safety profile, immunogenicity, pharmacokinetics, and monocyte saturation, as monocytes express the a5 ß1 receptor.
Results: To date, 16 pts have been enrolled and 15 pts have received M200. Baseline demographics for the 15 treated pts were median age=58 years (range 29–81 years), mean ECOG score=1 (range 0–2), and tumor types: colorectal (4), hepatoma (2), melanoma (2), bronchioalveolar carcinoma (1), thyroid (1), parotid (1), renal cell carcinoma (1) breast (1), esophageal (1) and neuroendocrine tumor of the pancreas (1). One pt received 0.5 mg/kg, 2 received 1 mg/kg, 3 received 2.5 mg/kg, 3 received 5 mg/kg, and 6 received 10 mg/kg. No dose-limiting toxicities have been observed. The adverse events that were possibly-related to study drug were mild to moderate nausea (5), fever (2), vomiting (2), headache (2), anorexia (2), and asthenia (2). No pts had an infusion reaction. Two of 16 (13%) pts developed HACA, but there were no apparent associated adverse events. A dose of 10 mg/kg was well-tolerated, achieved monocyte saturation, and a mean trough level of 82 mcg/mL two weeks after the 1st dose, which is above the minimum effective *in vitro* concentration of 2–3 mcg/mL. Thus, 10 mg/kg every 2 weeks is the recommended dosage regimen for subsequent clinical trials. The response outcomes were: SD (9) and PD (6), with 5 of 6 pts who received 10 mg/kg having SD.
Conclusions: M200 appears to be well tolerated at doses up to 10 mg/kg. As dose-limiting toxicity has not been observed, dose escalation is continuing with additional patients to be enrolled into the 15mg/kg cohort. Final data from this study will be presented.

167 POSTER

Combination with PI3 kinase inhibitors allows drastic dose reduction of tumor necrosis factor

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Both in patients treated using the isolated limb perfusion technique and in animal models, Tumor Necrosis Factor (TNF), alone or in combination treatment, displays a very potent antitumor effect. The severe shock-inducing effect resulting from a systemic application of the high doses of TNF needed to obtain the antitumor activity, however, limits its use to locoregional treatment. In mice, we previously could show that the antitumor and shock-inducing activities are not inevitably linked (Cauwels et al., Immunity 13:223, 2000). In order to allow systemic treatment, lowering of the dose of TNF required for tumor destruction is nevertheless necessary. Using transgenic mouse technology, we could show that TNF exerts its antitumor activity by a selective destruction of the tumor vasculature, rather than by a selective cytotoxic effect on transformed cells. This is triggered by an interaction of TNF with the TNF-R1 receptor on endothelial cells. Since PI3kinase (PI3K) is a central component of survival pathways in neovascular endothelial cells, we investigated whether they could be sensitised to the angiodestructive effects of TNF by PI3K inhibitors.
C57BL/6J mice bearing an established tumor (B16, LLC, EL4 or PG19) were treated with daily paralesional injections of murine TNF in combination with wortmannin, or either agent alone, for 10days. In the presence of the latter PI3K inhibitor (0.25 mg/kg, 1h before TNF), the dose of TNF required to obtain complete tumor destruction dropped from 0.35 mg/kg to 0.04 mg/kg. Also human TNF, that, in mice, is a selective TNF-R1 agonist, has a much faster clearance, and can not induce regression of the tumor, turned to be able to cause complete tumor destruction in mice in combination with wortmannin (as was previously shown for the combination of hTNF and IFN-gamma). No synergism was observed when TNF-R1−/− mice were used, while the synergism was retained when tumor cells were used that were rendered insensitive to TNF by a transfection with a dominant negative mutant of TNF-R1. This indicates again that the synergism targets the vasculature rather than the cancer cell in strict sense. Selected key experiments were repeated using the reversible PI3K inhibitor LY 294002, resulting in similar
Conclusions: In groups where higher doses of TNF were used, wortmannin but not LY294002 increased the toxicity of TNF. At lower doses of TNF, effective in the combination treatment, no increase of the toxicity was observed. The PI3K inhibitors used had no or only a marginal growth retarding effect on the tumors, when used alone at the same doses.
Together with some enhancement of the previously established maximal tolerated dose, that could be obtained by supportive measures and/or inhibitors of the toxicity, the order of magnitude of this dose reduction, when also present in human cancer patients, is likely to result in effective doses that are of the same order of magnitude as the tolerated ones. In this respect, it is worthwhile to refer to our recent results, showing that low doses of TNF could enhance the uptake and the effect of doxorubicin encapsulated in long-circulating liposomes (Brouckaert et al., Int. J. Cancer 109:442, 2004), indicating that further combinations with chemotherapeutic agents could result in additional synergisms.

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168 POSTER

PRL-3 promotes invasion in tumor epithelial cells and tube formation in normal endothelial cells

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Phosphatase of Regenerating Liver 3 (PRL-3) has become a drug target in cancer since it was first identified as a marker of colon tumor endothelium (St. Croix et al., Science, 2000) and subsequently recognized as a marker of colon tumor metastasis (Saha et al, Science, 2001; Bardelli et al, Science, 2003). Because of the presence of PRL-3 in two cellular compartments of the tumor, the endothelial and epithelial compartments, future inhibitors of PRL-3 promise a two-pronged attack against tumors. Anti-PRL-3 compounds are expected simultaneously to induce a regression of the tumor vasculature and to interfere directly with epithelial malignancy. We sought to identify the function of PRL-3 in cultured endothelial cells and tumor epithelial cells. We have previously reported that the phorbol ester PMA stimulated PRL-3 mRNA expression and induced proliferation, invasion and tube formation in human microvascular endothelial cells (HMVECs). Given the known upregulation of multiple genes by PMA, it was unclear at the time whether PRL-3 was a major driver of any of these three phenotypes. We now report that HMVECs infected with a PRL-3-expressing adenoviral vector express high levels of the PRL-3 protein and show increased tube formation. These data suggest that PRL-3 was a major driver of the PMA-stimulated tube formation and that it can on its own increase tube formation. These results implicate PRL-3 directly in tumor angiogenesis.
We have previously reported that PRL-3 exogenous expression promoted invasion in the DLD-1 human colorectal adenocarcinoma cell line. We also reported earlier that endogenous PRL-3 mRNA expression correlated positively with invasiveness in the MCF-7 and SKBR3 human breast cancer cell lines and in the SKNAS and IMR-32 human neuroblastoma cell lines in the Matrigel invasion assay. We now report that a PRL-3 siRNA reduces invasion down to 20% of control in the transfected DLD-1 cell model. These data provide additional evidence that PRL-3 stimulates tumor epithelial cell invasion. We also report that the expression of MMP-3 enzyme correlates positively with invasiveness and with PRL-3 protein expression in the transfected DLD-1 cell model as well as in the MCF-7, SKBR3, SKNAS and IMR-32 cell lines, which express PRL-3 endogenously. These data strengthen the evidence indicating that PRL-3 promotes tumor epithelial cell invasion. In addition, these results implicate a possible coregulation of PRL-3 and MMP-3 as a mechanism by which PRL-3 stimulates invasion.
Our studies confirm the involvement of PRL-3 in both tumor angiogenesis and epithelial malignancy. Specifically, our results shed light upon two different functions for PRL-3, depending on which cell type expresses the protein. PRL-3 seems to promote tube formation in endothelial cells, while stimulating invasion in tumor epithelial cells.

169 POSTER

Maximizing the anti-tumor and anti-proliferative effects of 2ME2 by maintaining levels above a threshold concentration for a defined period of time

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2-Methoxyestradiol (2ME2), an endogenous metabolite of estradiol, is an inhibitor of both tumor and endothelial cell proliferation. 2ME2 is currently in oncology clinical trials under the name Panzem®. Various new formulations of Panzem® have been developed recently to increase absorption. These studies were undertaken in an effort to understand the pharmacodynamics of 2ME2, by defining the critical pharmacokinetic parameters associated with inhibition of tumor growth.
MDA-MB-231 human breast carcinoma or Lewis lung carcinoma cell lines were incubated with media alone or increasing concentrations of 2ME2 and inhibition of proliferation was determined by cell counts. Following 48 hr exposure the IC50 values of 2ME2 in MDA-MB-231 and LLC cells are 0.8 μM and 3 μM, respectively. Further studies in which MDA-MB-231 cells were exposed to 2ME2 for limited periods of time each 24 hours demonstrated that a 6 hr incubation with 0.6 μM 2ME2 most closely approached the level of growth inhibition observed following continuous exposure. The level of growth inhibition following exposure of MDA-MB-231 cells to 0.6 μM 2 ME2 for 6 hr could not be mimicked by comparable exposures (AUCs) obtained by altering 2ME2 concentration and incubation time. Moreover, incubation of MDA-MB-231 cells with high concentration

of 2ME2 (10 or 3 μM) for short times (1 hr) did not inhibit cell growth. Taken together, these experiments suggest time above a critical threshold concentration, and not simply Cmax or AUC, is an important parameter for maximizing 2ME2-mediated tumor growth inhibition.
The importance of these results was assessed in vivo. Determination of plasma drug concentrations and exposure times required for the inhibition of tumor growth was carried out in a variety of models including the Lewis lung carcinoma (LLC) model of experimental metastases and an orthotopic human xenograft model in nude rats. These studies were used to assess the relationship between PK and PD of 2ME2. The data support the importance of time above a threshold concentration for optimal anti-tumor activity. This pharmacodynamic model of 2ME2 activity is being further explored in additional tumor models and is being used to assist in the design, selection, and evaluation of dosing regimens for future clinical trials.

170 POSTER

Å6, a urokinase plasminogen activator (uPA)-derived peptide: a phase I trial in patients with advanced gynecologic cancer

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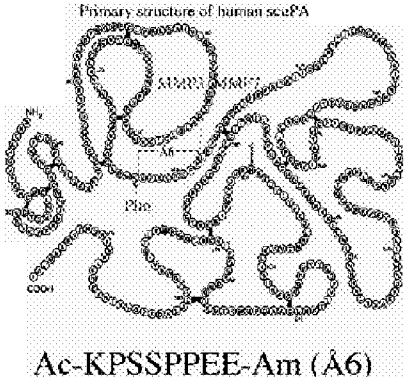
Background: Å6 is an 8 amino-acid peptide (Acetyl-Lys-Pro-Ser-Ser-Pro-Pro-Glu-Glu-NH_2) derived from uPA that has anti-angiogenic, anti-migratory, anti-invasive, and anti-metastatic, but not anti-proliferative properties preclinically. A phase I trial of Å6 in patients (pts) with advanced gynecologic cancer was conducted to define the toxicity, maximum tolerated dose (MTD), maximum feasible dose (MFD), and pharmacokinetics (PK), and to explore anti-tumor activity and the effects of Å6 on biomarkers of the urokinase system (serum uPA, soluble uPAR, and PAI-1 levels).
Methods: Previously treated advanced gynecologic cancer pts with adequate organ and hematologic function, elevated CA-125 levels and measurable disease received Å6 subcutaneously (SC). The first cohort received Å6 at 150 mg SC daily for 14 consecutive days every 28 days. The second cohort received Å6 at 150 mg SC daily for 28 consecutive days/cycle, and the third cohort received Å6 at 300 mg SC daily for 28 consecutive days/cycle. Three pts were evaluated at each dose level, with a provision to expand to 6 pts if a dose-limiting toxicity (DLT) was observed. If no DLT occurred, 6 additional pts would be accrued at the MFD. PK studies were performed during cycle 1 using HPLC-MS/MS.



Results: 16 pts with a median age of 63 years (range 45–76) have been treated. Tumor types included ovarian (11), endometrial (3), primary peritoneal serous (1), and cervical (1). Median number of prior regimens was 6 (range 3–9). Four pts were treated in cohort 1 (<1, 1, 2, 2 cycles), one of whom was replaced for early disease progression, 3 in cohort 2 (2, 3, 6+ cycles), and 9 in cohort 3 (1, 1, 2, 2, and 5 pts still on study for up to 4 cycles). All serious adverse events have been due to disease progression. No serious drug-related adverse events or dose-limiting toxicity occurred at the MFD, and the MTD was not achieved. Drug-related side effects were limited to grade 1 local injection site reactions and possibly grade 1 diarrhea. One pt (in cohort 3) has experienced a confirmed decrease in CA-125 of >50% with stable disease on CT scan after 2 cycles, and one pt (in cohort 2) has continued on Å6 for >6 months with stable tumor measurements. PK and biomarker data are pending.
Conclusions: Å6 given daily continuously is well tolerated at all dose levels, without any drug-related adverse events or dose-limiting toxicity. A phase II trial is underway in ovarian cancer.

171 POSTER

Unnatural small molecule mimic of VLA-4-binding VCAM-1 epitope inhibits the intravascular adhesion and proliferation of metastatic melanoma cells

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Background: Alpha4-beta1 integrin (VLA-4) is a leucocyte ligand for vascular cell adhesion molecule-1 (VCAM-1) that contributes to the adhesion of metastatic cancer cells to endothelium and stromal cells. Inhibition of VLA-4/ VCAM-1 interactions has therapeutic potential. However, due to the challenging relative efficacy/safety ratio of antibodies and disintegrin-based therapies, strategic alternatives in the forms of small molecule antagonists are being sought.
Material and Methods: Here, nonpeptidyl small molecule antagonists to VLA-4 were generated and tested in a prometastatic model of inflammation involving VLA-4/VCAM-1 interaction-dependent adhesion between cytokine-stimulated B16 melanoma (B16M) cells and hepatic sinusoidal endothelium (HSE) cells inflamed by tumor-conditioned medium (TCM).
Results: [(2S, 3R, 4S, 5S)-3-(1-(S)-benzyloxy-2-methyl-butyl)-4-nitro-5-phenyl] prolylglycine was the most potent inhibitor for VLA-4 binding to VCAM-1. Preincubation of B16M cells with this compound abrogated their adhesion to TCM-activated HSE. It also abrogated interleukin-18-dependent B16M cell adhesion to immobilized recombinant VCAM-1. Both intrahepatic microvascular retention of B16M cells and metastasis decreased by 50% in mice receiving VLA-4 antagonist-pretreated B16M cells. Metastatic growth of VLA-4 antagonist-pretreated B16M also decreased as compared to untreated cells. The rank order of potency for this VLA-4 antagonist in vitro was consistent with that observed in vivo, which confirms that their efficacy is likely via blockade of alpha4beta1/VCAM-1 interactions.
Conclusions: These data support the utility of small molecule alpha4beta1 antagonists in the chemoprevention of inflammation-dependent metastasis promoted by cytokine-dependent VLA-4/VCAM-1 interactions, such as hepatic melanoma metastasis.

172 POSTER

Preclinical profile of ABP309, a potent 2nd generation VEGF receptor tyrosine kinase inhibitor belonging to the class of aminonicotinamides

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The inhibition of angiogenesis offers the potential for a new therapeutic approach to malignancy, with the prospect of chronic, well-tolerated therapies stabilizing or preventing recurrence of disease. Vascular endothelial growth factor (VEGF) is a dimeric angiogenic factor that is overexpressed by many cancer cells. VEGF drives the angiogenic cascade in vascular endothelial cells by promoting the expression of invasive proteases, the activation of integrins and the induction of migration and mitosis. Furthermore, VEGF increases vascular permeability, characteristic of tumor vessels (Manley P, et al. *BBA* 2002;1697:17–27). VEGF binds to VEGFR-1 (Flt-1) and VEGFR-2 (KDR, flk-1) receptors, two high affinity transmembrane receptor tyrosine kinases, expressed on vascular endothelial cells. The phthalazine derivative PTK787/ZK222584 (co-developed by Novartis and Schering AG) is a potent multi VEGF receptor tyrosine kinase inhibitor. This compound possesses good oral bioavailability and has demonstrated anti-angiogenic and anti-tumor activity in a range of animal models (Wood JM, et al., *Cancer Res.* 2000;60:2178–2189; Bold G, et al. *Drugs Future* 2002;27:43–55).. PTK/ZK is currently in Phase **III** clinical trials in metastatic colorectal cancer. Here we report on a different chemical class of potent 2nd generation VEGFR-2 tyrosine kinase inhibitors and discuss the preclinical properties of ABP309, as a model compound. ABP309, 2-[[(1,6-dihydro-6-oxo-3-pyridinyl)methyl]amino]-N-[3-(trifluoromethyl) phenyl]-3-pyridinecarboxamide, selectively inhibits the receptor tyrosine kinases VEGFR-2/KDR (IC_{50} 0.037 μM) and VEGFR-3/Flt-4 (IC_{50} 0.33 μM). In addition, ABP309 shows modest activity against VEGFR-1/Flt-1, c-Kit, PDGFRß and c-Fms receptors (IC_{50} 0.81-, 0.4-, 1.7-, 1.8 μM). In human umbilical vein endothelial cells and Chinese hamster ovary cells expressing KDR, ABP309 inhibits VEGF-induced KDR tyrosine phosphorylation with IC_{50}