associated with well-defined endothelial cells such as HMVECs and HUVECs. The potential contribution of EPCs to tumor neovascularization defines them as an additional target for drug intervention therapy that may lead to a reduction in tumor vasculature or prevention of metastasis.

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The protein tyrosine kinase inhibitor SU5614 inhibits FLT3 and induces growth arrest and apoptosis in AML cells expressing a constitutively activated FLT3

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Activating mutations of the protein tyrosine kinase (PTK) FLT3 can be found in approximately 30% of patients with acute myeloid leukemia (AML) thereby representing the most frequent genetic alterations in AML. These mutations occur in the juxtamembrane (FLT3ITD) and the catalytic domain (FLT3D835/836) of FLT3 and confer IL-3 independent growth to Ba/F3 cells. In the mouse BMT model, the FLT3ITD mutants induce a myeloproliferative syndrome stressing their transforming activity *in vivo*. In this study we analyzed the pro-proliferative and anti-apoptotic potential of FLT3 in FLT3ITD/D835 transformed Ba/F3 cells and AML cells expressing an endogenous activated FLT3 receptor by using the PTK inhibitor SU5614. SU5614 has inhibitory activity for FLT3 and induces growth arrest, apoptosis and cell cycle arrest in Ba/F3 and AML cells expressing a constitutively activated FLT3. No cytotoxic activity of SU5614 was found in leukemic cell lines which express a nonactivated FLT3 or no FLT3 protein. At the biochemical level, SU5614 downregulated the activity of the hyperphosphorylated FLT3 receptor and its downstream targets STAT3, STAT5 and MAPK and the STAT5 target genes BCL-XL and p21. Our results show that SU5614 is an PTK inhibitor of FLT3 and has potent anti-proliferative and pro-apoptotic