and effect of antimetabolite treatment was assessed by urogenital and prostate weights, tumor grade and proliferation, and apoptotic markers. To study possible roles for FAS in prostate cancer, we examined the genetic alterations, which are associated with FAS protein expression and activity knockdown. We conducted a gene microarray analysis of prostate cancer cells with FAS knockdown by FAS gene specific siRNA in comparison to control treated cells.
**Results:** The anti-tumor efficacy of FAS inhibitors c75 and Orlistat was dose dependent and demonstrated a strong correlation to inhibition of akt phosphorylation and FAS pathway activity, reduced prostate and urogenital weights and decreased tumor grade compared to vehicle treated mice. Additional anti-tumor mechanistic studies demonstrated inhibition of tumor cell proliferation and induction of apoptosis. Our gene array data revealed that numerous genes are altered in expression including many proliferation and apoptotic genes with FAS knockdown that play significant roles in many pathways including cell growth, development, and cell signaling. These data suggest the upregulation of FAS expression plays a key role in tumorigenesis and provide insight into dysregulation of this gene in cancer.
**Conclusions:** These results indicate that the antitumor activity of FAS inhibitors may be mediated by direct effects on tumor cell growth or survival mechanisms.

**403** POSTER

**Cell death pathways as therapeutic targets for cancer**

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Proper execution of cell death plays an essential role in tumor suppression. Apoptosis is a form of regulated cell death. Defects in this process are a hallmark of cancer and contribute to chemotherapy resistance. Our laboratory recently determined that the proapoptotic protein Bim determines tumor responsiveness to taxanes and that Bim inactivation by MAPK-mediated phosphorylation and degradation in proteasomes confers chemotherapy resistance. The same studies also revealed a mechanism by which addition of a proteasome inhibitor reactivates apoptosis and abrogates MAPK pathway-dependent resistance to taxanes enabling tumor regression. These preclinical studies are now being translated to a clinical trial of taxane and bortezomib combinatorial treatment for solid tumors with activated MAPK pathway, and set an example of rationally designed tumor genotype-specific chemotherapy.
An alternative to reactivation of apoptosis is to divert apoptosis-resistant tumor cells to an alternate pathway of cell death such as type II programmed cell death (autophagy). Beclin1 is a key regulator of autophagy and defective autophagy plays a role in mammary oncogenesis since beclin1 haploinsufficiency is common in human breast carcinomas. We have developed a novel mouse mammary epithelial model for studying the mechanisms regulating breast tumorigenesis and are applying this model to determine the role of autophagy in breast cancer progression and treatment responsiveness. Primary mouse mammary epithelial cells (MMECs) were isolated from beclin1 +/− and beclin1 +/+ mice, immortalized (iMMECs) by inactivation of the retinoblastoma and p53 pathways, and their response to metabolic stress, capacity for 3D-morphogenesis, and tumorigenicity were compared. Allelic loss of beclin1 in iMMECs increased susceptibility of iMMECs to metabolic stress, indicating that autophagy is indeed a survival, and not a cell death, mechanism in mammary epithelial cells. Furthermore, beclin1 +/− iMMECs were more tumorigenic than beclin1 +/+ iMMECs after orthotopic injection demonstrating that beclin1 haploinsufficiency promotes mammary tumorigenesis. Thus, autophagy may function as a tumor suppression mechanism by mitigating metabolic stress, thereby preventing the accumulation of damaged tumor cells that can promote tumor progression. These findings also suggest that autophagy inhibitors may be a means to drive apoptosis resistant tumor cells to cell death in response to metabolic stress.

**404** POSTER

**Pharmacodynamics (pd) of xl880, a novel spectrum selective kinase inhibitor (SSKI), administered orally to patients (pts) with advanced solid tumors (AST)**

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**Background:** XL880 is a sub-nM inhibitor of the hepatocyte growth factor receptor (Met) and vascular endothelial growth factor (VEGF) family receptor tyrosine kinase (RTK), with low in vitro nM inhibition of PDGFRβ, KIT, FLT3, Tie-2 and Ron. It is the 1st orally bioavailable small molecule Met inhibitor to enter the clinic. An ongoing phase I study of XL880 in pts w/ASTs showed that XL880 is well tolerated up to 3.6 mg/kg, with definition of the maximum tolerated dose ongoing. Two dose limiting toxicities have been observed (1 grade 3 lipase and 1 grade 3 transaminase). Manageable hypertension and edema have been seen in pts treated at the highest doses for prolonged times. Two pts w/spontaneous papillary renal cell carcinoma (SPRC) have a PR (1 unconfirmed), 2 pts w/carcinoid and melanoma have had MRs and a pt w/medullary thyroid cancer showed tumor reduction by physical exam and decreased cortisol levels while receiving XL880.
**Methods:** Blood samples were collected from all pts. Tumor and normal (surrogate) tissues from selected pts were collected at baseline and following administration of XL880. Plasma samples were analyzed for ligands and soluble receptors via ELISA. Selected blood samples and tumor biopsies, including diagnostic (archival) paraffin embedded tumor sections, were analyzed for mutation of Met at known mutation hotspots. Tumor, skin, and hair follicles were processed for extensive IHC analyses.
**Results:** Staining of Met, phospho-Met (pMet), RON, pRON, pERK, and pAKT was detected in normal tissue, skin, and tumor tissue from a pt with melanoma who experienced a MR. Administration of XL880 decreased tumor staining of pMet, pRON, pERK, and pAKT, but staining for Met and RON was unchanged. Decreased tumor cell proliferation (Ki67 staining) and increased tumor cell apoptosis (TUNEL) were also observed. No hotspot mutations were observed in SPRC pts who exhibited PRs. However, when compared to adjacent normal renal tissue, staining for Met, pMet, RON, pRON and Ki67 was elevated in untreated tumor tissue. Additional pt tumors and tumor vasculature are under analysis.
**Conclusions:** In pts with solid tumors, administration of XL880 is associated with decreased activation of Met and RON, decreased activity of associated signaling pathways (AKT and ERK), decreased tumor cell proliferation, and increased tumor cell death. These data from clinical human samples are consistent with preclinical data demonstrating that XL880 exhibits potent anti-tumor activity by targeting MET. The responsiveness of SPRC pts to XL880 was not associated to mutational activation of Met. In the absence of clinical evidence of target inhibition with this novel SSKI, the tumor staining confirms activity against Met at doses at or below VEGF receptor and PDGFRβ inhibition.

**405** POSTER

**A phase I dose-escalation study of the safety and pharmacokinetics of a XL184, a VEGFR and Met kinase inhibitor, administered orally to subjects with advanced malignancies**

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**Background**: XL184 is an orally available small molecule inhibitor of multiple receptor tyrosine kinases involved in tumor cell growth and angiogenesis. The primary targets of XL184 are Met, VEGFR2/KDR, and additional targets include KIT, FLT3, and Tie-2. The purpose of this study is to define the maximum tolerated dose (MTD) and pharmacokinetics (PK) of XL184. In addition, exploratory pharmacodynamic (PD) assays are being evaluated using blood plasma samples.
**Methods:** Patients (pts) with advanced solid malignancies are enrolled in successive cohorts to receive XL184 orally as a single dose on day 1 with pharmacokinetic (PK) sampling, followed on day 4 by 5 consecutive daily doses with additional PK sampling and observation until day 21. In subsequent cycles, pts receive daily dosing for 5 days every 14 days. Tumor response is assessed every 8 weeks by RECIST criteria. PD blood samples were collected from all pts and plasma samples will be analyzed for ligands and soluble receptors via ELISA.
**Results:** To date, a total of 12 pts (carcinoid [3], mesothelioma [1], gastric [1], pancreatic cancer [1], breast cancer [1], parotid carcinoma [1], cholangiocarcinoma [1], T-cell lymphoma [1], angiosarcoma [1] and gastro/esophageal carcinoma [1]) have been treated across 3 dose levels: 0.08, 0.16, and 0.32 mg/kg. Currently, the maximum tolerated dose is not yet defined and dose escalation continues. Of 12 treated pts, 3 have had stable disease greater than 3 months (ongoing stability at 7, 6 and 4 months), including one patient with carcinoid carcinoma with liver metastases who has had approximately 20% reduction in tumor size (treated at 0.08 mg/kg). There have been no drug-related AEs or SAEs to date. Preliminary PK analysis (0.08–0.32 mg/kg) indicates that systemic drug exposure (area under the plasma concentration-time curve; AUC) and peak plasma levels (Cmax) tend to increase with increasing XL184 dose. Average Cmax values were 34.2±20.7, 70.0±51.6, and 189.3±49.6 ng/mL following the fifth dose at 0.08, 0.16 and 0.32 mg/kg, respectively. The terminal half-life was approximately 90 hours after 5 days of dosing, with levels as high as

111 ng/mL persisting for 96 hours following the fifth dose at 0.32 mg/kg. Plasma PD data will be analyzed in relation to PK data.
**Conclusions:** XL184 appears to have evidence of antitumor activity, even at doses not associated with toxicity. Its long terminal half-life may permit evaluation of alternative schedules.

**406** POSTER

**Establishment and in vivo evaluation of two human sarcoma xenograft models: results of tumor growth and chemotherapy sensitivity in models of mesenchymal chondrosarcoma (MCS) and leiomyosarcoma (LMS)**

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Soft tissue sarcomas are malignant tumors which originate from fat, muscle, nerve, blood vessel, and fibrous or deep skin tissues and occur in most parts of the body. Mesenchymal chondrosarcoma is a rare, chondrogenic neoplasm often developing in the spine, ribs and jaw and metastasizing to lungs, lymph nodes and other bones. Leiomyosarcoma is a malignant tumor originating from smooth muscle tissue, most often the retroperitoneum, internal organs and blood vessels. While occurrence of either tumor is quite rare, current therapies are limited and treatment potential for newly approved agents and novel combination regimens are largely unknown, primarily due to lack of evaluable models for each disease.
The goal of this project was to develop and establish transplantable models of disease for MCS and LMS and evaluate tumor growth inhibition (TGI) or delay (TGD) potential of newly approved agents and novel combination therapies. For model development, MCS and LMS tumors were removed from patients and fragments xenografted into male CD-1 nude mice; tumors were propagated and amplified until growth was stable and take rate $\geqslant$80%; molecular profiling was then performed on each tumor to establish baseline levels of relevant proteins and signaling molecules. Following establishment, these models were evaluated for sensitivity towards a panel of single agent and combination therapies, many including bevacizumab (Avastin®) with significant TGI ($p < 0.05$) and partial/complete responses reported in several regimens. Further, some treatments resulted in selective activity for MCS or LMS tumors, suggesting model specificity. From this project, we report two established sarcoma models and demonstrate significant, directed antitumor activity of novel single agent and combination treatment regimens. Further experiments are underway comparing molecular profiles of treated versus control tumors to determine mechanism in sensitive models and to identify additional useful combination therapies for treatment of MCS and LMS.

**407** POSTER

**New derivative of betulinic acid induces apoptosis by mitochondrial disruption and direct interaction with cytochrome c**

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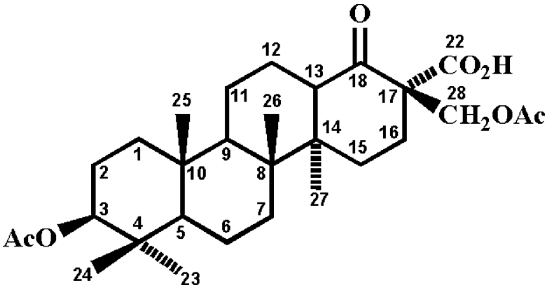
**Background:** The anti-tumour properties of lupane-derived triterpenoid compound were first discovered over 30 years ago when extract from the stem bark of various plants were tested for cytostatic ativity using different in vivo cancer model systems. We have recently developed a new synthetic betulinic acid derivative 3β,28-diacetoxy-18-oxo-19,20,21,29,30-pentanorlupan-22-oxic acid (JS8), that induces rapid apoptotic response in various tumor cells of different origin under in vitro conditions. The compound was effective in a concentration range $IC_{50}$ = 0.19−4.66 μM.
**Material and Methods:** For further study of the apoptotic machinery, we used the CEM-lymphoblastic leukemia cell line and various molecular biology methods as western blotting, flow cytometry techniques, mass spectrometry and UV/VIS and resonance Raman spectroscopy.
**Results:** Several lines of evidence indicate that JS8 induced apoptosis depends on mitochondria. We found that JS8 promotes elevation of intracellular reactive oxygen species which may affect the mitochondrial transmembrane potential and mitochondrial membrane permeability profoundly. After disruption of the mitochondrial transmembrane potential, cytochrome c is released from mitochondria into the cytosol and this leads to activation of the caspase cascade. Release of cytochrome c and apoptosis was completely prevented by the antioxidant N-acetyl-L-cysteine and KCN, an inhibitor of oxidative phosphorylation complex IV. These data suggest that the primary target of JS8 is in between complexes III and IV, where cytochrome c is located. Direct coincubation of JS8 with purified cytochrome c in vitro resulted in the formation of noncovalent complexes which were characterized using UV/VIS and resonance Raman spectroscopy and mass spectrometry techniques. Selectivity of JS8-cytochrome c interaction was further confirmed by low or absent capacity of the compound to bind other (un)related proteins: cytochrome $b_5$, cytochrome P450, myoglobin and lysozyme.




3β,28-Diacetoxy-18-oxo-19,20,21,29,30-pentanorlupan-22-oxic acid (code name JS8).

**Conclusions:** The results show that cytochrome c is the primary target for betulinic acid derivative JS8 and potentially for other cytotoxic triterpenoid compounds.
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**408** POSTER

**Human RNase 1 variants are effective anti-cancer agents**

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The ability to destroy RNA in a cell-selective manner would provide a new pathway for attacking cancer cells: inhibiting the cancer cell from making proteins and eventually resulting in cell death. RNA can be cleaved by a family of enzymes called ribonucleases, one of which is the widely studied and well-characterized bovine ribonuclease A (RNase A). As a member of the pancreatic RNase family, bovine RNase A is not active inside cells due to the presence of a cytosolic protein called ribonuclease inhibitor (RI). Previous reports have shown that bovine RNase A variants which have diminished binding to RI but retain ribonucleolytic function can kill cancer cells *in vitro* and *in vivo*.
Our primary objective was to develop variants of human RNase 1 with a positive safety and efficacy profile and to advance a candidate into the clinic. Variants of mammalian RNases with diminished binding to RI are called EVade™ RNases. Safety data as well as efficacy in xenograft models will be presented with an emphasis on the lead candidate that has been selected for IND-enabling studies.
The EVade™ RNases are currently expressed in inclusion bodies in *E. coli* and purified by traditional column chromatography. A FRET-based assay, utilizing a mixed oligonucleotide with quenching fluorophores at either end as the substrate, is used to determine enzymatic activity. A similar activity assay can be used to measure the binding of RNases to RI. Our RNase variants are tested for the inhibition of human tumor growth in tumors implanted in the flanks of nude mice (xenograft models).
QBI-188 is a variant of human RNase I that retains ~99% sequence identity with the native protein. QBI-188 has demonstrated tumor regression in a non-small cell lung cancer (A549) xenograft model. Additional variants that have significantly inhibited tumor growth (>60%) with broader efficacy will also be presented. Additional xenograft models to be presented are: prostate (DU145), pancreas (Bx-PC-3), and ovarian (SKOV-3) cancer. Xenograft study data for FDA approved therapeutics will also be provided as positive controls. In addition to efficacy data, pharmacokinetic data and clinical chemistry for EVade™ RNases will be provided.
EVade™ RNases created from the human pancreatic RNase 1 have potent anti-tumor activity *in vivo* compared to standard therapies and an RNase from another species. These data suggest that the engineered ribonucleases are strong candidates for development as therapeutic agents.