clearly involved in this feedback loop. Combination of rapamycin with the PI3K inhibitor LY294002 showed synergistic activity, which appears to be independent of the feedback activation of AKT. These findings might be of relevance for future therapeutic strategies with mTOR inhibitors in melanoma.

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Histone deacetylase inhibitors abrogate the levels and activity of estrogen receptor (ER) α and histone deacetylase (HDAC) 6 in human breast cancer cells (BCCs)

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Background: Since estrogen (E2) is mitogenic, depletion of the levels and/or activity of ERα is an effective therapy of ERα+ breast cancers. The heat shock protein (hsp) 90-based super chaperone machine binds and maintains ERα in an active conformation, allowing it to bind E2 and affect transcription of genes, e.g., progesterone receptor (PR) β and the class IIB HDAC6. Hydroxamic acid analogue pan-histone deacetylase inhibitors (HA-HDIs), e.g., LAQ824 and vorinostat, inhibit HDAC6 and induce hyper-acetylation of α-tubulin and hsp90, two known substrates of HDAC6. Hyper-acetylation was shown to inhibit ATP binding and chaperone function of hsp90 toward its client proteins, e.g., Her-2, AKT and c-Raf. Inhibition of hsp90 by HA-HDI led to depletion of hsp90 client proteins. Here, we determined the effect of HA-HDIs on ERα and HDAC6 levels and activity, and on the survival of ERα+ BCCs MCF-7 and BT-474.
Material and Methods: Cells were exposed to LAQ824 (25 to 250 nM) or vorinostat (0.5 to 2.0 μM) for 8 to 48 hours. ERα, HDAC6, p21, PRβ, p-AKT, AKT, c-Raf and p-ERK1/2 levels were determined by immunoblot (IB) analyses. Hsp90 acetylation and binding to ERα was determined by immunoprecipitation (IP) and IB analyses. ERα transactivation was determined by utilizing ER elements bound to luciferase cDNA reporter construct.
Results: Treatment of the ERα+ BCCs MCF-7 and BT-474 with HA-HDI for 8 to 24 hours induced histone acetylation and upregulated the levels of p21, and induced acetylation of α-tubulin and hsp90. Hsp90 acetylation led to decreased binding of ERα to hsp90 and polyubiquitylation of ERα. Depletion of ERα levels by HA-HDI treatment was restored by co-treatment with the 20S proteasome inhibitor bortezomib, indicating proteasomal degradation of ERα. Depletion of ERα levels was associated with abrogation of E2-induced luciferase expression and decline in PRβ and HDAC6 levels. Treatment with HA-HDI also depleted the levels of p-AKT, AKT, c-Raf and p-ERK1/2 levels in MCF-7 and BT-474 cells. This was associated with the accumulation of MCF-7 and BT-474 cells in the G2/M phase of the cells cycle, as well as induced the poly-ADP ribose polymerase (PARP) cleavage activity of caspases and loss of cell survival of MCF-7 and BT-474 cells.
Conclusions: These findings demonstrate that treatment with HA-HDIs abrogates ERα levels and activity, suggesting a potential role in the therapy of ERα positive breast cancers that are refractory to aromatase inhibitors.

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The dual specific Src/Abl kinase inhibitor AZD0530 inhibits in vitro growth and induces apoptosis in non-small cell lung cancer lines

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Background: Overexpression and activation of the non-receptor tyrosine kinase Src occurs in 50–80% of lung cancers. Src is activated by association with EGFR and in turn activates EGFR in a synergistic manner. A relationship between activated Src, cancer progression and metastatic disease has been demonstrated and provides a rationale for evaluating Src targeting agents in non-small cell lung cancer (NSCLC).
Methods: The efficacy of AZD0530, an orally available dual specific Src/Abl kinase inhibitor, was evaluated in a panel of 18 NSCLC cell lines characterized for EGFR mutation status. *In vitro* analyses included MTT assays in comparison with gefitinib, FACS analysis, western immunoblotting, and migration assays.
Results: AZD0530 IC50s were sub-micromolar, indicating physiologically relevant growth inhibition, in 11 cell lines, including 1 wild-type (wt) EGFR line insensitive to gefitinib (Table). Src gene and protein expression (Affymetrix arrays and western, respectively) did not predict sensitivity.
To date, 6 lines with sub-micromolar AZD0530 IC50 have been further analyzed: 4 EGFR mutant lines including H1975, and 2 EGFR wt lines including the line H1703 with gefitinib IC50 > 10 μM. 24-hour treatment with 1 μM AZD0530 induced G1 arrest (increases of 21–32% and 13–23% in EGFR mutant and wt lines, respectively), and western analysis showed reduced p-Src, cyclin D, p-EGFR (Tyr845), p-STAT3, and p-ERK1/2. Boyden chamber migration (1 μM AZD0530, 24 hours) was inhibited 50–60% in EGFR mutant lines H3255 and H1975, and in EGFR wt line H1703. Migration was not inhibited in EGFR wt line H358. FACS analysis demonstrated apoptosis of 33–48% after 48 hours' treatment with 1 μM AZD0530 in lines with activating EGFR mutations.

Cell lines	IC50 (μM)	
	AZD0530	Gefitinib
Activating EGFR mutations (5 lines)	0.036−0.177	0.004−0.018
H1975 (harboring T790M EGFR-TKI resistance mutation)	0.939	8
EGFR wt, gefitinib IC50 < 0.3 μM (4 lines)	0.177−0.740	<0.3
EGFR wt, gefitinib IC50 ⩾ 10 μM (8 lines)	0.72 (1 line)	
	1–3 (4 lines)	⩾10
	7–10 (3 lines)	

Conclusions: In mutant and wt EGFR NSCLC lines, AZD0530 inhibited growth, induced G1 arrest and apoptosis, inhibited downstream signaling through STAT3 and ERK1/2, and reduced migration. These data support a rationale for Src-targeting agents in the treatment of patients with NSCLC. Further assays are planned to investigate invasion inhibition by AZD0530 in this panel of cell lines.

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Inhibition of mTOR and targeting insulin-like growth factor I synergistically enhance taxol-induced cytotoxicity in Her-2 overexpressing breast cancer cells

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Background: The ErbB2 (Her-2/Neu) receptor tyrosine kinase is over-expressed in approximately one-third of human breast tumors, and this phenotype correlates with a poor clinical prognosis. ErbB2-overexpression confers resistance to taxol-induced apoptotic cell death through inhibition of p34(Cdc2) activation via ErbB2-mediated upregulation of p21(Cip1). In addition, crosstalk between Her-2 signaling and the insulin-like growth factor-I receptor (IGF-IR) pathway activates the phosphatidylinositol 3′-kinase (PI3K)/Akt and ERK, and the downstream mammalian target of rapamycin (mTOR). The IGF binding protein-3 (IGFBP-3) is an antagonist of IGF-IR signaling. IGF-IR blockade prevents rapamycin (a specific inhibitor of mTOR)-induced Akt activation and sensitizes tumor cells to inhibition of mTOR.
This study was designed to explore the ability of rhIGFBP-3 and rapamycin to enhance paclitaxel efficacy in Her-2 overexpressing breast cancer cells.
Material and Methods: MCF-7/Her2−18 cells were cultured with taxol (1−50 nM) alone or in combination with rhIGFBP-3 (20 μg/ml) and rapamycin (5 & 10 nM) for 72h. Cell survival was measured using the MTT assay. For Western blot analysis, MCF-7/Her-2 18 cells were cultured in serum-free medium for 24h and subsequently treated with IGF-I (50 ng/ml), rhIGFBP-3 (20 μg/ml), rapamycin (10 nM) alone or in combination with taxol (25 nM) for 30min.
Results: Treatment with taxol, rhIGFBP-3 and rapamycin, as single agents, inhibited the growth of Her-2 overexpressing cells in a dose-dependent manner. The triple combination of various concentrations of taxol, with constant doses of rhIGFBP-3 (20 μg/ml) and rapamycin (5 & 10 nM) exhibited greater dose-dependent inhibition of cell proliferation than each agent alone or the respective double combinations. Synergistic interactions were obtained with all the double or triple combinations as evaluated by the isobologram method (CI < 1). Western blot analysis revealed that the combination of taxol, rhIGFBP-3 and rapamycin caused a further decrease of p-Her-2 and the downstream p-Akt and p-MAPK.
Conclusions: The different combinations showed strong synergistic interactions in the Her-2 overexpressing breast cancer cells. Furthermore, we demonstrate that, rhIGFBP-3 abrogated the rapamycin and taxol-induced MAPK activation. Further analysis of the effects of these combinations on mTOR downstream components, 4E-BP1 and S6K1, apoptosis, such as caspase activation, and cell cycle progression are ongoing.