Patients and Methods: Eligible patients had pathologically proven, measurable unresectable or metastatic HCC, performance status $\leqslant$1, Cancer of the Liver Italian Program (CLIP) score $\leqslant$3, and adequate organ functions. For cycle 1 (14 days), B was given alone intravenously at 10 mg/kg on day 1. For cycle 2 and beyond (28 days/cycle), B was given at 10 mg/kg on days 1 and 15, gemcitabine was administered at 1000 mg/m^2 as dose rate infusion at 10 mg/m^2/minute followed by oxaliplatin at 85 mg/m^2 on days 2 and 16. A dynamic first-pass perfusion CT was performed after intravenous injection of 70 ml of iodinated contrast at 7 cc/s. The data were analyzed to calculate tissue blood flow (BF), blood volume (BV), mean transit time (MTT), and permeability surface area product (PS). CT perfusion and circulating endothelial cells (CECs) assays were performed at baseline and on days 10−12 following B administration during cycle 1.
Results: Of the thirty-three patients enrolled, 23 patients had CT perfusion scans and 21 patients had CECs performed at both baseline and 10−12 days following the B administration during cycle 1. Compared to the baseline, a significant decrease in the estimated tumor perfusion parameters including BF, BV, and PS and an increase in MTT were seen following B administration alone (Table 1). Two patterns of CEC changes were seen: 12 patients had increased CECs and 9 patients had decreased CECs following B administration. Changes in CT perfusion scan parameters and CECs did not correlate with time to tumor progression.

Table 1. CT perfusion scan parameters at baseline and following bevacizumab administration

Parameter	Baseline	Post-bevacizumab	*P*-value
Blood flow (mL/100 mg/min)	105±92.9	50±28.8	0.014
Blood volume (mL/100 mg)	5.4±3.9	2.7±1.1	0.009
Mean transit time (sec)	7.3±2.8	8.8±2.3	0.009
Permeability surface (mL/100 mg/min)	34.3±14	21.9±8.2	0.003

Conclusion: Bevacizumab can induce a decrease in BF, BV and PS and an increase in MTT in HCC. Two patterns in CEC changes were seen following B administration. While the changes in CT perfusion scan parameters and CECs may reflect the antiangiogenic effects of B, they did not correlate with time to tumor progression in this study.

55 POSTER

Cell surface proteomic analysis of human renal tumor endothelium

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Targeting tumor angiogenesis is a new anti-cancer strategy that has gained widespread support, but inadequate molecular information is available for human tumor vascular endothelium as it exists *in vivo*. This is in part due to technical limitations imposed by the small percentage of endothelial cells in tissue. By employing mass spectrometry (MS) as a tool to identify proteins that are over-expressed in cancer cells relative to normal cells, we aimed to discover new targets that could be utilized in tumor vasculature therapy. We focused our studies on cell surface proteins as they play a vital role in a plethora of cellular processes. Membrane proteins from endothelial cells of cancerous and matched normal human tissues were isolated through positive selection with known endothelial markers (>95% purity). Proteins were then preferentially captured, digested with trypsin and subjected to MS analysis. Peptides were first quantified followed by identification of differentials. To date, we have identified numerous proteins expressed on endothelial cells including known markers such as CD31 and CD146, several extracellular matrix proteins and a set of proteins with uncharacterized function. Moreover, initial studies have identified >20 proteins expressed at least 5-fold higher by MS in renal tumor-associated endothelium compared to normal endothelium.
Independently, MS based discovery of proteins over expressed on the epithelium of human solid tumors identified proteins associated with the endothelium. IHC analyses of these targets across a panel of multiple tumor types identified 3 with elevated expression in tumor endothelium. Interestingly, siRNA targeting of specific targets in human umbilical vein endothelial cells resulted in the inhibition of proliferation and induction of apoptosis. Further experiments are in progress to validate our differentially expressed renal tumor endothelial targets by IHC and their relevance to proliferation and apoptosis. These studies highlight the distinction between human tumor and normal endothelium *in vivo* and that, our large scale proteomic mapping capabilities can provide a platform for identification of novel therapeutics.

56 POSTER

Distribution of sunitinib and its active metabolite in brain and spinal cord tissue following oral or intravenous administration in rodents and monkeys

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Introduction: Sunitinib malate (SU11248; SUTENT®) is an oral multitargeted receptor tyrosine kinase inhibitor of KIT, PDGFR, VEGFR, RET, CSF-1R, and FLT3. It was recently approved by the US FDA – and has received a positive CHMP opinion for EU approval – for the treatment of advanced RCC and of GIST after disease progression on or intolerance to imatinib mesylate therapy. We report here the results of preclinical studies investigating the distribution of sunitinib and its active metabolite SU12662 in brain and spinal cord tissue.
Methods: Mice received sunitinib at 9 mg/kg via tail vein injection. Brain and plasma concentrations were measured to assess the ability of sunitinib to cross the blood brain barrier. Rats received a single oral dose of [^{14}C]-sunitinib at 15 mg/kg and tissue distribution was evaluated using quantitative whole body autoradiography. Female monkeys were treated with sunitinib at 0, 6, or 12 mg/kg/day for up to 56 days. The concentrations of sunitinib and SU12662 in brain and other tissues collected at terminal sacrifice at 24 hours to 6 weeks post last dose, were assayed via LC-MS/MS, with tissue concentrations compared to plasma concentrations at 24 hours.
Results: In mice, brain penetration was rapid with drug concentrations 7-fold greater than plasma concentrations at 5 and 60 minutes. Drug concentrations in both brain and plasma declined rapidly and in parallel over time. At 3, 6, and 24 hours after a single dose of ^{14}C-sunitinib in the rats, brain concentrations of total drug-related materials were generally 30–40% of the plasma concentrations. Brain concentrations fell to background levels beyond 24 hours post dose. In monkeys, at 24 hours post last dose, the brain concentrations of sunitinib and its major and active metabolite, SU12662, were similar to the plasma concentrations (C_{24}), with the brain to plasma concentration (T/C_{24}) ratios of approximately 1 to 3.
Conclusions: Sunitinib or its metabolite penetrate the CNS with rapid clearance in all three species but does not appear to accumulate. These nonclinical results are suggestive of a favorable potential for anti-tumor activity in the brain, but further evaluation is required to determine the optimal target drug concentrations in the clinical setting.

57 POSTER

Correlation of receptor tyrosine kinase (RTK) activity and apoptosis with response to sunitinib treatment in patients with gastrointestinal stromal tumor (GIST)

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Background: Approximately 85% of GISTs exhibit *KIT* gene mutations and another 5–7% have activating mutations of *PDGFRA*. Imatinib mesylate, a KIT and PDGFR kinase inhibitor, has considerable activity as first-line treatment for advanced GIST; however, ~12−14% of patients have primary resistance to imatinib and >40% develop secondary resistance after a median of 25 months. Sunitinib malate (SU11248; SUTENT®) is an oral multitargeted RTK inhibitor of KIT, PDGFR, VEGFR, RET, CSF-1R, and FLT3. Sunitinib was recently approved by the US FDA – and has received a positive CHMP opinion for EU approval – for the treatment of GIST after disease progression on or intolerance to imatinib mesylate therapy and of advanced renal cell carcinoma. This study examined the effects of sunitinib treatment on tumor and endothelial cell apoptosis, and correlated these changes with clinical benefit (CB) in patients with imatinib-resistant or -intolerant GIST. Prior data have shown that sunitinib exerts its antiangiogenic effect, in part, by inhibiting VEGFR-2 activity.
Methods: In a phase I/II trial, 97 such patients received sunitinib per 1 of 3 schedules: 25, 50, or 75 mg/day for 2 weeks followed by 2 weeks off treatment (2/2 schedule), 50 mg/day (4/2 schedule), or 50 mg/day (2/1 schedule). Tumor biopsies were obtained from 20 patients at baseline and after $\geqslant$11 days of treatment during cycle 1. Immunofluorescence coupled with laser scanning cytometry was used to quantify RTK activity and apoptosis of tumor and endothelial cells. Changes in RTK activity and apoptosis were correlated with CB (partial response [PR] or stable disease [SD] >6 months evaluated using RECIST).
Results: PDGFR-β phosphorylation significantly decreased ($P = 0.006$) in patients with clinical benefit and increased in patients with progressive disease (PD; Table 1). These effects were most pronounced in tumor-associated endothelial cells. Sunitinib-associated CB was also associated

with significant increases in tumor cell and endothelial cell apoptosis ($P \leqslant 0.05$).

Table 1. Correlation of change in p-PDGFR-β activity with clinical benefit.

Clinical outcome	No. of patients	Change in p-PDGFR-β activity
CB	8	18.2% decrease (P = 0.006) (42% decrease [P = 0.008])*
PR	2	26.1% decrease (P = 0.001)
SD	6	13.9% decrease (P = 0.04)
PD (SD < 6 months)	12	9.9% increase (P = 0.06) (23% increase [P = 0.443])*

*Change in p-PDGFR-β activity in tumor-associated endothelial cells.

Conclusions: PDGFR-β phosphorylation was significantly lower in tumor biopsies from patients with GIST who experienced CB but not in biopsies from patients with PD. Sunitinib appears to exert its antiangiogenic effects by inhibiting PDGFR-β activity in tumor-associated endothelial cells, in addition to inhibiting VEGFR-2 activity. Endothelial cell PDGFR-β phosphorylation may be a sensitive marker of sunitinib biological activity.

58 POSTER

Antiangiogenic and anti-invasive activities of the kinase inhibitor sunitinib malate on experimental human glioblastoma in vitro and in vivo

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Background: Angiogenesis inhibitors appear to be promising therapies for highly vascularized tumors such as glioblastoma multiform (GBM). Sunitinib (SUTENT®, SU11248) is an oral multitargeted tyrosine kinase inhibitor with both antiangiogenic and antitumor activities due to selective inhibition of various receptor tyrosine kinases, including those important for angiogenesis (VEGFRs and PDGFRs).
Material and Methods: Here we evaluated the antitumor activities of sunitinib on orthotopic models of GBM *in vitro* and *in vivo*.
Results: Sunitinib potently inhibited angiogenesis which was stimulated by implantation of U87-MG and GL15 cells into organotypic brain slices at concentrations as low as 10 nM. At high dose (10 μM), sunitinib induced direct antiproliferative and proapoptotic effects on GL15 cells and decreased invasion of these cells implanted into brain slices by 49% ($P < 0.001$). Treatment was also associated with decreases in src (60%) and FAK (73%) phosphorylation. However, anti-invasive activity was not observed *in vivo* at the highest dose level utilized (80 mg/kg/day). Survival experiments involving athymic mice bearing intracerebral U87-MG GBM demonstrated that oral administration of 80 mg/kg sunitinib (5 days on, 2 days off) improved median survival by 36% ($P < 0.0001$). Sunitinib treatment caused a 74% reduction in microvessel density ($P < 0.05$), an increase in tumor necrosis, and a decrease in number of MIB-1-positive GBM cells.
Conclusions: The main finding of the present study is that sunitinib exhibited potent antiangiogenic activity which was associated with a meaningful prolongation of survival of mice bearing intracerebral GBM. This data supports the potential utility of sunitinib in the treatment of GBM.

59 POSTER

Adiponectin as a novel therapy for the suppression of liver cancer growth and metastasis

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Background: Recently, adipocyte-derived factor – adiponectin has been demonstrated to be able to suppress angiogenesis in addition to its anti-inflammatory function. It will have great clinical impact to explore the possibility of the application of adiponectin in liver cancer therapy, together with the underlying liver diseases, such as liver cirrhosis and NASH. In the present study, we aim to investigate the effect of adiponectin in the suppression of liver cancer growth and metastasis.
Material and Methods: The orthotopic liver tumor nude mice models with different metastatic potential were applied. 5×10^6 MHCC97H or MHCC97L cells were injected subcutaneously into the right flank of the mice. Once the subcutaneous tumor reached 1 cm in diameter, it was removed and cut into about 1–2 mm cubes which were implanted into the left liver lobe of another group of nude mice. Ad-adiponectin (1×10^8) (treatment group) or Ad-luciferase (control group) was injected via portal vein after tumor implantation. The animals were sacrificed at day 30, 40 and 50 after tumor implantation. The tumor growth and proliferation (Ki67) and local/distant metastases were compared among the groups. Hepatic stellate cell activation in the tumor tissue was detected by α-SMA staining. Cell signaling related to invasion, migration (ROCK-Rho, CAK and FAK) and angiogenesis (VEGF) were compared. The effect of adiponectin on hepatic stellate cell was also investigated by in vitro functional study.
Results: The tumor growth was significantly inhibited by adiponectin treatment at different time points accompanied with the lower incidence of lung metastasis compared to the control groups at different time points. The hepatic stellate cell activation by α-SMA staining in the liver tumors was suppressed by adiponectin treatment. The treatment group got lower incidence of Ki67 positive tumor cells. Protein expression of CAK and FAK was down-regulated in the adponectin treatment groups by immunostaining. Gene and Protein expression of Rho, ROCK and VEGF in the liver tumors was also suppressed.
Conclusion: Adiponectin treatment significantly inhibited liver tumor growth and metastasis by suppression of hepatic stellate cell activation in tumor and down-regulation of cell invasion and angiogenesis pathways.

60 POSTER

Targeting the chemokine receptor CXCR4 and ligand SDF-1/CXCL12 in tumor vasculature and stroma

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Background: The chemokine receptor CXCR4 is expressed at high levels in human cancers. The CXCR4 ligand, SDF-1 (stromal-derived factor 1) is a pro-angiogenic factor secreted by stromal cells. Tumor stroma consists of a variety of cell types including endothelial cells (EC), fibroblasts, progenitor cells, and pericytes. Tumor vasculature may be targeted by therapeutics directed against this pathway.
Materials and Methods: Gene expression analysis was performed on cell lines and databases generated from normal and tumor tissues. Flow cytometry assessed CXCR4 expression; ELISA quantified SDF-1 secretion. Inhibitors were tested in tube formation assays. Progenitor cells were co-injected with colon carcinoma cells to further explore the CXCR4-SDF-1 axis in vivo.
Results: RT-PCR analysis of a 61-cell line panel revealed CXCR4 mRNA expression in EC were comparable to human carcinoma cells. Gene expression analysis confirmed CXCR4 expression in healthy artery tissue, bulk bone marrow, white blood cells, and EC that were isolated from normal lung, brain, breast, and colon samples; CXCR4 was overexpressed 2-fold in EC derived from tumors of the same patients. Comparison of CXCR4 expression in tumor tissue vs. normal counterpart revealed a 2–9-fold increase in CXCR4 mRNA expression in many tumor types. Secreted SDF-1 levels in cultured media were measured by ELISA. MSC and HDF secreted the highest levels of SDF-1 compared to pericytes and EC. Flow cytometry indicated that HUVEC, HMVEC, pericytes, and fibroblasts in vitro express CXCR4 while mesenchymal stem cells (MSC) do not.
HUVEC tube formation on Matrigel was inhibited by antibodies against SDF-1 or CXCR4. Pericyte tube formation was also affected by an antibody against CXCR4 and AMD3100. Immunohistochemistry performed on tumors arising from the co-injection of MSC that secrete SDF-1 and CXCR4-positive colon cancer cells indicate that MSC contribute not only to the stroma, but associate with EC, suggesting that SDF-1 production by MSC can influence both cancer cells and developing blood vessels.
Conclusions: While CXCR4 overexpression in malignant cells is becoming more widely recognized, the tumor vasculature offers additional therapeutic targets. Secretion of SDF-1 by fibroblasts or MSC enhances angiogenesis through the recruitment of CXCR4+ EC, progenitors, and pericytes. A dual approach with antagonists against CXCR4 and/or its ligand SDF-1 against cancer cells and stroma may provide clinical benefit.

61 POSTER

Participation of paxillin in the inhibition by 4-hydroxycoumarin of experimental melanoma metastases

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During the metastasic process, cancerous cells change their adhesiveness and increase their motility. Paxillin is a multidomain adapter protein that interacts with integrins as well as with cytoskeletal proteins, having a crucial participation in the reorganization of the cytoskeleton needed for adhesion and migration. Therefore, changes in paxillin expression or activation correlate with the metastatic potential of cancerous cells. Previously, we