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.

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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.

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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.

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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