cycles (median 2, range 1–8+) of therapy. Three of 9 patients at Sarasar 75 mg BID/ C 75 mg/m2/G 1000 mg/m2 experienced DLT (1 pt: gr 3 N/V (Sarasar and C both given day 1, 2 pts: gr 4 ANC). Other at least possibly drug-related toxicities have included gr 3–4 thrombocytopenia, gr 2–3 nausea and vomiting, gr 2 fatigue/asthenia, gr 2 diarrhea, gr 1 transaminitis, and gr 1 tinnitis. The extent of myelosuppression appears to be enhanced in patients with extensive prior therapy, whereas N/V has been ameliorated with oral antiemetics administered for several days following therapy with C. Two previously treated patients with breast cancer have demonstrated confirmed clinical responses, one CR (chest wall disease) lasting for 7 cycles and one PR (soft-tissue) that is ongoing at 8+ cycles of therapy. These patients received their first cycles at doses of Sarasar/C/G of 75/75/1000 (d1, 8, 15) q 4 weeks, respectively, while all subsequent cycles have been at Sarasar/C/G doses of 75/75/750, respectively. Based upon tolerability over several cycles, accrual is ongoing at the Sarasar 75 mg/m2 BID/ C 75 mg/m2/G 750 mg/m2 (d1, 8) dose level on the every three-week schedule. These results suggest that this novel combination might be active in the treatment of metastatic breast cancer, a tumor type that has demonstrated single-agent responses (albeit modest) to farnesyl transferase inhibitors. Pharmacokinetic and biologic correlative analyses, including farnesyl transferase functional inhibition and surrogate assays, will be presented.

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Genetic validation of activated polyamine catabolism as a novel therapeutic strategy targeting prostate cancer

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Background: Depletion of intracellular polyamine pools invariably inhibits cell growth and thus, represents a viable therapeutic/prevention strategy. Although this is usually accomplished by inhibiting biosynthetic enzymes, we propose that it might be more effectively achieved by activating polyamine catabolism at the level of spermidine/spermine N^1-acetyltransferase (SSAT), an enzyme known to be inducible by various drugs and compounds. Our previous studies have confirmed this strategy in MCF-7 breast carcinoma cells (Vujcic et al., J. Biol. Chem. 275:38309, 2000) . On the basis of unique polyamine homeostatic responses in the prostate gland, we have reason to believe that tumor cells derived from it may be particularly sensitive to this approach.
Methods and Results: SSAT was conditionally over-expressed in LNCaP prostate carcinoma cells via a tetracycline-repressible system. Tetracycline removal resulted in a ~20-fold increase in SSAT mRNA and enzyme activity and a massive accumulation of SSAT acetylated polyamines. This, in turn, led to sustained growth inhibition that unexpectedly, was not associated with spermidine and spermine depletion. Rather, polyamine pools were maintained by a compensatory increase in biosynthetic enzyme activities that gave rise to heightened metabolic flux through polyamine biosynthetic and catabolic pathways. Treatment with the biosynthetic inhibitor α-difluoromethylornithine during SSAT induction interrupted flux and prevented growth inhibition, thus, demonstrating a cause-and-effect relationship. Of the various underlying mechanisms investigated, flux-induced growth inhibition correlated closely with a 50% depletion in the SSAT cofactor, acetylCoA as measured by capillary electrophoresis. Having demonstrate the antiproliferative potential of this approach, we next examined the *in vivo* consequences of SSAT overexpression in mice genetically predisposed to develop prostate cancer. TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) female mice were cross-bred with male transgenic mice that systemically over-express SSAT. At 30 wk of age, the average genitourinary tract weight of TRAMP/SSAT mice was 75% smaller than that of TRAMP mice and by 36 wk, it was ~92% smaller. SV 40 large T-antigen expression in the prostate epithelium were similar in TRAMP and TRAMP/SSAT mice. Consistent with an 18-fold increase in SSAT activity in the TRAMP/SSAT bigenics, prostatic putrescine and acetylated spermidine pools increased remarkably relative to the TRAMP mice while spermidine and spermine pools were minimally affected due to a compensatory increase in biosynthetic activity similar to that seen in LNCaP cells. This heightened metabolic flux resulted in >70% reduction in acetyl-CoA in TRAMP/SSAT prostate tumors while having only a minor effect on acetylCoA levels in the liver. A role for SSAT in fat metabolism is indicated by markedly reduced levels of abdominal and subdermal fat in SSAT transgenic and bigenic mice. Taken together, the antitumor activity deriving from activated polyamine catabolism appears to be related to downstream effects on acetylCoA and fat metabolism.
Conclusions: In addition to elucidating the overall antitumor effects of SSAT overexpression in prostate cancer and defining previously unrealized metabolic consequences, the present findings provide *in vitro* and *in vivo* genetic support for the discovery and development of specific small molecule inducers of SSAT as a novel therapeutic strategy targeting prostate cancer. Given the known high responsiveness of this enzyme system to various anticancer drugs, polyamine analogs, and other agents, such a molecule should not be difficult to identify.

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The cytotoxic effects of 17-AAG, an inhibitor of Hsp90 are enhanced by combination with the PI-3-kinase inhibitor LY294002 in non-small cell lung cancer (NSCLC) cell lines

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NSCLC cells rely on multiple genetic abnormalities that result in several aberrant signaling pathways that in turn mediate cancer maintenance and progression.
Because of cellular signaling redundancy it is anticipated that interruption of a single signaling network or transforming molecule will not significantly affect tumor growth.
As an alternative approach, we have examined the effects of 17AAG a derivative of geldanamycin, a drug that alters the function of heat shock protein 90 (Hsp90), a ubiquitously expressed molecular chaperone that appears to play an essential role in malignant transformation by regulating the stability and activity of multiple oncogenic growth factor receptors and proteins important in promoting tumor proliferation and survival such as EGFR and p-Akt.
A549 (PTEN wild-type) and H157 (PTEN mutant) cell lines with low and high constitutive activated Akt expression respectively were used for these experiments. Exposure of A549 and H157 cells to 17AAG resulted in inhibition of cell growth as measured by MTT assay with IC_{50} concentrations of 5 μM and 500 nM respectively at 72 hours. Flow cytometry at 24 and 48 hours revealed G1/S and G2/M arrest respectively.
We reasoned that using 17AAG to destabilize Hsp90 proteins, while simultaneously targeting directly one of the most dominant signaling pathways, the PI-3 kinase pathway might result in improved tumor cytotoxicity. LY294002, a PI-3 kinase inhibitor inhibited growth at IC_{50} concentrations of 30–40 nM for both cell lines.
Indeed simultaneous exposure for 72 hours to equitoxic concentrations (ratios of IC_{50}) led to supra-additive cytotoxic effects for the A549 and synergistic effects for the H157 cells with inhibition achieved at suboptimal concentrations of the individual drugs.
The effects of the combination on apoptosis and cell cycle were also evaluated and will be presented.
Depletion of target client proteins was examined by immunoblot analysis. Dramatic decreases in p-Akt, p-GSK3β, pERK1/2, c-Raf and cyclin D1 were observed in H157 and to a lesser degree in A549 cells, while induction of apoptosis as evidenced by PARP cleavage, and detection of caspases, 3, 9 and 8 was also seen.
Studies are underway to clarify further the molecular determinants of interaction between Hsp90 and PI-3 kinase inhibitors, aiming at identifying the mechanisms of differential sensitivity and predictors of response. The information obtained from the present study could have direct clinical applications in the treatment of NSCLC (supported by the ASCO CDA and P50CA91007–02).

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Expression of genes relevant for tumour aggressiveness in endometrial cancer

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Objective: The aim of this study was, to analyse the expression of identified genes in endometrial cancer tissue, witch might be responsible for tumour aggressiveness.
Methods: After identification and analysis of differential displayed cDNA in matched paired patients with endometrial cancer with and without metastatic recurrence, three cDNA samples (named edi1, edi-2 and edi-3) were reamplified and sequenced. An NCBI-database request on homologies on these three sequences was done. To measure and to analyse the expression of these three cDNA samples TAQMAN-assay was used on 54 cases (42 without and 12 with recurrent disease). Statistical analysis was done by using Mann-Whitney – U-Test, Cox-regression model and Kaplan-Maier concerning to overall survival and recurrence free interval.
Results: The mean age of patients in this study population was 68 years (range 34–89), the mean weight was 75kg (range 55–132). 71.9% of patients showed no evidence of disease, 17.2% died on disease. In this