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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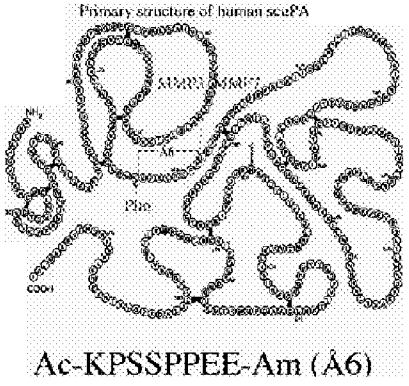
Background: Å6 is an 8 amino-acid peptide (Acetyl-Lys-Pro-Ser-Ser-Pro-Pro-Glu-Glu-NH_2) derived from uPA that has anti-angiogenic, anti-migratory, anti-invasive, and anti-metastatic, but not anti-proliferative properties preclinically. A phase I trial of Å6 in patients (pts) with advanced gynecologic 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}

values of 0.002 and 0.01 μM, respectively. In mice, ABP309 is well absorbed following an oral dose of 50 mg/kg attaining a C_{max} of 15 μM after 0.5 hours, with 32% bioavailability and elimination half-life of 1.8 hours. Furthermore, ABP309 inhibits VEGF-induced angiogenesis in a murine growth factor inplant angiogenesis model and exerts anti-tumor activity in a range of tumor models. The preclinical pharmacokinetic profile of this 2nd generation VEGFR inhibitor will be presented in more detail, covering the use of different formulations and of salt forms.

173 POSTER

Prostate specific membrane antigen (PSMA) expression in the neo-vasculature of non-prostate cancers: in vitro target validation and in vivo imaging

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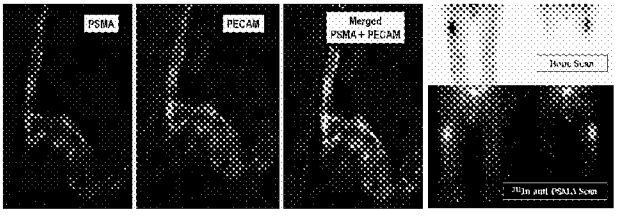
Background: Prostate specific membrane antigen (PSMA), a transmembrane folate hydrolase consisting of 750 amino acids, has been consistently detected in normal and hyperplastic prostate tissues, in pre-cancerous lesions and prostate cancer (PCa) using immunohistochemistry and other techniques. The expression of PSMA in Non-PCa is currently being pursued as a target for diagnostic imaging and anti-cancer antibody therapeutics.
Methods: PSMA expression was measured on a series of fresh frozen and formalin-fixed paraffin embedded non-PCA malignancies by transcriptional profiling (TP) using cDNA microarrays on nylon membranes, RT-PCR (Taqman), in situ hybridization (ISH), western blotting, dual co-localization immunofluorescence (IF) and immunohistochemistry (IHC) both before and after laser capture microdissection (LCM) using both internal domain (7E11) and external domain (MLN591) antibodies. In vivo imaging was performed using ^{111}I-conjugated Anti-PSMA (J591) in patients with primary lung, breast, colorectal and renal carcinomas.
Results: PSMA mRNA expression was localized to the neo-vasculature in 55% of a series of breast, colon, lung and ovarian cancers using an S-35 labeled probe and ISH. PSMA mRNA expression was localized to the endothelium of the tumor vessels after microdissection using Taqman™ RT-PCR. 40% of the same carcinomas were positive for PSMA immunoreactivity of the tumor vasculature by IHC on frozen sections with the MLN591 antibodyDual IF studies using the MLN591 antibody and anti-CD31 (PECAM-1) localized PSMA expression to the endothelium of neo-vasculature in carcinomas of the breast, colon, lung and ovary, in Wilm's tumors and neuroblastomas (Figure). Using the 7E11 antibody on paraffin sections, PSMA staining was observed in 9/10 clear cell renal cell carcinomas, 7/10 infiltrating ductal breast cancers, 6/10 invasive colorectal cancers and 4/10 non-small cell lung cancers. Patients with lung, breast, colorectal and renal cancers (image) were successfully imaged in vivo with the radio-labeled anti-PSMA conjugate.
Conclusion: PSMA expression is regularly associated with the neo-vasculature of many non-PCas and co-localizes with endothelial cell markers. A variety on non-PCas can be detected in vivo by anti-PSMA radiolabeled imaging. Further studies of PSMA in non-PCa as a target for both diagnostic imaging and anti-cancer antibody-based therapies appear warranted.



174 POSTER

Correlation between protein kinase C-beta expression and patient survivals in primary tumors – implications for clinical drug development

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PKC-β is an isoform of protein kinase C, a family of serine-threonine kinases involved in a wide range of signal transduction pathways such as cell proliferation, cell differentiation and apoptosis. Recent evidence implicated the role of PKC-β in signal cascade of vascular endothelial cell growth factor (VEGF), B cell function and B cell receptor signal pathway. PKC-β was also shown to be one of the most prominently overexpressed genes in fatal/refractory DLBCL patients. Therefore, its role in tumor development and angiogenesis makes it a potential therapeutic target in cancer. LY317615 (Enzastaurin HCl) is a potent and selective inhibitor of PKC-β. The compound exhibited antiangiogenic activity in a preclinical animal model and is well tolerated in toxicology studies. In this study, we first analyzed NCI 60 cell line gene expression profiles to identify genes that show correlation with cells' response to LY317615 for growth inhibition. We then analyzed public gene expression profiling data on different types of cancer to investigate if PKC-β gene expression is correlated with patient survival. Our analysis has demonstrated that high PKC-β expression has a strong correlation with poor patient outcome in DLBCL, confirming the observations published in previous publications on these datasets. A similar demonstration of a correlation between PKC-β expression and poor survival was observed in glioblastomas. When we performed similar analyses in other subtypes of lymphoma, such as MCL, CLL as well as other solid tumors, including NSCLC, we did not find a correlation between PKC-β expression and survival. Analysis of microarray data on DLBCL has also indicated that expression of genes in cell survival signaling and proliferation pathway that are closely related with PKC-β expression, consistent with previous findings that PKC-β plays an essential role in these pathways. Taken together, these results suggest that PKC-β in DLBCL and glioblastomas is associated with poor survival suggesting that inhibiting this molecule in patients with such malignancies may provide a clinical benefit.

175 POSTER

Pseudolarix acid B inhibits angiogenesis and reduces hypoxia-inducible factor 1alpha by promoting proteosome-mediated degradation

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Angiogenesis plays a critical role in tumor progression. Vascular endothelial growth factor (VEGF), which can be secreted from neighboring tumor cells, potently stimulates tumor angiogenesis. The inhibition of tumor angiogenesis including inhibition of VEGF signaling pathways has been one of the promising strategies in the development of novel anticancer therapy. Pseudolarix acid B (PAB), the naturally occurring diterpenoid isolated from the root bark of Pseudolarix kaempferi Gordon tree (Pinaceae), possesses potent antifungal and pregnancy-terminating effects that may be tightly associated with angiogenesis. This study was to examine its angiogenic inhibition, impact on VEGF secretion from tumor cells, and the possible molecular mechanism of its action. Results showed that PAB inhibited VEGF-stimulated proliferation and migration, and fetal bovine serum (FBS)-stimulated tube formation of human umbilical vein endothelial cells (HUVECs) in a concentration-dependent manner. The chicken chorioallantoic membrane (CAM) assay further revealed that PAB (10 nmol/egg) significantly suppressed in vivo angiogenesis. ELISA data also showed that PAB could abrogate hypoxia-induced VEGF secretion from human breast cancer MDA-MB-468 cells via reducing the protein level of hypoxia-inducible factor 1α (HIF-1α). Further analysis using LY294002, a phosphatidylinositol 3-kinase (PI3K) inhibitor and U0126, a MEK inhibitor, showed that the increase in HIF-1α protein level was highly dependent on PI3K and p42/p44 mitogen-activated protein kinase (MAPK) activities in hypoxic MDA-MB-468 cells. However, PAB treatment did not affect the active (phosphorylated) forms of Akt and Erk. Interestingly, the selective proteosome inhibitor MG-132 completely reversed the reduction of HIF-1α protein in the PAB-treated MDA-MB-468 cells. Together, the results reveal that PAB displays the dual activities of directly inhibiting endothelial cells and abrogating paracrine stimulation of VEGF from tumor cells. Additionally, PAB accelerates HIF-1α protein degradation probably by stimulating the proteosome pathway in MDA-MB-468 cells. Further studies on the molecular mechanism of its stimulatory effect on the proteosome pathway may well generate new therapeutic opportunities.

176 POSTER

In vitro human metabolism of BAY 57-9352: a novel VEGFR-2/PDGFR kinase inhibitor

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VEGFR-2 and PDGFR are key mediators of tumor angiogenesis. Disruption of signal transduction by these receptors inhibits tumor growth in preclinical