cells was compared with control sequence transfected cells by Time Lapse Video Microscopy (TLVM). HSP27 was suppressed with specific siRNA and antisense (OGX-427). Normal human bronchial epithelial cells (NHBE) were used as controls. Viability was measured with quantification of absolute cell numbers. Wortmannin and LY290042 were used to inhibit PI3K and the effect on motility determined.
**Results:** HSP27/Phospo-HSP27 were overexpressed in 65%/61% of tumor tissues. HSP27 expression was higher in 30% of matched metastases in comparison to the primary tumor and lower in only 3% ($p < 0.0001$). HSP27 was overexpressed in 92% of Stage IIIB/IV tumors in comparison to only 65% in Stages I-IIIA ($p = 0.048$). Preliminarily HSP27 expression correlation with survival did not reach statistical significance ($N = 163$), but a trend as a negative prognostic marker was evident and particularly clear for squamous cell histology. Additional staining of TMAs (pre-specified goal $N = 500$) is ongoing.
HSP27 overexpressing cells showed significantly increased cell motility in comparison to control transfected cells. HSP27 suppression with specific siRNA and OGX-427 antisense lead to growth inhibition and tumor cell death. There was no toxic effect on NHBE cells. In addition HSP27 suppression with OGX-427 HSP27 specific antisense markedly decreased cell motility/migration and membrane ruffling. PI3K inhibition with both Wortmannin and LY290042 abrogated the pro-motility effects of HSP27 overexpression.
**Conclusions:** HSP27 is a promising novel target in NSCLC with a prominent role for migration/cell motility, and membrane ruffling, and is PI3K dependent. In addition increased expression in NSCLC metastases suggest a role of HSP27 in the metastatic process. HSP27 inhibition is feasible and leads to cell death as well as near complete abrogation of surrogate markers of metastasis – cell motility/migration, and membrane ruffling. A phase I trial of OGX-427 is scheduled to start this year.

**382** POSTER

**Disulfiram inhibits the E3 ligase activity of breast cancer associated gene 2 (BCA2) and the growth of BCA2-expressing breast cancers in vitro and in vivo**

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We have isolated a novel monomeric RING-finger ubiquitin E3 ligase BCA2 from invasive breast cancer cells by subtractive hybridization cloning. BCA2 is overexpressed in more than 50% of invasive breast cancers compared to normal tissues. Overexpression of BCA2 increases proliferation of normal cells, whereas small interfering RNA inhibits the growth of BCA2-expressing breast cancer cells. A binding partner of BCA2 is Rab7. Rab7 is known to regulate endocytic trafficking of the epidermal growth factor (EGF)/EGF-receptor complex. Overexpression of BCA2 was found to inhibit Rab7-mediated EGF degradation. This suggested to us that the BCA2 E3 ligase represents a target for mechanism-based drug development. Here, we evaluated means to inhibit the catalytic activity of BCA2. To examine the structural requirements for its E3 ligase activity and for small molecule inhibitors, we created RING-finger mutants and assessed their ubiquitination activity. Mutation of the $Zn^{2+}$-complexing cysteine residues in the RING-finger completely abolished ubiquitination activity. Consequently, we tested 10 compounds, which have been described to release zinc from zinc-finger proteins. Only one agent, namely disulfiram was able to inhibit BCA2. The latter translated into inhibition of growth of the BCA2-expressing breast cancer cell lines MCF-7 and T47D. Cells lacking BCA2 expression such as MDA-MB-231 were insensitive. In MTT assays performed with T47D and MCF-7, the TGI of disulfiram was 0.5 and 1 μM respectively. Other zinc ejectors were inactive (TGI>100 μM). MCF-7 cells were treated at $IC_{50}$ (0.25 μM) and TGI concentrations of disulfiram and whole cell lysates evaluated for endogenous BCA2 expression by Western blotting. Exposure of BCA2 to disulfiram yielded bands identical to mutant RING-finger protein. In vivo activity of disulfiram was assessed in advanced stage MCF-7 xenografts grown in nude mice. Disulfiram was given orally for 5 days at doses of 50 mg/kg/d and 25 mg/kg/d. The treatment was well tolerated. Marked tumor growth inhibition (T/C=58%, $p = 0.018$) was observed at 50 mg/kg/d, which was accompanied by minor remissions. However, after treatment was terminated, the tumors grew back. Ongoing experiments are evaluating disulfiram combinations with standard cytotoxic agents and will also be reported. Our data indicate that BCA2 is novel a target for the treatment of breast cancer and that it might be possible to develop specific inhibitors of BCA2 based on disulfiram.

**383** POSTER

**A Phase I Study of dasatinib, a Src and multi-kinase inhibitor, in patients (pts) with GIST and other solid tumors**

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**Background:** Dasatinib (BMS-354825) is a potent, orally active, inhibitor of several kinase signaling molecules including members of the SRC family of kinases, KIT, PDGFR, EPHA2 and BCR-ABL. We report the results of a Phase I study evaluating the safety, tolerability, and pharmacologic profiles of dasatinib in pts with treatment-resistant GIST and other refractory solid tumors.
**Methods:** Pts with adequate hematologic, renal, cardiac and liver function, received dasatinib orally BID for 5 days followed by a 2-day break, every week. A continuous twice-daily (CTD) schedule was also explored. Pts were assessed continuously for safety. Pharmacokinetics (PK) were evaluated on days 1, 8, 26. Pharmacodynamic (PD) biomarkers were assessed on week 1 and on day 26. Serial imaging with CT was performed at least every 8 weeks, with FDG-PET on weeks 1, 4, and 8.
**Results:** 48 pts [M=27, F=21] ECOG PS $\leqslant$1 (1 pt PS=2) with GIST ($n = 18$) or other solid tumors ($n = 30$) were treated at 1 of 7 escalating dose levels: 35, 50, 70, 90, 100, 120 and 160 mg BID. Dose-limiting toxicity (DLT) was observed in 2 of 4 pts treated at the 160 mg BID on the 5 on/2 off schedule consisting of grade (gr) 2 rash requiring dose reduction and gr 3 asymptomatic hypocalcemia requiring calcium supplementation. Pts were then enrolled in the CTD schedule at 70 mg and 90 mg BID where 2 of 6 pts reported a DLT (recurrent gr 2 rash, and discontinuation due to gr 2 nausea and vomiting and lightheadedness in 1 pt each). 100 mg BID on the CTD schedule is currently explored with no DLTs reported in first 2 evaluable pts. No significant myelosuppression was observed; non-hematologic toxicities including fluid retention, gastro-intestinal intolerance, skin rash, headache and bleeding were infrequently reported; they were mostly gr 1/2 causing dose reductions in 15% of pts, mostly at 160 mg. No objective responses on CT have been reported. Activity has been noted as mixed responses on FDG-PET; treatment with dasatinib was continued for $\geqslant$3 months in 4 GIST and 3 sarcoma pts and 1 pt each with biliary tract cancer, mesothelioma and melanoma. Dasatinib substantially inhibited p-SRC, implying *in vivo* modulation of SRC kinase activity. Inhibition of p-Src correlated with the PK of dasatinib. A $\geqslant$50% inhibition for $\geqslant$16 hours was achieved at 90 mg BID.
**Conclusions:** Dasatinib can be safely administered at doses of up to 120 mg BID on a 5-days on, 2-days off, weekly schedule. Dose escalation continues at 100 mg BID on the CTD schedule.

**384** POSTER

**Smac mimetics selectively induce apoptosis in cancer cells but not in normal cells**

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**Background:** X-linked inhibitor of apoptosis protein (XIAP) surpresses apoptosis in cells by binding to and inhibiting of an initiator caspase-9 and effectors caspase-3 and -7. XIAP represents a promising new molecular target for anticancer drug design. We have designed and synthesized a novel class of non-peptide Smac mimetics that bind to XIAP with extremely high affinity and function as highly efficient antagonists of XIAP in cell-free assays.
**Material and Methods:** We present herein our characterization of these potent Smac mimetics for their activity in cell growth and apoptosis induction in the MDA-MB-231 human breast cancer cell line. In addition, we elucidate in detail their molecular mechanism of action in induction of apoptosis.
**Results:** Our fluorescence-polarization-based binding assays show that our designed Smac mimetics bind to XIAP with low nanomolar affinity ($IC_{50}$ values = 1–5 nM), being 1000-times more potent than the Smac AVPI peptide. Our most potent Smac mimetics inhibit cell growth with $IC_{50}$ values of less than 1 nM in the MDA-MB-231 cancer cell line and induce robust apoptosis at 1 nM or less within 12 hours. Consistent with their mode of action, they induce rapid and strong activation of caspase-9, -3 and -7 within 6 hours at 1–10 nM and cleavage of PARP. They have no or very little toxicity to normal cells, displaying over 1000-fold selectivity for cancer cells over normal cells. We also found that these Smac mimetics are extremely potent and effective in induction of apoptosis in a panel of cancer cell lines at 1–10 nM.
**Conclusions:** Our studies show that potent Smac mimetics can be highly effective in induction of rapid apoptosis in cancer cells as single agent at

extremely low concentrations, while displaying an outstanding selectivity over normal cells. Our results suggest that our potent Smac mimetics warrant extensive evaluation as a new class of anticancer agents for the treatment of human cancer by overcoming apoptosis resistance of cancer cells.

**385** POSTER

**Targeting p53-independent apoptosis in refractory breast cancers**

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"Triple-negative" or "basal-like" breast cancers represent a tumor subtype that express neither estrogen/progesterone receptors nor Her2 protein and have a relatively poor prognosis despite conventional therapies. To date little progress has been made in identifying specific molecular pathways associated with these refractory cancers that may be effectively targeted for therapeutic intervention. Our previous work demonstrated that p63, a member of p53 gene family, is upregulated in certain epithelial tumors and is required to promote tumor cell survival through its ability to bind and inhibit the pro-apoptotic activity of the related family member p73. The importance of p63 in normal mammary development implies that dysregulation of p63 might also contribute to breast cancer pathogenesis. Here, we demonstrate that p63 and p73 are exclusively expressed in a subset of primary triple-negative breast carcinomas that exhibit frequent mutational inactivation of p53. Consistent with these findings, we find that p63 and p73 mRNA and protein are also co-expressed in a subset of triple-negative breast cancer cell lines including MDA-MB-468 and HCC1937. To determine the functional role of p63 in this subtype of breast cancer, we tested the effect of endogenous p63 knockdown by lentiviral small hairpin RNA (shRNA) expression in HCC1937 and MDA-MB-468 cells. Inhibition of endogenous p63 in these cells induced the pro-apoptotic bcl-2 family members Puma and Noxa, followed by apoptosis. In contrast, no effect was observed using control shRNA constructs in these cells, nor was any effect observed following expression of p63-directed shRNAs in MCF-7 and Saos2 cells that do not express p63. The induction of Puma, Noxa and apoptosis in triple-negative breast cancer cells is highly dependent on p73 function, as inhibition of p73 by lentiviral shRNA in HCC1937 cells completely abrogated apoptosis following knockdown of p63. These results suggest that p63 promotes survival of breast cancer cells by inhibiting the pro-apoptotic activity of p73. Consistent with these findings, we demonstrate that p63 directly interacts with endogenous p73 and that expression of p63 blocks p73-dependent transcription in a dose dependent manner. Together these findings demonstrate p63 and p73 mediate an essential and tumor-specific survival pathway in triple-negative breast cancers. Therefore, targeting p63 and/or p73 may represent an attractive therapeutic strategy against these refractory tumors.

**386** POSTER

**Knockdown of PRL levels by siRNA influences response to etoposide in pancreatic cancer cells**

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**Background:** We previously identified several genes involved in stress response as differentially regulated in pancreatic cancer cells following PRL-1 or PRL-2 silencing with siRNA (Proc Am Assoc Cancer Res 2005; 46: 5508). We sought to further investigate the role that PRL phosphatases might play in regulating stress response by treating cells in combination with PRL targeting siRNAs and chemotherapeutic agents as inducers of stress.
**Material and Methods:** PRL or non-targeting siRNA treated MIA PaCa-2 and PANC-1 cells were treated with the chemotherapeutic agents etoposide and bortezomib. Cell cycle profiles as well as Caspase-3 activity were then evaluated to determine the effect of PRL knockdown.
**Results:** Etoposide treated MIA PaCa-2 (and PANC1 to a lesser degree) cells with PRL-1 and/or PRL-2 knockdown were morphologically distinct from their non-targeting siRNA control counterparts. Cell cycle analysis confirmed that cell cycle distribution was significantly altered in the etoposide treated cells with PRL knockdown compared to the control siRNA treated cells. However, when cells were treated with the proteasomal inhibitor bortezomib, significant differences in morphology or cell cycle distribution were not observed.
**Conclusions:** Our results indicate that PRL-1 and PRL-2 knockdown might affect how pancreatic cancer cells respond to certain chemotherapeutic agents. We are currently evaluating cell cycle distribution and induction of apoptosis in these cells lines using other chemotherapeutic agents with known activity against pancreatic cancer (gemcitabine, erlotinib and 5-fluorouracil). This should aid in identifying chemotherapeutic agents, which might successfully be used in combination with PRL inhibitors.

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**387** POSTER

**A phase Ib trial of ARQ 501, a checkpoint pathway activator, in combination with docetaxel in patients with advanced solid tumors**

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ARQ 501 is a DNA damage checkpoint pathway activator whose effect is to induce selective cell death in cancer cells through E2F1 pathway, independent of cell's p53 status. In preclinical models, ARQ 501 demonstrates potent anticancer activity with a high therapeutic index, an effect greatly enhanced by the addition of a variety of cytotoxic agents, including taxanes. Therefore, a phase Ib dose escalation study began in patients with advanced solid tumors to determine the maximum tolerated dose (MTD), pharmacokinetics, and preliminary antitumor activity of ARQ 501 in combination with docetaxel. In all arms of the study, docetaxel (50, 75 or 100 mg/m$^2$) was given every 21 days. Within this 21 day cycle, several schedules of ARQ 501 administration were investigated. These included: (1) ARQ 501 given days 1–5 and docetaxel administration on day 3 of each cycle; (2) ARQ 501 infused over one or three hours followed by an infusion of docetaxel on day 1 of each cycle. In this schedule, additional weekly infusions of ARQ 501 were added, as tolerated, to achieve a final schedule of weekly ARQ 501. With the first schedule, MTD was reached at a dose of 120 mg/m$^2$/day (600 mg/m$^2$ per 21 day cycle). With weekly ARQ 501 administration, maximum doses administered to date are 390 mg/m$^2$ for the one hour infusion and 550 mg/m$^2$ for the three hour infusion times. In all cases, anemia has been the major adverse event, which has limited dose escalation. Evidence to date suggests that this is due to hemolysis of circulating mature red cells as a result of oxidative stress (unrelated to the checkpoint activation). Other adverse events have been generally mild and include neutropenia, hyperglycemia, hypomagnesemia, fatigue, pyrexia, naseau, and hyperbilirubinaemia.
As of May 30, 2006 38 patients have been enrolled with monitored data available for 31 patients. Nine of 11 patients enrolled in schedule 1 were evaluable for efficacy. Of these, 5 patients achieved a best response of stable disease (SD) or better (9.4 to 23.6 weeks). Of note, 2/4 ovarian cancer patients, who had failed prior therapies with platinum and taxanes, achieved a partial response (PR) per decrease in CA 125 levels. One also showed a 42% reduction per RECIST, but no confirmatory scan was performed. To date, 27 patients have been treated on schedule 2. Twelve patients remain on study, and 18 patients are evaluable for efficacy. Fourteen patients achieved a best response of SD or better (6 to 32 weeks). Three patients achieved a PR (unconfirmed), including a 47.6% regression at week 12 in a patient with head and neck cancer, a 51% regression at week 6 in a patient with pancreatic adenocarcinoma, and a 33.9% regression in a patient with ovarian cancer.
These data suggest that the combination of the checkpoint pathway activator ARQ 501 with a taxane is well tolerated and has encouraging signs of anti-tumor activity, particularly in ovarian cancer. Phase II investigation in this condition is warranted.

**388** POSTER

**Expression profile of histone deacetylases and histone H4 acetylation in selected B- and T-cell lymphomas**

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**Background:** Histone deacetylase inhibitors (HDACi) are in clinical trials for a variety of malignant diseases. Interestingly, in hematological malignancies the HDACi SAHA and depsipeptide have shown remarkable efficacy in cutaneous T-cell lymphoma (CTCL) with relatively fewer responses in diffuse large B-cell lymphoma (DLBCL). The reason for this suggested class effect of HDACi in B- and T-cell malignancies is unknown. In a study on breast cancer, low levels of histone H4 acetylation prior to treatment with an HDACi were predictive of response. In the present investigation, we have examined the expression of selected HDACs and the acetylation of histone H4 in CTCL and DLBCL.
**Material and Methods:** CTCL (n = 75) and DLBCL (n = 31) samples were examined for expression of HDAC1, HDAC2, HDAC6, and acetylated H4 by immunohistochemistry. Stained samples were grouped in three expression categories (negative/low, moderate, high) based on the proportion of positive cells and staining intensity in each sample. Comparisons were done using Chi-square tests with exact probabilities.