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