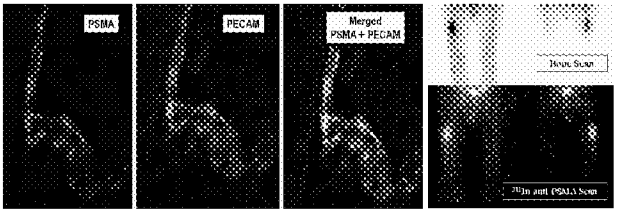
values of 0.002 and 0.01 μM, respectively. In mice, ABP309 is well absorbed following an oral dose of 50 mg/kg attaining a C_{max} of 15 μM after 0.5 hours, with 32% bioavailability and elimination half-life of 1.8 hours. Furthermore, ABP309 inhibits VEGF-induced angiogenesis in a murine growth factor 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

J. Ross[1,2], c. Sheehan[1], D. Schenkein[2], I. Webb[2], G. Gray[2]. [1]*Albany Medical College, Pathology and Laboratory Medicine, Albany, USA;* [2]*Millennium Pharmaceuticals, Oncology Development, Cambridge, USA*

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

D. Li[1], M. Phong[1], M. Lahn[2], D. Thornton[2], L. Brail[2], G. Ganji[1], B. Liao[1]. [1]*Eli Lilly & Company, Integrative Biology, Indianapolis, USA;* [2]*Eli Lilly & Company, Medical Oncology, Indianapolis, USA*

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

M. Li[1], Z. Miao[1], W. Tan[1], J. Yue[2], C. Zhang[1], L. Lin[1], X. Zhang[1], J. Ding[1]. [1]*Shanghai Institute of Materia Medica, Division of Anti-tumor Pharmacology, Shanghai, China;* [2]*Shanghai Institute of Materia Medica, Division of Phytochemistry, Shanghai, China*

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

S. Walle[1], M. Radtke[1], A. Kern[1], K. Schmeer[1], D. Seidel[1], P. Adams[2]. [1]*Bayer HealthCare AG, Department of Drug Metabolism and Isotope Chemistry, Wuppertal, Germany;* [2]*Bayer HealthCare, Department of Pharmacokinetics and Drug Metabolism, West Haven, USA*

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

models using human tumor xenografts of a variety of histological origins. Efforts to identify inhibitors of these receptor tyrosine kinases led to the identification of BAY 57-9352. This compound is a potent, orally active inhibitor of VEGFR-2 and PDGFR tyrosine kinase activity, cellular receptor autophosphorylation and VEGF- and PDGF-stimulated mitogenesis *in vitro*. The present studies describe the investigation of major metabolic pathways of BAY 57-9352 following incubations with liver preparations, the elucidation of metabolizing human cytochrome P450 (CYP) isoforms involved and the inhibitory and inductive potential of BAY 57-9352 on major human CYP isoforms. The main phase I metabolic pathways in man were assessed *in vitro* using human liver microsomal preparations. Additionally, phase II biotransformations were assessed in a sandwich model with cultured hepatocytes. CYP 3A4, 2C8, 2C19 and 1A2 and glucuronosyl transferase were found to be the major metabolizing enzymes. These data indicate that BAY 57-9352 metabolism is mediated by multiple enzymes.
The potential of BAY 57-9352 to induce the human P450 isoforms CYP 1A2 and 3A4 was investigated in cultured human hepatocytes of five different donors. No inductive effects on CYP 1A2 and 3A4 were observed after treatment of human hepatocytes with concentrations up to 7 μM BAY 57-9352 in the hepatocyte medium. These results provide evidence that BAY 57-9352 is not an inducer of CYP1A2 and 3A4 up to a concentration, which is markedly higher than therapeutic plasma concentrations of BAY 57-9352 in preclinical models. In addition, no major CYP enzyme inhibition was observed. Taken together, the data indicate that clinical drug-drug interactions through inhibition or induction of CYP enzymes by concomitantly administered drugs are unlikely. Based on a favorable pharmacological and *in vitro* metabolic profile, BAY 57-9352 has advanced to Phase 1 clinical trials as an anti-angiogenic agent.

177 POSTER

Tyrosine kinase inhibition as a strategy to suppress growth of primitive endothelial cells

V. MacDonald[1], E.B. Dickerson[1], N. Akhtar[1], J.F. Modiano[2], S.C. Helfand[1,3]. [1]*University of Wisconsin-Madison, School of Veterinary Medicine, Madison, USA;* [2]*University of Colorado Health Sciences Center, Immunology and Cancer Center, Denver, USA;* [3]*University of Wisconsin, Comprehensive Cancer Center, Madison, USA*

Blockade of receptor tyrosine kinases (RTKs) is an increasingly important area of cancer research because RTKs are ubiquitous, and mutated forms often give rise to cells that are constitutively activated. Imatinib, an inhibitor of the split RTK family including c-kit and vascular endothelial growth factor receptor (VEGFR), has had a major impact on the treatment of several cancers associated with dysregulated activation of RTKs. Angiogenesis, the formation of new blood vessels from established vasculature, results from proangiogenic stimuli, such as vascular endothelial growth factor (VEGF), stem cell factor (SCF), and others, acting on mature endothelial cells. To begin to test the hypothesis that blocking RTKs on endothelial cells could suppress angiogenesis in cancer, we developed a unique endothelial xenograft model utilizing neoplastic canine endothelial cells (SB-HSA) derived from a malignant angiosarcoma in a dog (Akhtar et al, Neoplasia 6:106, 2004). All studies were done with prior approval of the UW Animal Care Committee. Canine angiosarcomas are comprised of primitive endothelial cells and recapitulate many features characteristic of mitotically active endothelial cells (Fosmire et al, Lab Invest 84:562, 2004). The cells used in our xenograft model express VEGFR-2 and c-kit, as well as their ligands, VEGF and SCF. NOD-SCID mice injected with SB-HSA cells and treated with imatinib developed statistically smaller tumors compared to untreated control mice. Therefore, blocking RTKs in this model had an important therapeutic benefit in arresting the growth of mitotically active endothelial cells. To extend these findings and to begin to learn more about the pathways affected, we have examined spontaneous angiosarcomas from multiple dogs and found them to express c-kit uniformly. We are pursuing investigations intended to clarify expression and activation of other RTKs including VEGFR-2 and platelet-derived growth factor as possible targets of imatinib in this model. Taken together, we have shown a promising effect of RTK inhibition in a novel angiogenesis model that may have potentially broader applications in cancer. Furthermore, the natural occurrence of canine angiosarcoma may provide a unique opportunity to evaluate RTK inhibition as a strategy to suppress the angiogenesis of malignancy in an out bred animal model. (Supported by NIH CA86264, Morris Animal Foundation D03CA-71, Canine Health Foundation 2025, and a UW Companion Animal Fund grant)

178 POSTER

Toxin-VEGF fusion protein inhibits tumor growth

T. Hu[1], C. Hamby[2], J. Backer[1], M. Backer[1]. [1]*SibTech, Inc., Newington, USA;* [2]*New York Medical College, Microbiology and Immunology, Valhalla, USA*

Growth of primary tumor and metastatic lesions beyond few millimeters requires neovascularization that combines angiogenesis and vasculogenesis. The critical question in attacking neovascularization is how to target the tumor-associated vasculature while sparing the normal quiescent vasculature. Endothelial cells in tumor vasculature overexpress VEGFR-2, a major receptor for vascular endothelial growth factor (VEGF), which is expressed only at low levels in quiescent vasculature. To target only those endothelial cells that overexpress VEGFR-2, we have constructed a novel toxin-VEGF fusion, named SLT-VEGF by fusing the catalytic subunit A of Shiga-like toxin I (SLT) to the 121 amino acid-long splice variant of VEGF. To obtain an "FDA-friendly" protein, the SLT-VEGF coding sequence was cloned into the pET29a(+) bacterial expression vector (Novagen) carrying a kanamycin resistance gene. The resulting protein contains 293 aa of the full-length SLT subunit A fused to 121 aa of the human $VEGF_{121}$ via a 7-aa linker GTDDDDK. SLT-VEGF is purified from inclusion bodies to >95% purity with a yield of >5 mg/L. SLT-VEGF selectively kills (IC_{50} ~0.07 nM) growing endothelial cells overexpressing VEGFR-2, but not quiescent endothelial cells, or cells expressing low levels of VEGFR-2. SLT-VEGF retains full activity after storage for more than a year at −70ºC, one month at +4ºC, or seven days at room temperature.
SLT-VEGF activity *in vivo* was tested using PC3 human prostate tumors in Ncr nu/nu mice and 4T1 mouse mammary carcinoma tumors in Balb/c mice. Mice (n = 15) received five bi-weekly injections of SLT-VEGF, 0.05 mg/kg/injection (1 μg/mouse/injection). This SLT-VEGF treatment significantly inhibited tumor growth in both models while inducing only transient discomfort in mice and a low-titer anti-SLT antibody response. Mice bearing 4T1 tumors were also treated with different SLT-VEGF treatment regimens and tumor growth for every individual mouse was analyzed. By plotting tumor growth rates for individual mice as linear regression curves, responder and non-responder mice could be clearly distinguished in each group. We found that the proportion of mice whose tumors respond to SLT-VEGF treatment increases with an increasing cumulative dose of SLT-VEGF.
To establish the mechanism of SLT-VEGF action, the protein was injected into 4T1 and PC3 tumors that were excised 24 h later, sectioned and stained for apoptotic cells with a TUNEL kit and for markers of endothelial cells, such as VEGFR-2 and PECAM. Judging by co-localization of apoptotic and endothelial markers, SLT-VEGF induces apoptosis preferentially in endothelial cells overexpressing VEGFR-2.
We expect that the combination of highly potent and selective cytotoxicity *in vivo*, convenience of FDA-friendly production, and excellent stability would make SLT-VEGF a valuable candidate for clinical development.

179 POSTER

Augmentation of radiation response in upper aero-digestive tract tumors with the vascular targeting agent ZD6126

T. Hoang[1], S. Huang[2], E. Armstrong[2], P. Harari[2]. [1]*University of Wisconsin Medical School, Medical Oncology, Madison, Wisconsin, USA;* [2]*University of Wisconsin Medical School, Human Oncology, Madison, Wisconsin, USA*

Background: Epithelial tumors of the upper aero-digestive tract result in particularly high morbidity and mortality. Angiogenesis plays an essential role in epithelial tumor growth and metastasis. We have performed preclinical studies to investigate the anti-tumor effects of radiation combined with ZD6126, a novel agent targeting the microtubules of endothelial cells, in head and neck (H&N) and lung cancer cell lines. The hypothesis is that tumor control may be enhanced by simultaneously targeting tumor (radiation) and tumor vasculature (ZD6126).
Methods: To characterize specific cellular effects and anti-tumor activity of the investigational regimen, a series of *in vitro* studies using endothelial cells (HUVEC), and *in vivo* studies in athymic mice bearing human lung (H226, A549) and H&N (SCC1) tumor xenografts were performed.
Results: Exposure to ZD6126 results in clear morphologic changes in HUVEC and inhibits HUVEC growth in a dose dependent manner. ZD6126 also inhibits the process of capillary-like network formation in HUVEC. Flow cytometry analysis using propidium iodide staining indicates that ZD6126, with or without radiation, results in cell cycle arrest in G2/M. ZD6126 augments radiation-induced apoptosis in HUVEC as measured by caspase assay. *In vivo* matrigel angiogenesis assay demonstrates that the anti-vascular effect of ZD6126 is enhanced by combination with radiation. In tumor xenografts, extensive necrosis is observed in H226 tumors as early as 24 hours following the injection of ZD6126. Experiments in athymic mice bearing SCC1 tumor xenografts demonstrate that the combination of